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MASTER THESIS

Nutrient contents analysis of locally available feedstuffs and formulated diets for Nile Tilapia (*Oreochromis niloticus*) production in small-scale cage culture in Kenya, East Africa

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Abstract

In times of overfishing, rapid population growth and environmental disasters, aquaculture becomes more and more of importance and provides an encouraging opportunity to appease global hunger. Aquaculture represents an auspicious source of animal protein for the crisis-ridden Africa.

This master thesis was written in the framework of the EU-funded research project BOMOSA (www.bomosa.org, 2011). The aim of BOMOSA was to introduce small-scale cage culture of indigenous species such as Nile Tilapia (*Oreochromis niloticus*) [LINNAEUS, 1758] to East Africa, whereby increasing food security and poverty alleviated by providing employment and additional income.

Based on the high tolerance to a wide range of environmental influences and the resistance to stress and disease, Nile Tilapia (*Oreochromis niloticus*) represent an excellent candidate for aquaculture, especially in tropical and subtropical regions. As herbivores/omnivores the costs for the fish feed are considerably lower than for other species.

Another goal of BOMOSA was to only use ingredients, which do not compete with human food sources, or agricultural products for the livestock. The challenge in developing countries is to produce cost-effective feeds using locally available, inexpensive and unconventional resources. Unused water bodies, such as reservoirs and irrigation channels for rice fields, were used for rearing the fish.

The main objective of this master thesis was to produce cost-effective feeds using locally available and inexpensive agricultural by-products and to analyse the nutrient composition of these feeds. In total 36 mixtures were produced and analysed in the laboratory on their content of ash, ether extracts, crude fibre and crude protein.

Some of the feed ingredients, such as brewery waste and Mexican sunflower (*Tithonia rotundifolia*) were chosen based on the results of previous research work [HEIMBERGER, 2010], which has shown the excellent qualification of brewery waste as feed, especially with respect to crude protein.

For the production of the feed exclusively dried leaves of Mexican sunflower (*Tithonia rotundifolia*), sweet potato (*Ipomoea batatas*) and cassava (*Manihot esculenta*) were collected in the surroundings of the experimental site and after desiccating, ground. Thereafter each of the leaves was mixed with brewery waste, a by-product from beer production, in different proportions, binders were added and pelletized. In addition to the conventional binder gelatine, the tasteless vegetable gellant agartine was chosen in deference to the religious beliefs of the many Muslims living in Africa. Various amounts of binder were added to the mixtures to find out whether or not, this parameter has a bearing on the descent rate of the pellets.

In the course of another study [STÖGER, 2010] the acceptance of 13 selected mixtures by Nile Tilapia (*Oreochromis niloticus*) was analysed by means of video recording. Two tanks, each stocked with five fish with a weight of 60-80 g, were observed. The fish were fed three times a day and the feedings were filmed in sequences of eight minutes through a glass pane. Afterwards the feeding behaviour was analysed and interpreted. As reference feed, the standard fish feed at the Sagana Aquaculture Centre “Sagana diet” was used.

The analysis of the single feed components and the 36 mixtures has shown that sweet potato leaves could not reach the levels of ether extracts (see table 4.6) recommended by EL-SAYED [2006]. On the other hand all feed components met the requirements of Nile Tilapia (*Oreochromis niloticus*) in terms of crude protein content. In particular, Mexican sunflower and cassava have shown great potential as an alternative feed.

Zusammenfassung

In Zeiten von Überfischung, rasantem Bevölkerungswachstum und Umweltkatastrophen gewinnt Aquakultur immer mehr an Bedeutung und bietet eine ermutigende Möglichkeit den weltweiten Hunger zu stillen. Aquakultur stellt eine viel versprechende Proteinquelle für das krisengeschüttelte Afrika dar.

Diese Masterarbeit wurde im Rahmen des EU-finanzierten Forschungsprojektes BOMOSA (www.bomosa.org, 2011) geschrieben. Das Ziel von BOMOSA war die Einführung von Kleinkäfigaquakultur einheimischer Arten, wie zum Beispiel dem Niltlapia (*Oreochromis niloticus*) [LINNAEUS, 1758], in Ostafrika, wodurch die Nahrungssicherheit gesteigert und die Armut durch Schaffung von Arbeitsplätzen und zusätzlichem Einkommen verringert werden sollte.

Aufgrund der hohen Toleranz gegenüber einer großen Auswahl an Umwelteinflüssen und der Resistenz gegenüber Stress und Krankheiten stellt der Niltlapia einen hervorragenden Kandidaten für die Aquakultur dar, besonders in tropischen und subtropischen Regionen. Als Pflanzenfresser/Allesfresser sind die Futtermittelkosten beträchtlich geringer als für andere Arten.

Ein anderes Ziel von BOMOSA war die ausschließliche Verwendung von Inhaltstoffen, die nicht mit menschlichen Nahrungsmitteln oder landwirtschaftlichen Produkten für die Viehzucht in Konkurrenz stehen. Die Herausforderung in Entwicklungsländern besteht darin, kosteneffektives Futter aus lokal verfügbaren, billigen und unkonventionellen Ressourcen herzustellen. Für die Aufzucht der Fische wurden nicht genutzte Wasserkörper, wie Reservoirs und Bewässerungskanäle für Reisfelder, verwendet.

Das Hauptziel dieser Masterarbeit bestand in der Herstellung von kosteneffektivem Futter, unter Verwendung lokal verfügbarer und günstiger landwirtschaftlicher Nebenprodukte und der Analyse der Nährstoffzusammensetzung dieses Futters. Insgesamt wurden 36 Futtermischungen hergestellt und im Labor auf ihren Anteil an Asche, Rohfett, Rohfaser und Rohprotein untersucht.

Einige der Futtermittelbestandteile, wie Biertreber und die mexikanische Sonnenblume (*Tithonia rotundifolia*) wurden aufgrund der Ergebnisse früherer Forschungen [HEIMBERGER, 2010] ausgewählt, die die ausgezeichnete Qualifikation von Biertreber als Futtermittel, besonders bezüglich Rohprotein, gezeigt hat.

Für die Produktion des Futters wurden ausschließlich getrocknete Blätter der mexikanischen Sonnenblume (*Tithonia rotundifolia*), der Süßkartoffel (*Ipomoea batatas*) und von Cassava (*Manihot esculenta*) in der Umgebung des Untersuchungsstandortes gesammelt und nach dem Trocknen, zerkleinert. Danach wurde jede Pflanze mit Biertreber, einem Nebenprodukt aus der Bierherstellung, in verschiedenen Verhältnissen vermischt, Bindemittel hinzugefügt und pelletiert. Zusätzlich zu dem herkömmlichen Bindemittel Gelatine wurde das geschmacklose, pflanzliche Geliemittel Agartine, in Rücksichtnahme auf die religiösen Überzeugungen der vielen Muslime, die in Afrika leben, ausgewählt. Außerdem wurden den Futtermischungen verschiedene Mengen an Bindemittel hinzugefügt, um herauszufinden ob dieser Faktor Einfluss auf die Sinkgeschwindigkeit der Pellets hat.

Im Zuge einer anderen Untersuchung [STÖGER, 2010] wurde die Akzeptanz des Niltlapia gegenüber 13 ausgewählten Futtermischungen mit Hilfe von Videoaufnahmen untersucht. Dafür wurden zwei Becken mit jeweils 5 Fischen in der Größe von 60-80 g besetzt und

beobachtet. Die Fische wurden dreimal täglich gefüttert und die Fütterungen in Sequenzen von acht Minuten durch eine Glasscheibe hindurch gefilmt. Danach wurde das Fressverhalten untersucht und interpretiert. Als Referenzfutter wurde „Sagana diet“, das Standardfischfutter des Sagana Aquaculture Centre, verwendet.

Die Analyse der einzelnen Futtermittelkomponenten und der 36 Futtermischungen hat gezeigt, dass die Süßkartoffelblätter nicht die von EL-SAYED [2006] empfohlenen Werte an Rohfett (siehe Tabelle 4.6) erreichen konnten. Auf der anderen Seite konnten alle Futtermittelkomponenten den Anforderungen des Niltlapia, bezüglich des Gehaltes an Rohprotein, gerecht werden. Im Besonderen haben die mexikanische Sonnenblume und Cassava großes Potenzial als alternatives Futtermittel gezeigt.

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List of Abbreviations

AC	ash content
AIDS	acquired immunodeficiency syndrome
AOAC	Association of Official Agricultural Chemists
BOMOSA	B oku University, M oi University, S agana Aquaculture Centre
BW	brewery waste
C	cassava
°C	degree Celsius
CF	crude fibre
cm	centimetre
CO ₂	carbon dioxide
CP	crude protein
DM	dry matter
DO	dissolved oxygen
EE	ether extracts
FAO	Food and Agriculture Organization of the United Nations
FRDC	Fisheries Research and Development Corporation
g	gramme
GME	Gelatine Manufacturers of Europe
GMO	genetically modified organisms
ha	hectare
H ₃ BO ₃	boric acid
HCl	hydrochloric acid
HIV	human immunodeficiency virus
H ₂ SO ₄	sulphuric acid
IFAD	International Fund of Agricultural Development
kJ	kilo joule
kcal	kilocalorie
km ²	square kilometres
m	metre
M	moisture
MDG	Millennium Development Goal
mg	milligramme
mg/l	milligramme per litre
ml	millilitre
m _o	organic mass
MSF	Mexican sunflower
mt	megaton
m _{tot}	total mass
NaOH	sodium hydroxide
NfE	nitrogen-free extract
NH ₃ -N	ammonia nitrogen
NH ₄	ammonium
No	number
NO ₂	nitrogen dioxide
NO ₃	nitrate
pH	potentia hydrogenii
ppt	parts per trillion

SD	standard deviation
SP	sweet potato
SSA	Sub-Saharan-Africa
SW	sample weight
t	tonnes
t/ha	tonnes per hectare
UN	United Nations
V	volt

1 Problem Definition

Aquaculture especially cage culture is a relatively young branch of Kenya's economy and therefore a lot of research is still required. It is more important to develop sustainable production systems, which enhance the local economic conditions, than concentrating on the maximum of production [FAO, 2012].

The main objective of small-scale culture is to produce high quality animal protein, easily accessible for everyone. Therefore, low-input aquaculture tries to abstain from costly animal protein, such as fish meal or freshwater shrimp meal, which are common ingredients in many fish feed, such as in "Sagana diet", and rather uses plant protein. The goal is to utilise valuable natural protein sources and at the same time not increasing nutrient loads within the water body.

Since fish feed is one of the most costly components in fish production (more than 50 % of the total costs) it is very important to find cost-effective diets. The research of this master thesis concentrated on feed for Nile Tilapia (*Oreochromis niloticus*), an indigenous fish species to Africa [EL-SAYED, 2006].

The main focus of this research was to analyse the nutrient contents of self produced, low-cost fish feeds, by using locally available ingredients (agricultural by-products), which do not directly compete with human utilisation and to avoid conflicts of interest. The ingredients of 36 different fish feeds were analysed for their content of ash, ether extracts, crude fibre and crude protein. The laboratory work of this master thesis was conducted between the beginning of February and the end of April 2009 at the Sagana Aquaculture Centre in Kenya, East Africa.

This thesis was mainly based on the results of previous research completed by HEIMBERGER [2010].

The feed formulation was adapted to locally available ingredients in the surroundings of the Sagana Aquaculture Centre. The main feed component was brewery waste, a by-product of the brewery process. In addition to brewery waste, leaves from three species of plants were collected in the surroundings of the Sagana Aquaculture Centre, processed and analysed:

- Mexican Sunflower (*Tithonia rotundifolia*)
- Sweet Potato (*Ipomoea batatas*)
- Cassava (*Manihot esculenta*)

The leaves of these plants were mixed with brewery waste and turned into pellets, using two binders; first the conventional binder gelatine, and secondly the tasteless vegetable binder agartine.

At the same time of the research of this master thesis, STÖGER [2010] researched feeding 13 selected mixtures to Nile Tilapia (*Oreochromis niloticus*) in two observatory tanks and analysed the feeding behaviour of the fish by video recording to find out whether the feeds are accepted by the fish.

The following questions were researched in the course of the on site fieldwork of this thesis:

- Are the locally available agricultural by-products and resultant mixtures meeting the nutrient requirements of Nile Tilapia (*Oreochromis niloticus*) and do they qualify to supersede the previous standard feed “Sagana diet” in terms of nutrient contents?
- Does the binder have an influence on the nutrient composition of the mixtures?
- Does the amount of used binder affect the descent rate and rigidity of the pellets and consequently are the pellets suitable for cage culture?
- Which mixture can be recommended, regarding both nutrient composition and acceptance by Nile Tilapia (*Oreochromis niloticus*)?

Although questions concerning the descent rate or the acceptance of different feed mixtures by Nile Tilapia (*Oreochromis niloticus*) are necessarily part in this master thesis, because they deal with the production of these mixtures, they have actually been researched in depth by STÖGER [2010].

The research findings in the last chapter of this thesis show which of the produced feed mixtures are suitable for small-scale cage culture in Kenya.

2 Introduction

2.1 Global Nutrition Situation

Eventhough the global food production suffices to supply everybody with enough food many people do not have access to it. Chronic hunger causes undernourishment, which is especially dangerous to the health of children under the age of five and women. As shown in figure 2.1, the number of undernourished people has slightly declined over the past years. Nevertheless the first Millennium Development Goal (MDG) with the objective of halving the proportion of those who suffer from hunger between 1990 and 2015 will not be accomplished. Figure 2.2 depicts the number of undernourished people relating to region [FAO, 2013].

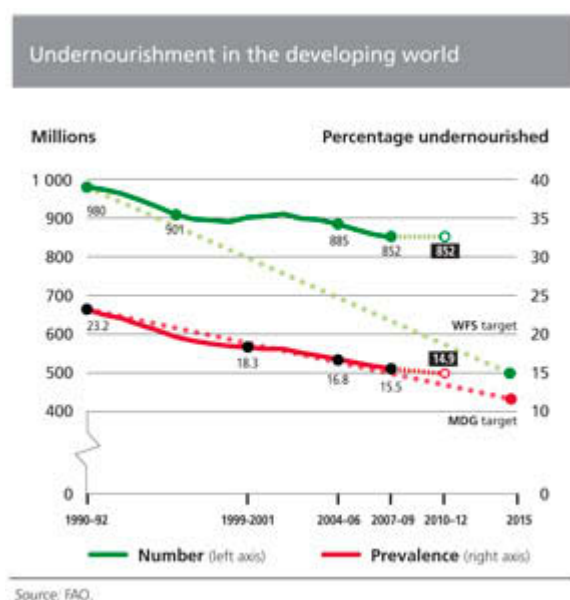


Figure 2.1: Undernourishment in the developing world [FAO, Hunger statistics, 2013]
<http://www.fao.org/hunger/en/>

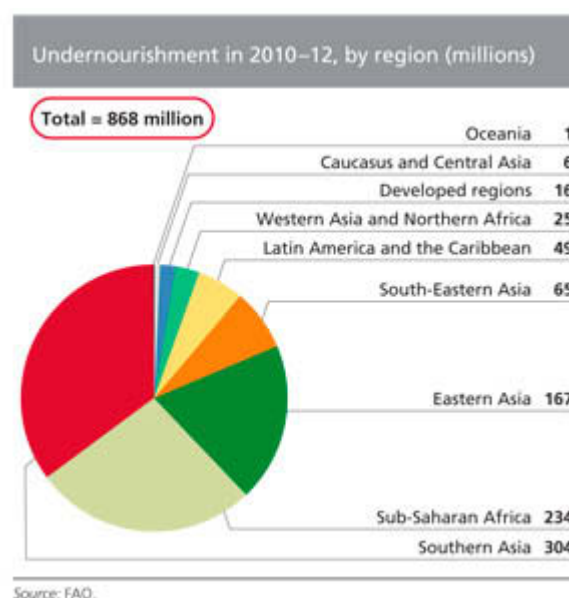


Figure 2.2: Undernourishment in 2010-2012, by region [FAO, Hunger statistics, 2013]
<http://www.fao.org/hunger/en/>

The Food and Agriculture Organization of the United Nations (FAO) defines undernourishment as “the status of persons, whose food intake regularly provides less than their minimum energy requirements”. Generally applied to energy deficiency, it may also relate to vitamin and mineral deficiencies. Undernourishment weakens the immune system and leaves the human body more susceptible to disease and infection.

As per FAO food security exists “when all people at all times have both physical and economic access to sufficient, safe and nutritious food that meets their dietary needs for an active and healthy life”. The FAO promotes two strategies to reduce hunger:

- enhancing the productive activities of the poor to improve food availability and incomes
- giving direct and immediate access to food to the most needy people.

Simultaneously, the right to adequate food should be legally defined.

Ironically, the most food-insecure people are often those, who produce the food. On the one hand the rural poor cultivate crops on small plots of land, which they often do not own, with no access to safe drinking water, sanitation or electricity. On the other hand, the urban poor are not producing food and do not have the funds to purchase food. One of the greatest problems to be solved in the future is the access to affordable food in the expanding cities [FAO, 2013].

As shown in figure 2.3, the global rising food prices between 2007 and 2008 caused by the financial crisis, posed a serious problem for the poor, who already spend a high percentage of their income (50-60 % in Low Income Food Deficit Countries) on food.

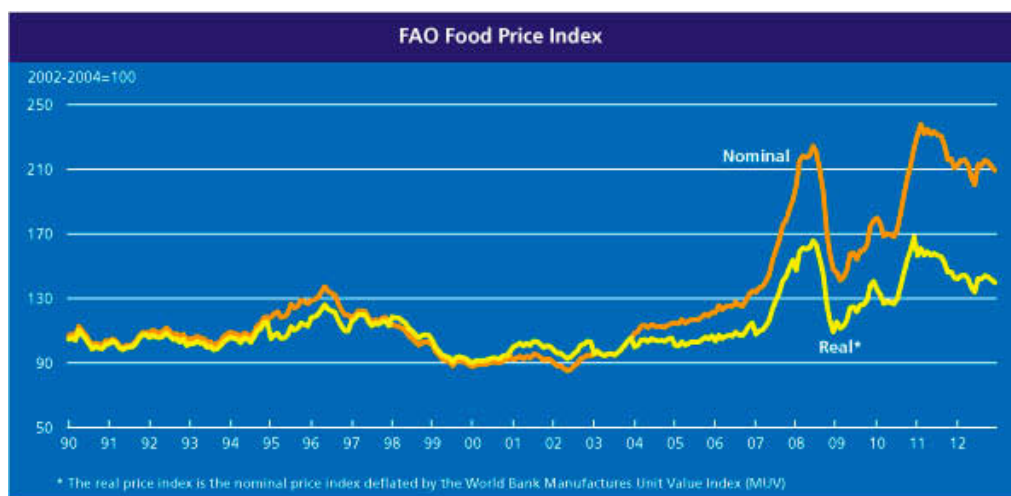


Figure 2.3: FAO Food Price Index [FAO, World Food Situation, 2013]
<http://www.fao.org/worldfoodsituation/wfs-home/foodpricesindex/en/>

As a result of rising food prices the poor are forced into diets of lower quality (reduced micronutrient intake), reduced diversity and at worst, quantity and safety of diets (calorie consumption) are affected. As per FAO more than 41 million children under the age of five were underweight prior to the rise in food prices in 2008 and the crisis has exacerbated the situation. Children, pregnant and lactating women are particularly affected by hunger and food insecurity. However, nutrition security also requires access to safe water, hygiene and sanitation, as well as access to quality health-care services and environmental health [THE AFRICAN, 2009].

2.1.1 Nutrition Situation in Africa

Africa epitomises recurring famines, acute food shortages, chronically low dietary intakes and food insecurity. The attempt of reducing hunger and malnutrition in Africa has so far failed for reasons of inadequate food production (both quantity and quality) and the unequal distribution of the food that actually is produced [THE AFRICAN, 2009].

More than 300 million people in Africa suffer from major nutrition problems. Especially affected by malnutrition are both urban and rural poor, smallholders, the landless, female-

headed households and children under five years of age living in such households. Figure 2.4 shows trends in childhood stunting in Sub-Saharan Africa.



Figure 2.4: Levels and trends in childhood stunting in Sub-Saharan Africa [PRB, Population Reference Bureau, 2013]

<http://www.prb.org/Articles/2008/stuntingssa.aspx>

The consequences of malnutrition are manifold:

- increased mortality
- reduced ability to resist infections
- reduced ability to work and/or go to school
- reduced employment and income
- impaired physical and mental development in young children.

Africa's malnutrition is caused by both man-made and natural disasters. Man-made factors are underdevelopment linked to political, economic, socio-cultural constraints and demographic dynamics leading to war and civil conflicts, population growth and pressure on natural resources. Natural disasters including droughts, floods, land-slides, earthquakes and bush fires add to the food insecurity in Africa. Figure 2.5 shows the number of people affected by diverse natural disasters in which part of Africa. Inappropriate cropping systems and expansion of agriculture onto fragile ecological lands cause accelerated soil erosion, which abolishes any chance of future development in food production in that area [THE AFRICAN, 2009].

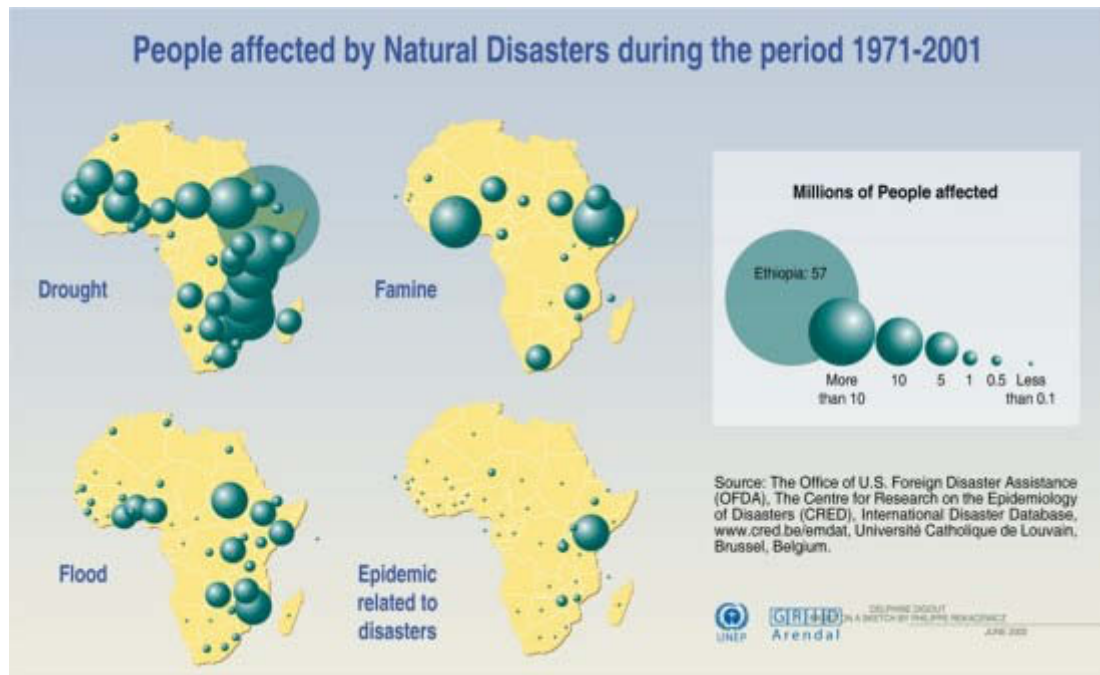


Figure 2.5: People affected by natural disasters during the period 1971-2001 [UNEP, United Nations Environment Programme, 2012]
http://www.grida.no/graphicslib/detail/people-affected-by-natural-disasters-during-the-period-1971-to-2001_cea3

2.2 Global Aquaculture

While capture fisheries production stopped growing in the mid 1980s, world aquaculture production has grown significantly in the last 50 years with an annual growth rate of 8.7 % worldwide since 1970. The aquaculture production has increased from less than 1 million tonnes in the 1950s to 51.7 million tonnes (36 % of total production by weight) in 2006. Thereby it accounted for 47 % of the world's fish food supply in 2006. No other animal food-producing sector can register such significant growth. The top ten countries for aquaculture production in 2006 are shown in table 2.1 [FAO, The State of World Fisheries and Aquaculture, 2008].

Table 2.1: Top ten producers in terms of quantity, 2006 [FAO, *The State of World Fisheries and Aquaculture*, 2008]

Top ten producers in terms of quantity, 2006			
Country	2004 [tonnes]	2006 [tonnes]	Average annual growth rate [%]
China	30 614 968	34 429 122	+ 6.05
India	2 794 636	3 123 135	+ 5.71
Vietnam	1 198 617	1 657 727	+ 17.60
Thailand	1 259 983	1 385 801	+ 4.87
Indonesia	1 045 051	1 292 899	+ 11.23
Bangladesh	914 752	892 049	- 1.25
Chile	665 421	802 410	+ 9.81
Japan	776 421	733 891	- 2.78
Norway	636 802	708 780	+ 5.50
Philippines	512 220	623 369	+ 10.32

Inland waters aquaculture represents the largest percentage of aquaculture production with 61 % by quantity (58 % freshwater environments), followed by marine aquaculture with 34 % [FAO, *The State of World Fisheries and Aquaculture*, 2008].

As shown in figure 2.6 the growth of world aquaculture production has not been uniform. Latin America and the Caribbean region show the highest average annual growth (22.0 %), followed by the Near East region (20.0 %) and the African region (12.7 %).

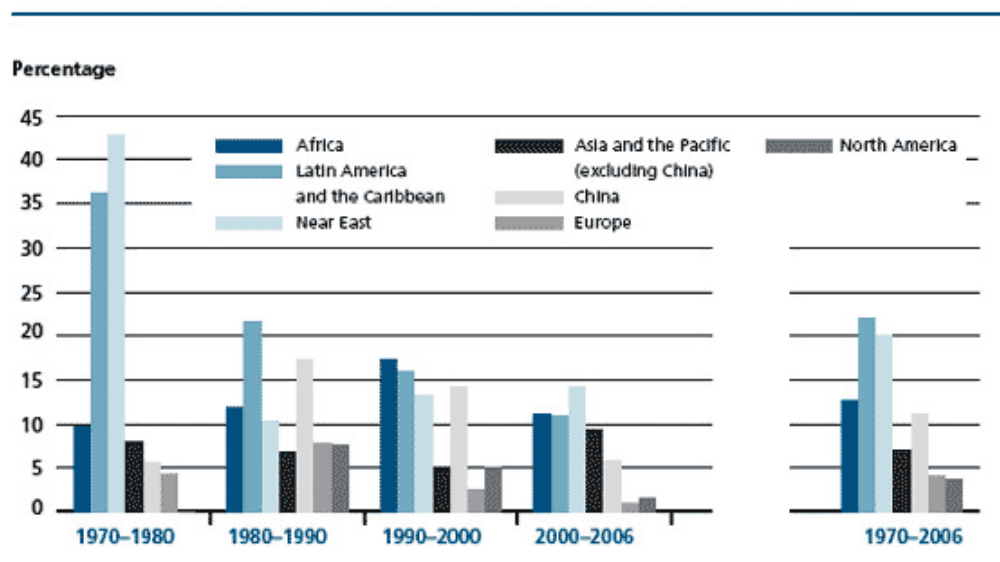


Figure 2.6: World aquaculture production: change in growth by region since 1970 [FAO, *The State of World Fisheries and Aquaculture*, 2008]
<http://www.greenfacts.org/en/fisheries/figtableboxes/figure-11.htm>

A recent global FAO study found that there is a strong public mistrust against aquaculture in all regions except Africa and Eastern Europe. This bias against aquaculture is caused by the notion that aquaculture harms the environment, an idea that is mainly based on misinformation or particular attributes of aquaculture. In order to increase public support for

aquaculture, FAO has drawn up guidelines for aquaculture certification, covering topics including; animal health, food safety and quality, environmental and social responsibility. When it comes to genetically modified organisms (GMOs) opinions tend to differ significantly among aquaculturalists. Proponents highlight the enhanced performance and profitability of GMOs implicating food security, while opponents point out significant risks to the environment and, possibly, to human health. To date no GMO products from aquaculture are available on the markets [FAO, The State of World Fisheries and Aquaculture, 2008].

2.2.1 The Role of Aquaculture in Rural Development

Rural development plays an important role in promoting food security, poverty alleviation and healthy nutrition. Given that many people work in agriculture in rural areas, aquaculture's role can become very important too, especially in tropical and subtropical regions. Aquaculture can play diverse roles in these regions: it can supply fish for domestic consumption, raise the producer's income and create employment opportunities. Developing countries produce more than 85 % of the world aquaculture production. In times of increasing population, malnutrition and environmental degradation, aquaculture gains in importance [EL-SAYED, 2006].

In rural areas, small-scale aquaculture is only one part of the many farming activities. Rural aquaculture often means the producer is also the consumer. It provides good quality animal protein at low costs, which means money can be saved for other essential necessities. Also, efficiency can be increased as farming by-products are used for small-scale aquaculture. Unused water bodies such as rice fields, can be used for aquaculture, which offer economic and environmental benefits [EL-SAYED, 2006].

2.2.2 Aquaculture in Africa

Especially in times of overfishing freshwater and coastal marine resources, aquaculture gains in importance. The growing demand for fish in Africa can not be supplied by capture fishery alone. There is significant potential for aquaculture in the East African regions [www.bomosa.org, 2011].

The population of Africa is projected to grow rapidly, with the highest population growth rates occurring in Sub-Saharan Africa [UN, 1999]. Supplying people with enough food represents a great challenge to this region. Fish is an essential source of food, supplying at least 25 % of the total animal protein in developing countries, and it provides important nutritional and health benefits [FRDC, 2001].

Although aquaculture production in Sub-Saharan Africa (SSA) still has not reached its full natural potential, there are some encouraging signs in the continent. For example Nigeria, with a production of 85 000 t of Catfish, Tilapia and other freshwater fishes, is by far the most dominant fish-producing country in the region. In North Africa, Egypt is even the second largest producer of Tilapia worldwide after China. For the aquaculture production in Africa in 2008 see figure 2.7.

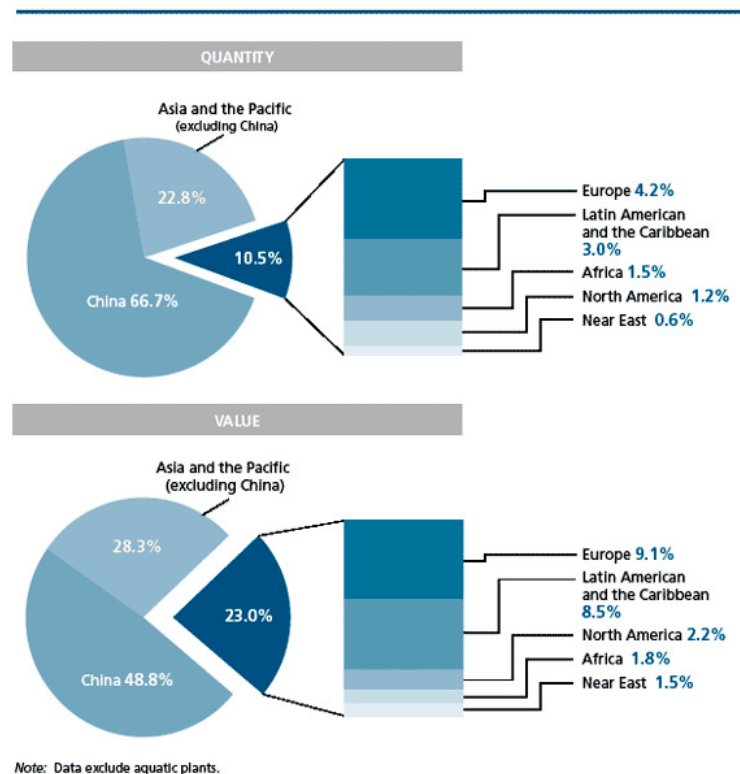


Figure 2.7: Aquaculture production by region [FAO, *The State of World Fisheries and Aquaculture*, 2008]
<http://www.greenfacts.org/en/fisheries/figtableboxes/figure-10.htm>

Africa has high production potential for warmwater fish, however aquaculture practices to date are still very limited. FAO reported in 2008 that 35 % of Africa's fish needs could be produced by small-scale fish farming. Severe political instabilities in many African countries, poor infrastructure and lack of knowledge are only some of the reasons why the full potential has not yet been exploited. Estimated 95 % of African aquaculture production comes from small-scale fish farming. Tilapia and Catfish are the predominantly farmed species. Nile Tilapia (*Oreochromis niloticus*) provides more than 30 % of the total fish production in Africa. Freshwater aquaculture in small ponds remains the most common aquaculture system in Africa. Tanks, cages and raceways are also used but at much smaller scales. [EL-SAYED, 2006]

2.2.2.1 Aquaculture in Kenya

For many years aquaculture in Kenya has stagnated at an annual production of approximately 1 000 t. In 2003 for example, the total production of the three main fish species farmed in Kenya (Nile Tilapia, Rainbow Trout and North African Catfish) accounted for 948 t, amounting to an income of US\$ 2 153 000. Due to the efforts of the Department of Fisheries to increase food security, improve the dietary nutrition and generate new employment and additional sources of income in rural areas, Kenya's aquaculture production was increased to 12 000 t in 2010 (see figure 2.8).

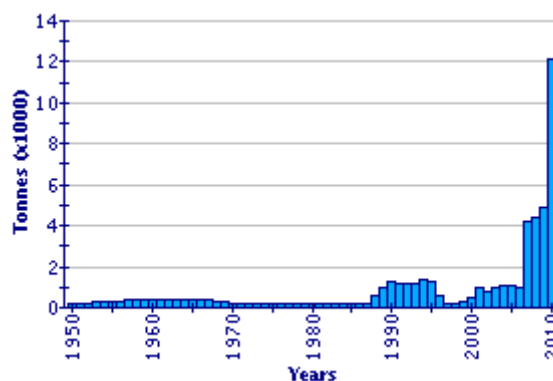


Figure 2.8: Aquaculture production in Kenya [FAO; Fishery Statistics, 2013]
http://www.fao.org/fishery/countrysector/naso_kenya/en

Different species of Tilapia, often kept in polyculture with the North African Catfish (*Clarias gariepinus*), form about 90 % of farmed fish in Kenya. Aquaculture in Kenya is characterised by low levels of pond production including intensive, semi-intensive and extensive systems. Most of the aquaculture production in Kenya can be ascribed to semi-intensive systems, with an average production of about 3 t/ha, contributing more than 70 % of the total production from aquaculture. Small-scale farming of Tilapia is mainly conducted by poor households in inland areas, where fish is stocked and harvested periodically. Cage culture has also been attempted with some degree of success. Aquaculture is often done in small hand-dug “kitchen ponds”, fairly large earth ponds, dams and other impoundments used for storing water [FAO, Fisheries and Aquaculture Department, 2012].

Fish farmed in Kenya, has promising chances of success on the domestic market. Aquaculture has become a source of healthy animal protein in many parts of Kenya, even in areas where fish was not primarily farmed, such as the Central and Eastern Provinces. In the coming years, aquaculture will most likely make a significant contribution to both food security and foreign exchange earnings in Kenya [FAO, Fisheries and Aquaculture Department, 2012].

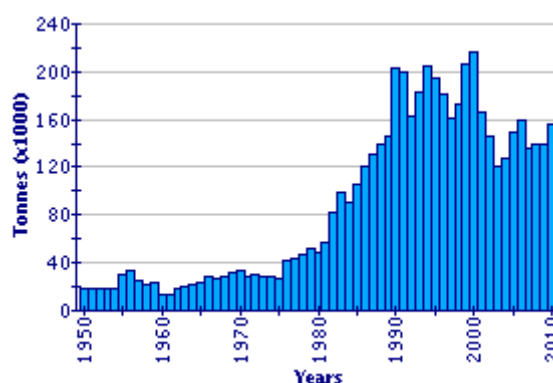


Figure 2.9: Fisheries production in Kenya (including mariculture, aquaculture and other kinds of fish farming)
 [FAO, Fishery Statistics, 2013]
http://www.fao.org/fishery/countrysector/naso_kenya/en

2.3 Global Tilapia Production

With 806 000 t of farmed Nile Tilapia (*Oreochromis niloticus*) China leads the global production in 2003, followed by Egypt with a reported production of nearly 200 000 t, the Philippines (111 000 t), Thailand (97 000 t) and Indonesia (72 000 t) [FAO, Fisheries and Aquaculture Department, 2012]. The global aquaculture production of Nile Tilapia (*Oreochromis niloticus*) is shown in figure 2.10.



Figure 2.10: Main producer countries of Nile Tilapia (*Oreochromis niloticus*) [FAO, Fishery Statistics, 2006]
http://www.fao.org/fishery/culturedspecies/Oreochromis_niloticus/en

Tilapia is ranked number two of the worldwide most important farmed fish after Carp, and the most widespread of any farmed fish. Due to an increasing market for Tilapia, especially in the USA (number eight of the most popular seafood) and Europe, the production of Tilapia is predicted to increase even more. Most of this predicted production is expected to be Nile Tilapia (*Oreochromis niloticus*). The resistance to poor water quality and disease, but also the fast growth on low protein feeds has led to commercialisation of Tilapia production in more than 100 countries all over the world [FAO, Fisheries and Aquaculture Department, 2012].

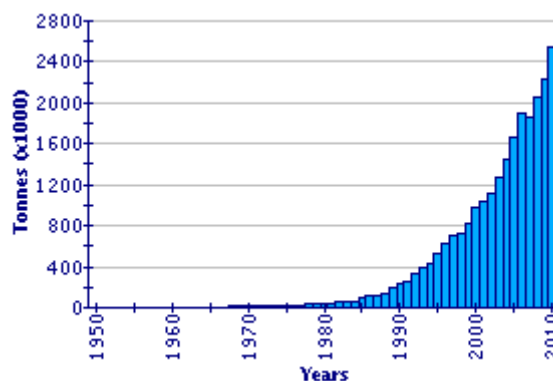


Figure 2.11: Global aquaculture production of Nile Tilapia (*Oreochromis niloticus*) [FAO, Fishery Statistics, 2013]
http://www.fao.org/fishery/culturedspecies/Oreochromis_niloticus/en

Nile Tilapia (*Oreochromis niloticus*) was originally introduced to developing countries to increase food security through local production of inexpensive animal protein for rural population. In the meantime, more and more people in Europe have discovered Tilapia on the basis of low price, year-round supply, mild flavour and simple preparation. Hence the market has veered from frozen whole fish, towards fresh or frozen fillets, which are favoured by the industrialised countries [FAO, Fisheries and Aquaculture Department, 2012].

2.4 BOMOSA

The research project “Integrating BOMOSA cage fish farming system in reservoirs, ponds and temporary water bodies in Eastern Africa” was funded especially for East African conditions by the European Commission. BOMOSA is an acronym for **B**oku University Vienna, **M**oi University Kenya and **S**agana Aquaculture Centre [DREXLER S.-S., WAIDBACHER H., 2009].

Like most African countries, Kenya faces deficits in high protein food supply and poverty alleviation, as well as problems in sustainable management of sensitive and dynamic ecosystems. One of BOMOSA’s aims is to increase food security and to improve the dietary nutrition standards by providing high quality animal protein at low costs for rural families. BOMOSA cage fish farming systems provide employment and generate additional income for fish farmers in the rural areas. In doing so, at least 50 % of employment positions are secured by women within each plot community [www.bomosa.org, 2011].

Another goal of BOMOSA is to increase economic viability and social acceptance of small-scale fish farming in otherwise under-utilised water bodies not needed for human consumption [DREXLER S.-S., WAIDBACHER H., 2009].

14 BOMOSA plots have been set up in Kenya, Ethiopia and Uganda to optimise a cost-effective processing technology for small water bodies. BOMOSA has developed a “hub and plot” cage-based fish farming system. Cage culture means rearing fish in cages, which can be applied in existing unused water bodies such as lakes, large reservoirs, farm ponds, ditches, irrigation channels and naturally occurring temporary water bodies formed during the rainy season that otherwise would not be suitable for aquaculture. The net-like BOMOSA cages can be folded, easily transported and handled by two persons without mechanical aid. A central fish farm (hub) is providing rural fish farming sites with fish fingerlings. The plot farmers are trained to rear the fish in cages in their plots, harvesting them for personal consumption as a source of high protein diet or of additional income by selling the fish. The BOMOSA cages are stocked with indigenous species such as Nile Tilapia (*Oreochromis niloticus*) [DREXLER S.-S., WAIDBACHER H., 2009].

The BOMOSA cage construction has gone through many amendments. The first cages were placed on the water surface and fixed at the base. To improve handling at stocking, a feeding and sampling pier was built and the cages fixed on the edges. Ultimately they were converted into floating piers which are moving with the water level. A ferry transports people and items to those floating piers and a sinker weight avoids drifting [www.bomosa.org, 2011].

Several pros and cons of cage culture are listed in table 2.2.

Table 2.2: Advantages and disadvantages of cage culture [www.bomosa.org, 2011]

Advantages	Disadvantages
adjournment of the breeding cycle of Nile Tilapia (<i>Oreochromis niloticus</i>)	loss from broken cages or predators
mixed-sex population in one cage culture	necessity of nutritionally-complete diets
flexibility of management	greater risk of disease outbreaks
monitoring of feeding behaviour	less tolerance to poor water quality
simple harvesting	

Another of BOMOSA's aims is to produce high quality animal protein at a low costs by producing low protein fish feed. This fish feed should contain a minimum of 80 % locally available agricultural by-products [www.bomosa.org, 2011].

2.5 Experimental Site

2.5.1 Kenya

The most important facts and figures about Kenya are listed below – Kenya in a nutshell:

Table 2.3: Kenya in a nutshell [source: <http://www.britannica.com/EBchecked/topic/315078/Kenya>, 2013]

Kenya in a nutshell	
Official name	Jamhuri ya Kenya (Swahili); Republic of Kenya (English)
Form of government	unitary multiparty republic with one legislative house (National Assembly)
Head of state and government	President: Mwai Kibaki Prime Minister: Raila Odinga
Capital	Nairobi
Official languages	Swahili; English
Official religion	none
Monetary unit	Kenyan shilling (K Sh)
Population	43,013,000 (2012 est.)
Total area (sq km)	582,646
Urban-rural population	Urban: 32.3 % (2009) Rural: 67.7 % (2009)
Life expectancy at birth	Male: 61.3 years (2011) Female: 64.2 years (2011)
Literacy	Male: 90.3 % (2008) Female: 82.8 % (2008)
GNI per capita (U.S.\$)	820 (2011 est.)

2.5.1.1 Origin of Name

Kenya is named after Mount Kenya, the highest mountain in Kenya and the second-highest in Africa. The Kikuyu people referred to Mt. Kenya as 'Mt. Kirinyaga', meaning 'mountain of whiteness' because of its snow capped peak. Representing the main landmark, 'Mt. Kirinyaga' became synonymous with the territory. The name was changed to Kenya due to the inability of the British to pronounce 'Kirinyaga' correctly [ENCYCLOPEDIA BRITANNICA, 2013].

2.5.1.2 History

In the mid-1880s Germany, Britain, and France were carving up East Africa. Among British acquisitions was the land we call Kenya today. A British trading company, Imperial British East Africa Company, was set up and posted to administer Kenya under the name British East Africa Protectorate. Kenya became fully independent on 12 December 1963. A year later, on 12 December 1964 the Republic of Kenya was proclaimed with Jomo Kenyatta as its first president [ENCYCLOPEDIA BRITANNICA, 2013].

2.5.1.3 Geography and Climate

Kenya covers an area of approximately 582,646 km² and the equator runs exactly across the country. Kenya is bordered to the north by Sudan and Ethiopia, to the east by Somalia, to the west by Uganda, to the south by Tanzania, and to the southeast by the Indian Ocean. For details see figure 2.12 below.

Swaths of the country, especially in the north and east, are arid or semi-arid. In the low-lying districts along the coast, the climate is tropical, hot and humid, while in the highlands the climate is more temperate. There are two rainfall periods; the long rains from April to June and the short rains from October to November [ENCYCLOPEDIA BRITANNICA, 2013].

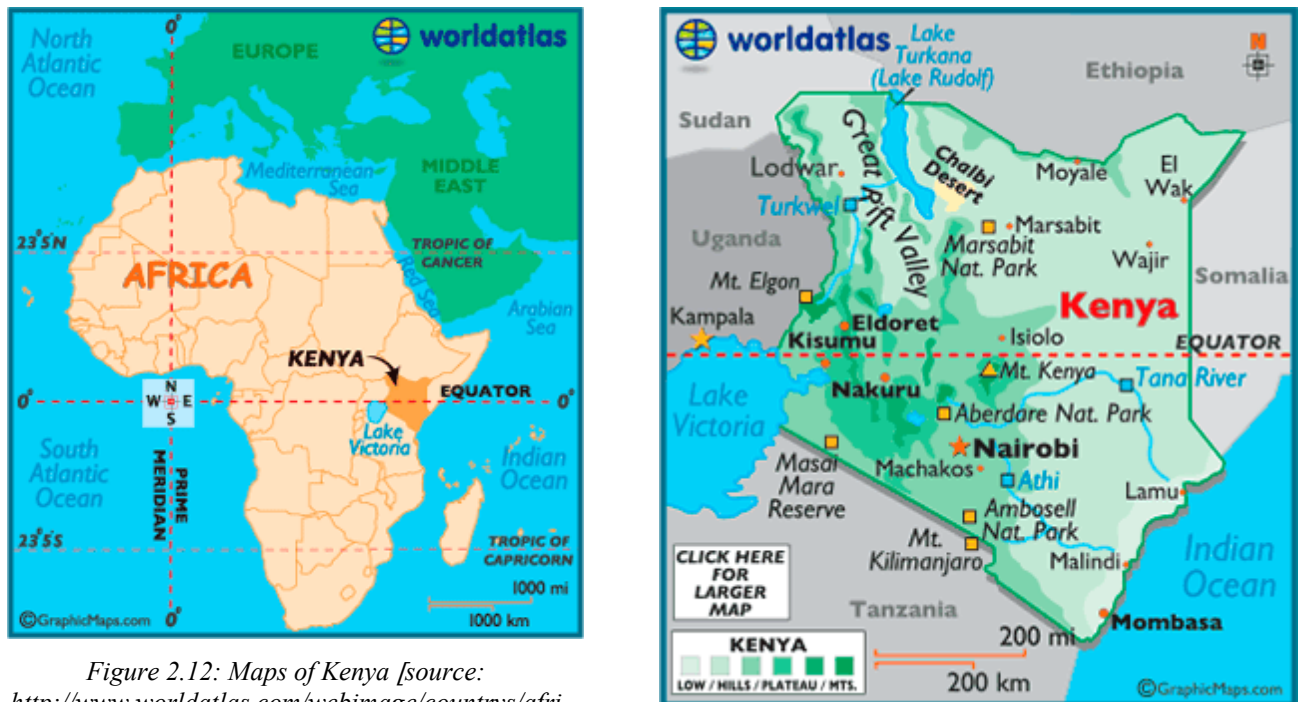


Figure 2.12: Maps of Kenya [source: <http://www.worldatlas.com/webimage/countrys/afrika/ke.htm>, 2013]

2.5.1.4 Economy and Demography

As seen in figure 2.13, 80 % of Kenya are arid and semi-arid and only about 18 % of the land is characterised by areas with good agricultural potential. About 79 % of Kenya's population live on agriculture in rural areas. Next to agriculture, Kenya mainly depends on tourism. 75 % of the total agricultural production is produced by means of smallholder subsistence agriculture. The fertile plains, where most Kenyans live, are located in the centre and west, while the poorest communities are found in the arid zones in the north. Kenya is consistently affected by droughts, floods and environmental degradations due to over-exploitation of natural resources [IFAD, 2007].

Besides the effects of climate change Kenya also suffers from tribal conflicts between the diverse ethnic groups and from a great many diseases. HIV/AIDS, waterborne diseases and malaria caused a decline in life expectancy to 46 years in 2006, slightly rising since then. HIV/AIDS especially concerns young and middle aged Kenyans. The most vulnerable to poverty, namely orphans and women, are most affected [IFAD, 2007].

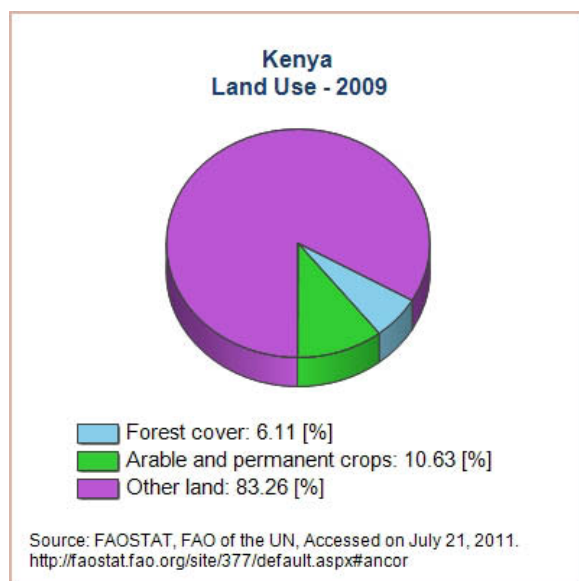


Figure 2.13: Land use, Kenya [FAO Statistics, 2011]
http://faostat.fao.org/CountryProfiles/Country_Profile/Direct.aspx?lang=en&area=114

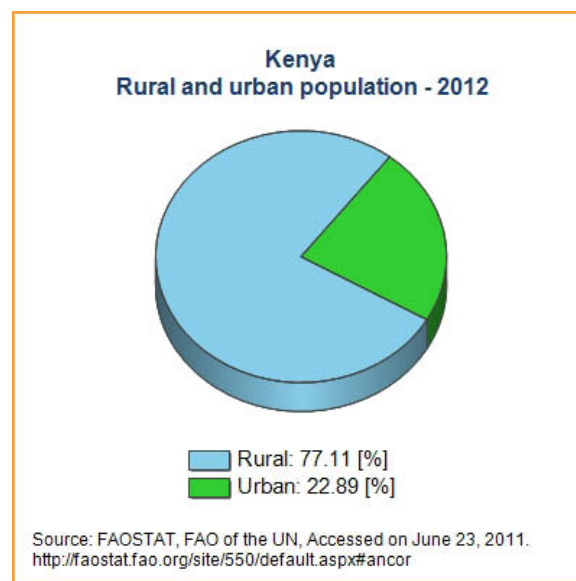


Figure 2.14: Rural and urban population, Kenya [FAO Statistics, 2011]
http://faostat.fao.org/CountryProfiles/Country_Profile/Direct.aspx?lang=en&area=114

According to the national population and housing census report of 2012, there are an estimated 43,013,000 people living in Kenya. Nearly 33 % of the total population is concentrated in the large cities of Nairobi, Mombasa and Kisumu and large towns such as Nakuru [ENCYCLOPEDIA BRITANNICA, 2013].

Although Kenya has one of the best-developed economies in Eastern Africa, nearly half of the population (43 million people) live below the poverty line, unable to meet their daily nutritional requirements. A third of the population, especially young children in the North Eastern, Eastern and Coastal Provinces, are undernourished. Kenya has one of the world's fastest population growth rates, which affects food security, income, employment, health, and education. Stagnation of food production, an unfavourable economic environment and poverty are the major causes of food insecurity in Kenya. These have contributed to the fall in life expectancy and the rise in mortality rates, particularly for infants and under-fives [IFAD, 2007].

2.5.2 Sagana Aquaculture Centre

The Sagana Aquaculture Centre is located within Sagana, a small township in the Kirinyaga district in the Central Province. Sagana is situated along the Nairobi-Nyeri highway, about 104 kilometres north-east of Nairobi, the capital of Kenya, at an altitude of about 1231 metres above sea level. It is named after Kenya's longest river, Tana River, which is also called Thagana [MINISTRY OF FISHERIES DEVELOPMENT, 2013].

The Sagana Aquaculture Centre promotes the following aims:

- Demonstration of warm fresh water fish farming
- Research and trial of warm water aquaculture
For the purpose of research work, a modern laboratory was built. Here scientific studies on various aspects of aquaculture, such as water quality, soil analysis and fish disease, can be conducted by students from local and foreign universities.
- Training of fish farming extension workers
In the course of this training, extension workers can be trained on the following subject areas:
 - Pond design and construction
 - Seed selection and stocking
 - General fish farm management
 - Fish feeding and feed formulation
 - Fish breeding and harvesting
 - Fish diseases and disease control
 - Fish handling and processing.
- Production of fingerlings for fish farmers
The Sagana Aquaculture Centre produces fingerlings of Nile Tilapia (*Oreochromis niloticus*) and African Catfish (*Clarias gariepinus*).

2.6 Nile Tilapia (*Oreochromis niloticus*) [Linnaeus, 1758]

The name '*tilapia*' originates from the African Bushman word, which means 'fish' [TREWAVAS, 1982].

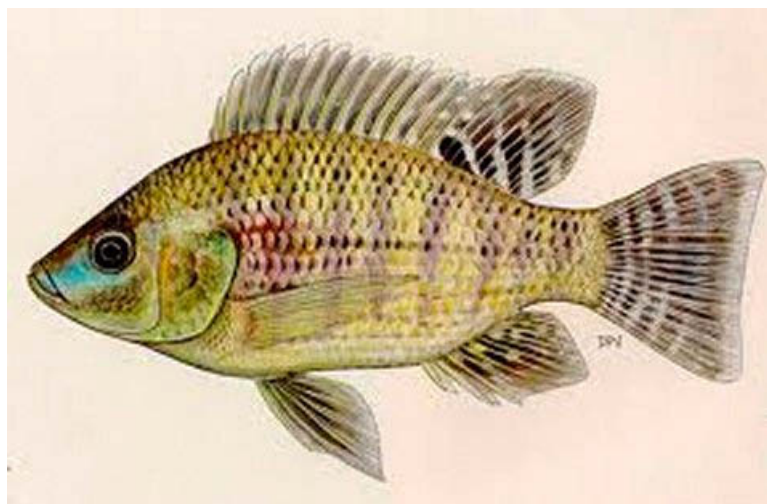


Figure 2.15: *Oreochromis niloticus* [source: <http://informedfarmers.com/bio-technical-data-on-the/>]

The below mentioned characteristics make Tilapia an excellent candidate for aquaculture [EL-SAYED, 2006].

- rapid growth at warm temperatures
- tolerance to poor water quality
- mostly resistant to stress and diseases
- ability to reproduce in captivity and early sexual maturity
- feed on a wide range of natural food organisms.

2.6.1 Taxonomy

Due to natural and anthropogenic crossbreeding it is difficult to identify the species of a fish [POPMA and MASSER, 1999]. Over 70 species of Tilapia have been described but due to the similarity of their morphological characteristics it is not clear whether or not these species are truly separate species [EL-SAYED, 2006].

Tilapia is a freshwater fish in the *Cichlidae* family endemic to Africa. In addition to being the most widely cultured fish in Africa, Tilapia has also been introduced to many tropical, subtropical and temperate regions all over the world. According to the Food and Agriculture Organization (FAO) there are Tilapia aquacultures in more than 100 countries.

Due to their reproductive behaviour, Tilapia can be distinguished into three genera [POPMA and MASSER, 1999]:

- Tilapia (nest builders)
- Sarotherodon (male or both male and female mouth breeders)
- Oreochromis (female mouth breeders).

Characteristic for the *Cichlidae* family is an interrupted lateral line. On their laterally compressed body they have long, heavily spined dorsal fins and spined anal and pelvic fins, which make them excellent swimmers. They are covered with large scales. Fry, fingerlings and occasionally adults have vertical bars down the side. Male Tilapia have gray or pink pigmentation in the throat region.

Tilapia have well developed sense organs, for example relatively large eyes which provide them with excellent visual capacity. In Africa, Tilapia inhabit a wide range of ecologically and geographically different habitats. This habitat diversity represents a high tolerance to a wide range of environmental conditions such as; temperature, salinity, water depth, turbidity, competition, dissolved oxygen and ammonia. Those physical, chemical and biological parameters will be discussed in detail later on. Tilapia and Sarotherodon species are spread all over West Africa, while Oreochromis is more established in Central and Eastern African regions [EL-SAYED, 2006].

2.6.2 Environmental Requirements

Water quality parameters may have a major effect on fish growth and health. Hence those parameters will be discussed in the following chapters.

2.6.2.1 Temperature

With regards to physiology, growth, reproduction and metabolism of Tilapia, water temperature is of vital importance, especially in temperate and subtropical regions, where seasonal fluctuations in water temperature occur. Tilapia are thermophilic fish and tolerate a wide range of water temperatures. For the normal development, reproduction and growth of Tilapia the temperature range should be 20-35 °C, with an optimum range of about 25-30 °C. Nonetheless large disparities in the growth and feed efficiency of Tilapia may occur even within the narrow optimum range of temperature. Studies showed twice the growth of the fish at 28 °C compared to the growth at 24 and 32 °C [EL-SAYED, 2006].

Tilapia tolerate relatively high water temperatures of up to 40-42 °C, while long term exposure to low temperatures of 7-10 °C lead to mass mortality. Reduced feeding starts below 20 °C and they stop feeding at about 16 °C. Since their tolerance of temperature can be highly diverse Tilapia species are found in different climatic regions. Studies show that Tilapia adapt to the local temperature. The level of temperature compatibility depends on the individual species, strains, size, exposure duration, environmental conditions and climatic region. Fingerlings for example are less hard-wearing to low temperature than adults. Regarding environmental conditions, increasing the pond depth by 100-200 cm (in earthen ponds) can bring significant improvements to growth and survival of the fish. It has also been found that water temperature is of vital importance on sex differentiation during early larval stages [EL-SAYED, 2006].

2.6.2.2 Salinity

Due to water shortage for both sanitary engineering and agriculture, aquaculture has been forced into brackish water and seawater. Supposed to descent from marine ancestors, Tilapia can normally grow and reproduce in brackish water at salinity levels of up to 15 ppt. Most Tilapia species tolerate a wide range of water salinity and grow and reproduce at very high water salinity. Once again species, strains, size, adaption time, environmental conditions and climatic region have a major effect on the tolerance of Tilapia to water salinity [EL-SAYED, 2006].

2.6.2.3 Dissolved Oxygen

Dissolved oxygen (DO) is one of the limiting environmental factors affecting fish feeding, growth and metabolism. The amount of DO available mostly depends on photosynthesis and respiration. Eventhough Tilapia on the one hand survive at zero DO concentration by surfacing, very low DO concentrations cause limitation in respiration and growth. On the

other hand, Tilapia tolerate oversaturation of DO, which can be ascribed to high photosynthesis.

Handling stress can double oxygen consumption, therefore the fish should be placed into water with high levels of DO after handling. Due to decreasing DO concentration at higher water temperature, increasing metabolism leads to higher oxygen consumption [EL-SAYED, 2006].

2.6.2.4 Ammonia

Ammonia is the primary waste product of fish, excreted basically through the gill tissue, but to a lesser extent also via the kidney. Ammonia can also accumulate from the decay of fish tissues, feed and other organic debris derived from protein. Ammonia ionises below pH 7.4 to ammonium, which is less toxic to fish. Above pH 8.0 most ammonia is ionised, and becomes more toxic. The toxicity of ammonia depends on DO, CO₂ and pH but also on fish species and size, acclimatisation time and culture system. The toxicity rises with increasing DO and decreases with lower CO₂ levels. According to research, the toxic level of NH₃-N ranges from 0.07 to 0.14 mg UIA-N/l and is recommended to be maintained below 0.1 UIA-N mg/l [EL-SAYED, 2006].

2.6.2.5 Nitrite

Nitrogen dioxide (NO₂) is an intermediate in the oxidation of ammonium (NH₄) to nitrate (NO₃). Nitrite is toxic for fish since it disrupts multiple physiological functions. It was found that nitrite toxicity to Tilapia depends on fish size [ATWOOD et al., 2001]. Small-sized fish show more tolerance to nitrite than large fish. Chloride concentration in water is considered one of the most important factors influencing nitrite toxicity to fish. A chloride-to-nitrite ratio of 1.5-to-1 has proven especially effective [EL-SAYED, 2006].

2.6.2.6 pH

Some Tilapia are known to tolerate a very wide range of water pH. Tilapia can survive in pH ranging from 5-10 but do best in a pH range of 6-9 [POPMA and MASSER, 1999].

Once again the fish size has a major influence on the tolerance of water pH. Studies have shown that fingerlings are less resistant to low pH than adults. Low or high water pH may cause different physiological changes, such as damage of gill epithelial cells, reduction in the efficiency of nitrogenous excretion, increased mortality and behaviour changes [EL-SAYED, 2006].

2.6.2.7 Water Turbidity

According to ARDJOSOEDIRO and RAMNARINE [2002] increasing water turbidity has an adverse influence on fish growth, feed efficiency and survival and should be kept below 100 mg/l in earthen ponds [EL-SAYED, 2006].

2.6.3 Nutrient Requirements

Nutrition is the most cost intensive component of aquaculture. Amounting to 50 % of the operating costs of aquaculture special attention is paid to feeding management. Especially in developing countries, cost effective feeds made of inexpensive and locally available resources are of great importance [EL-SAYED, 2006].

2.6.3.1 Proteins

Proteins are large biological molecules consisting of one or more chains of amino acids. Proteins perform a vast array of functions within living organisms. With 50 % of the total feed costs, protein is the most cost-intensive part of nutrition in aquaculture.

Protein requirements depend on fish size and age, protein source and energy content of the feed [POPMA and MASSER, 1999]. Protein requirements decrease with increasing fish size. While young fish require about 35-45 % protein in their feed for maximum growth, adults only require 20-30 % dietary protein for optimum performance [EL-SAYED, 2006].

Widely-used protein sources for aquaculture are fishmeal, fishery or terrestrial animal by-products, oilseed plants, such as soybean meal and cotton seed cake, aquatic plants and grain legumes [EL-SAYED, 2006].

2.6.3.2 Amino Acids

Tilapia require the same ten essential amino acids as other warm water fish [POPMA and MASSER, 1999]. There are two types of amino acids:

- Essential amino acids: These can not be synthesised by living organisms and therefore need to be contained in the diet.
- Non-essential amino acids: They can be synthesised by the organisms.

Although there is only a limited number of studies about the specific essential amino acid requirements of Tilapia, it is certain that they vary by species and amino acid source [EL-SAYED, 2006].

2.6.3.3 Lipids

Lipids are essential for innumerable physiological functions such as; energy production, protein sparing, absorption of fat-soluble vitamins, normal growth and development. Generally speaking, Tilapia are efficient lipid users. For maximum growth 10-15 % dietary lipids within the feed are recommended [EL-SAYED, 2006].

2.6.3.4 Carbohydrates

Carbohydrates are the least expensive source of dietary energy for fish and also the most available food source in the world. As herbivores, Tilapia can utilise up to 35-40 % digestible carbohydrates. Carbohydrate sources are mainly wheat bran, maize and rice. There are several factors affecting the carbohydrate utilisation of Tilapia, such as carbohydrate source, other dietary ingredients, fish species and size and feeding frequency. The fibre content is of great interest where the quality of dietary carbohydrates is concerned, given that it significantly influences fish performance [EL-SAYED, 2006].

2.6.3.5 Vitamins

There is very little information about vitamin requirements of Tilapia, since they ingest unknown amounts of vitamins with the natural food they feed on. Vitamin deficiency may lead to poor growth, nutrition-related diseases and increased susceptibility to infection [EL-SAYED, 2006].

2.6.3.6 Minerals

Very little is known about the mineral requirements of Tilapia, but clearly they are needed to maintain many of their functions such as; bones, teeth and soft tissues. Merely eight minerals have been detected to be vital for Tilapia namely; calcium, phosphorus, magnesium, zinc, manganese, potassium, iron and chromium. Fish size, mineral contents of the water and fish feed are crucial for mineral requirements of Tilapia [EL-SAYED, 2006].

2.6.4 Feeding Habits

Due to their increasing importance for aquaculture the feeding habits of Tilapia in their natural habitat are of great interest. It is only by understanding their food preferences and feeding regimes, that adequate diets and feeding regimes with respect to feeding frequency, timing and quantity can be developed for aquaculture systems.

Tilapia feed on plankton, aquatic macrophytes, planktonic and benthic aquatic invertebrates, larval fish, detritus and decomposing organic matter. They filter plankton from the water by secreting a mucous with the gills. This mucous assimilates the plankton and is then

swallowed. Tilapia digest plant protein more efficiently (30-60 % of the protein in algae) than animal protein. Although Tilapia favour the use of mash, most feeds are pelletised in order to reduce nutrient loss [POPMA and MASSER, 1999].

Since Tilapia are herbivorous/omnivorous, they range low on the aquatic food chain. Compared to carnivorous fishes the feeding costs are lower, nevertheless Tilapia are a high-quality protein source [EL-SAYED, 2006].

Species, size, time of day, water depth etc. have a major effect on the feeding habits and dietary preferences of Tilapia. Larvae prefer zooplankton like crustaceans, while juveniles and adults feed on all kinds of aquatic vegetation, phytoplankton, zooplankton and vegetable detritus. Depending on species and environmental conditions, Tilapia can be divided into the following feeding patterns:

- Oreochromis are so called “filter feeders” and favour phytoplankton, periphyton and detritus
- Sarotherodon are much more selective phytoplankton feeders
- Tilapia are primarily macrophyte feeders.

Tilapia can either have jaw teeth or pharyngeal teeth. As the small jaw teeth are used as scrapers they are flattened and arranged in one to five rows. Depending on the species pharyngeal teeth can either be fine, thin and hooked, or coarse and robust. Gill rakers may be long, thin and closely spaced, or fewer and large [EL-SAYED, 2006].

Diurnal and seasonal changes may also effect the feeding patterns of Tilapia. While fry feed at night in the darkness, all other sizes of fish feed on a daytime feeding circle. Zooplankton may be preferred in summer and autumn [SPATARU, 1978] while the feeding activity is generally greater in summer [DEWAN and SAHA, 1979].

Research has shown that food consumption decreases with increasing fish size and that large fish are less active at feeding. The method of ingestion changes with the size of the fish. While small fish are visual feeders, large fish are pump-filter feeders [HAROON et al., 1998].

The challenge for nutritionists in developing countries is to produce cost-effective feeds using locally available, inexpensive and unconventional resources.

2.6.4.1 Feeding Practices

Two major feeding regimes can be distinguished:

- daily feeding as a percentage of the body weight
- satiation, where the fish can feed as much as they like.

Based on their small stomachs, it is common to feed more frequently, whereas frequency should decrease with increasing fish size. Dividing the daily rations into more feedings can reduce feed loss and therefore result in better growth [EL-SAYED, 2006].

Apart from reduced work loads there are no known influences on fish performance in question of feeding methods (handfeeding, automatic feeding, demand feeders, etc.). While fingerlings and juveniles do better with mashed feed, adults grow better on pellet feed. There is no difference in acceptance of floating or sinking pellets [EL-SAYED, 2006].

2.6.5 Reproduction

Due to their mode of reproduction, Tilapia can be divided into two groups:

- Substrate Spawners (biparental breeding): fertilised eggs are guarded in the nest by a brood parent
- Mouthbreeders: fertilised eggs are immediately picked up from the nest and bred in the buccal cavities until hatched fry are several days old.

The group of mouthbreeders can be further divided into:

- *Oreochromis* (maternal mouthbreeders)
- *Sarotherodon* (biparental mouthbreeders).

For reproduction all male *Oreochromis* gouge a nest in the substrate and mate with multiple females. After spawning and fertilisation, the female incubates the eggs in the buccal cavity until they hatch and even several days afterwards [POPMA and MASSER, 1999]. Characteristic for Tilapia are low fecundity and relatively large egg size. It is also known that in some Tilapia species, like Nile Tilapia (*Oreochromis niloticus*), fertilisation of the eggs takes place in the female's mouth [EL-SAYED, 2006].

The point of puberty can diversify considerably within Tilapia species and is often down to age, size and environmental conditions. Sexual maturity of Nile Tilapia (*Oreochromis niloticus*) begins at about 10-12 months [POPMA and MASSER, 1999]. Feed, especially the protein level, may also influence sexual maturity and productivity of broodfish [EL-SAYED, 2006].

There are several adverse factors regarding seed production in Tilapia cultures. First of all the early maturation of Tilapia may cause overcrowding and stunting [EL-SAYED, 2006].

Therefore all-male cultures are often favoured. In addition, male fish grow twice as fast as females [POPMA and MASSER, 1999].

2.6.6 Diseases and Stress

Several diseases are known to cause illness and mortality in Tilapia and consequently economic loss to fish farmers. The diseases affecting Tilapia include; parasitic, bacterial, fungal and viral diseases as well as non-infectious diseases and disorders. At optimum temperatures, Tilapia are very resistant to viral, bacterial and parasitic diseases [EL-SAYED, 2006].

Stress is known to weaken the body's defences and increase the susceptibility to disease. Therefore, stress should be avoided at all times. It can be caused by nutritional differences, environmental factors, handling, overcrowding or transportation. Most notably, social stress caused by interactions and hierarchies, decreases growth rates [EL-SAYED, 2006].

2.6.7 Culture Systems

2.6.7.1 Intensive Culture System

Although Tilapia culture was traditionally extensive or semi-intensive, the tolerance of Tilapia with regard to high density and a wide range of environmental conditions as well as their resistance to stress, disease and handling has made them perfect candidates for intensive culture. Intensive culture means high production with 100 to more than 500 Mt/ha/year. Such systems completely depend on artificial feeding and water exchange, involve high operating costs and require high levels of technology [EL-SAYED, 2006].

2.6.7.2 Semi-intensive Culture System

Semi-intensive culture system means that fish is produced at low cost by means of lower stocking density but also lower production costs, which is especially cost-effective for small scale farmers. Natural food, through pond fertilisation, can be used as well as supplemental feed. Herbivorous and omnivorous fish, such as Tilapia are qualified for this system [EL-SAYED, 2006]. In many cases polyculture of Nile Tilapia (*Oreochromis niloticus*), African Catfish (*Clarias gariepinus*) and the common Carp (*Cyprinus carpio*) is practiced [FAO, 2013]. This system is optimal for food security in rural areas in developing countries [EL-SAYED, 2006].

2.6.7.3 Extensive Culture System

Extensive culture systems are characterised by the lowest management levels in aquaculture but also by low stocking densities and thus low yields [FAO, 2013]. Extensive culture in cages is mainly done in lakes, rivers, dams and water reservoirs where the fish are left to fend for themselves. The fish, as well as stocking densities are highly dependent on the natural productivity and the physical conditions of the water [EL-SAYED, 2006]. The main cultured

species besides *Oreochromis niloticus* (Nile Tilapia) are *Clarias gariepinus* (African Catfish) and *Cyprinus carpio* (common Carp) [FAO, 2013].

3 Methodology

3.1 Feed Production

3.1.1 “Sagana Diet“

“Sagana Diet” is the standard feed at the Sagana Aquaculture Centre and highly accepted by the fish. The feed consists of 25 % cottonseed meal, 12 % freshwater shrimp (*Caridinea niloticus*) and 63 % wheat bran. For the nutrient composition of “Sagana Diet” see table 3.1.

Table 3.1: Proximate nutrient composition of the “Sagana Diet” [cf. MUNGUTI et al., 2009]

Nutrient composition	“Sagana Diet“
Crude protein [% DM]	28.5
Ether extracts [% DM]	5.6
Nitrogen free extracts [% DM]	43.2
Crude fibre [% DM]	14.8
Ash [% DM]	7.9

3.1.2 Feed Components

In the course of the research for this thesis, fish feeds in varying compositions were tested. In order to provide the future fish farmers with inexpensive and easily available feed components, only feedstuffs which do not or only in restricted manner compete with human food sources, or agricultural products were used. Another requirement was local availability. The following plants and by-products were used for the feed production:

- Brewery waste
- Mexican sunflower leaves
- Sweet potato leaves
- Cassava leaves.

For the feed production exclusively dried leaves of plants listed above were used. The leaves were collected in the vicinity of the Sagana Aquaculture Centre, sun-dried on black plastic films and crushed. Nutrient analyses were conducted in order to find out whether or not the particular feed components were suitable as feed. Special attention was paid to the growth of the fish. Furthermore each feed component was researched with regard to its acceptance by Nile Tilapia (*Oreochromis niloticus*). In the experimental basins of the Sagana Aquaculture Centre the produced mixtures were fed to the fish and their feeding behaviour was analysed via video recordings [STÖGER, 2010].

3.1.2.1 Brewery waste

Brewery waste is a by-product from beer production, which is gained by solubilising starch from the grain and converting it into sugar. Brewery waste is a source of nutrients and micro elements and available in wet and dry form. In its dried form it can be stored for two years. As a result of its high crude protein and crude fibre contents, by far the most common use of brewery waste is as animal feed, primarily for cattle but also for pigs, goats, fish and any other livestock [TREBERKRAFT GMBH & CO KG, 2012].

A disadvantage of using brewery waste in aquaculture as fish feed is the fact that brewery waste has to be purchased and it is a widely-used feed at livestock breeding, thus competing with agriculture. Still considering the little quantity used in this research work it is quite affordable and available all year round.



Figure 3.1: Brewery waste from Kenya Breweries Ltd., Ruaraka, Kenya

Brewery waste was chosen as a feed component because conclusions from previous research showed the excellent qualification of brewery waste as a fish feed. Both nutrient composition [HEIMBERGER, 2010] and the acceptance by the fish [ASSMANN, 2009] were satisfying. For the feed production during the course of the research for this thesis, exclusively dry brewery waste from Kenya Breweries Ltd., which is located in Ruaraka, near the capitol of Nairobi, was used.

The composition of brewery waste from Kenya Breweries Ltd. is shown in table 3.2.

Table 3.2: Nutrient composition of dry brewery waste

Nutrient composition	Brewery waste
Crude protein [% DM]	28
Crude fibre [% DM]	15.3
Ether extracts [% DM]	15.2
Ash [% DM]	5.2

3.1.2.2 Mexican Sunflower Leaves (*Tithonia rotundifolia*)

Tithonia rotundifolia is a species of flowering plant of the *Asteraceae* family and commonly known as Mexican sunflower. As the Mexican sunflower originates from Mexico and Central America, it grows best in hot, dry climates and requires well drained soil.

Easily reaching up to 1.8 m in height and 1.2 m across, it has 3-lobed leaves, which are covered with a soft bloom. The flower heads of this annual plant are red-orange [FLORIDATA, 2012].

In Kenya, the Mexican sunflower is almost ubiquitous, for example as fence for farms, fields and along roads. Due to the contained bitterness, Mexican sunflower is not used as feed for livestock or as a human food source. The bitterness can be diminished by chopping and drying the aerial parts [WAIDBACHER, personal note, 2009]. Considering that the Mexican sunflower is accessible to everyone, and not used for any other purpose it generally represents an excellent feed. However, former studies have shown that the plant is hardly accepted by Nile Tilapia (*Oreochromis niloticus*) [ASSMANN, 2009]. Based on its high protein content it was nonetheless considered in this study.

For the nutrient composition of Mexican sunflower leaves see table 3.3.

Table 3.3: Nutrient composition of dry Mexican sunflower leaves

Nutrient composition	Mexican sunflower leaves
Crude protein [% DM]	30.4
Crude fibre [% DM]	12
Ether extracts [% DM]	14.2
Ash [% DM]	14.7

For this research work, Mexican sunflower leaves were collected in the surroundings of the Sagana Aquaculture Centre and dried in the sun on a black plastic film. Afterwards the leaves were chopped to minimise the natural bitterness. The leaves were separated from coarse parts of the leaf stalks as far much as possible.



Figure 3.2: Mexican sunflower leaves [source: HEIMBERGER, 2010]



Figure 3.3: Dried Mexican sunflower leaves on a black plastic film

3.1.2.3 Sweet Potato Leaves (*Ipomoea batatas*)

Ipomoea batatas, also called sweet potato belongs to the family *Convolvulaceae*. Presumably native to Central and South America, it is now grown throughout tropical and warm temperate regions. The sweet potato is one of the most important staple foods for several millions of people all over the world. Misleadingly assumed, the sweet potato is not related to the potato (*Solanum tuberosum*). The edible tuberous root is long and tapered and matures in two to nine months. The flesh of the root, whose colour ranges from yellow, orange, red to purple, is floury and contains a lot of sugar and starch. Some varieties are also high in beta-carotene. The perennial vine features alternate heart-shaped or palmate lobed leaves, single flowers and usually hairy sepals. The plant grows best at an average temperature of 24 °C and does not tolerate frost [WOOLFE, 1992].

The nutrient composition of sweet potato leaves is shown in table 3.4.

Table 3.4: Nutrient composition of dry sweet potato leaves

Nutrient composition	Sweet potato leaves
Crude protein [% DM]	28.1
Crude fibre [% DM]	15.1
Ether extracts [% DM]	10.7
Ash [% DM]	13.1

The sweet potato leaves were bought and harvested in Kibingoti, which is located about 10 km to the north of Sagana. After sun-drying the leaves on black plastic films, they were separated from the leaf stalks and chopped. The stems of the plant were not used because of their high crude fibre content.



Figure 3.4: Sweet potato plant



Figure 3.5: Dried sweet potato leaves

3.1.2.4 Cassava Leaves (*Manihot esculenta*)

Cassava, also called Manioc or Yucca, belongs to the family of *Euphorbiaceae* and is native to South America. Cassava is widespread as an annual crop in tropical and subtropical regions and therefore a major staple food for around 500 million people. Cassava is counted among the most drought-tolerant crops, capable of growing on marginal soils.

The perennial, woody shrub reaches up to 5 m in height and has palmate compound leaves, standing on up to 30 cm long leaf stalks. Its edible tuberous root is long and tapered and rich in starch but poor in protein and other nutrients. In contrast to the root, cassava leaves are a good source of protein. The firm white or yellowish flesh of the root is encased in a detachable rind, which is about 1 mm thick, rough and brown on the outside.

Both cassava roots and leaves should not be consumed raw because they contain toxins. Cassava can be categorised as either sweet or bitter according to the contained quantity of toxins. During drought, the amount of toxins is especially high. Since improper preparation can cause intoxication, societies that traditionally eat cassava know methods to detoxify the plant for example by soaking, cooking, fermenting and so on [O'HAIR, 1995].

For the nutrient composition of cassava leaves see table 3.5.

Table 3.5: Nutrient composition of dry cassava leaves

Nutrient composition	Cassava leaves
Crude protein [% DM]	28.8
Crude fibre [% DM]	16.9
Ether extracts [% DM]	15
Ash [% DM]	7.5

In the course of this study, cassava leaves were plucked in the surroundings of the Sagana Aquaculture Centre. To detoxify the plant it was chopped with a bushwhacker, soaked for several hours and dried in the sun on a black plastic film. Afterwards the leaves were separated from coarse leaf stalks.



Figure 3.6: Cassava leaves [source: STÖGER, 2010]



Figure 3.7: Chopping cassava leaves [source: STÖGER, 2010]

3.1.3 Binders

The binders gelatine and agartine were used to produce pellets from the different feed components. They were tested for their varying nutrient compositions, especially the crude protein content. Besides the function as binders they were also used to analyse whether the binders have a bearing on the descent rates of the pellets. Given that fish in cage culture are kept in cages in a pond the descent rates of the pellets are of prime importance. In case the pellets sink too fast, they fall through the net of the cage, be out of reach for the fish and therefore unsuitable for cage culture. The fish neither would have enough time to feed on the pellets nor the chance to collect some of the pellets from the ground later on. Furthermore a deterioration of the water quality is to be expected from too many nutrients in the water.



Figure 3.8: Dr. Oetker Gelatine powdered (white) & RUF Agartine (white) [source: HEIMBERGER, 2010]

3.1.3.1 Gelatine

Gelatine is a natural and tasteless collagen protein made of animal raw materials. The base material is the connective tissue of pigs, cattle or fish. In Europe about 80 % of gelatine is pigskin gelatine, another 15 % come from cattle hide split and the remaining 5 % is obtained from pig and cattle bones, and fish. For the production of gelatine, solely by-products from the slaughtering are used that have been examined by veterinarians. Gelatine is made of 84-90 % protein, 1-2 % mineral salts and water. Gelatine is devoid of any antidegradants and additives. It contains no fat, cholesterol or purine (= uric acid compounds).

For humans, protein is essential for survival. Gelatine consists of amino acids. There are nine amino acids which the human body cannot produce itself. Protein contains 18 amino acids, including eight out of the nine essential amino acids [GME, 2012].

The nutrient content of gelatine is shown in table 3.6.

Table 3.6: Nutrient composition of gelatine (per 100 g content) [DR. OETKER, 2012]

Nutrient composition	Gelatine
Calorific value [kJ]	1.411
Calorific value [kcal]	322
Protein [g]	83
Carbohydrate [g]	0
Fat [g]	0
Dietary fibre [g]	0
Natrium [g]	0.03

Because of its high protein content, gelatine (Dr. Oetker, powdered, white) was used to increase the protein content in the feed, which is of great importance for the growth of the fish. One bag contains 9 g of gelatine. In order to research the influence of the binder on the descent rate of the pellets as mentioned above, different amounts of gelatine were added to the mixtures. Thus differing amounts of gelatine were dissolved in water. This solution was mixed with miscellaneous feed components and pelletised.

3.1.3.2 Agartine

Agartine, a tasteless vegetable gellant, is counted among the food additives. Agartine consists of maltodextrin and the gellant “agar agar” (20 %), a polysaccharide which is especially found in red algae where it is leached from the cell walls by water. In Japan agar agar has been regarded as gellant since the 17th century. The long-chained polysaccharides cannot be used by the human body and therefore are counted among the dietary fibres [LEBENSMITTELLEXIKON, 2012].

Although it does not have the same binding characteristics and outstanding protein contents as gelatine, agartine (RUF, white) was chosen as an alternative to gelatine because of the many Muslims living in Africa. Given that gelatine is made of animal raw material of pigs, which is classified as impure to the Islam, an alternative was needed.

One bag contains 9 g agartine. Agartine was mixed and used in the same way as gelatine. In the course of this research work it was found that agartine dissolves faster than gelatine under the same circumstances.

For the nutrient composition of agartine see table 3.7.

Table 3.7: Nutrient composition of agartine (per 100 g content) [RUF, 2012]

Nutrient composition	Agartine
Calorific value [kJ]	1636
Calorific value [kcal]	387
Protein [g]	0.4
Carbohydrate [g]	89
Fat [g]	0
Dietary fibre [g]	14
Natrium [g]	0.9

3.1.3.3 Mixtures

Each of the above listed plants was mixed with the main feed component, brewery waste. While the gravimetric mixtures were weighed and mixed in various proportions, the single volumetric mixture was produced with equal volume fractions. The volumetric mixture was chosen to facilitate the procedure of mixing the feed for fish farmers. For those who have no direct access to a scale, a volumetric measurement (for example: one bucket of brewery waste and one bucket of cassava leaves) is the easiest method of measuring ingredients.

Each mixture was produced with both, gelatine and agartine due to reasons mentioned previously in chapter 3.1.3.

3.1.3.3.1 Mixtures with Mexican Sunflower Leaves

For the mixtures with Mexican sunflower leaves, 18 g of gelatine or agartine were dissolved in one litre of water. 200 ml of this solution and 200 ml of distilled water were added to each mixture.

The different mixtures that were produced are shown in table 3.8. The numbers in table 3.8 represent the percentage of the feed components in the mixture in %.

Table 3.8: Mixtures of brewery waste and Mexican sunflower leaves

Gelatine			Agartine		
	BW [%]	MSFL [%]		BW [%]	MSFL [%]
volumetric	50	50	volumetric	50	50
gravimetric	70	30	gravimetric	70	30
	60	40		60	40
	50	50		50	50
	40	60		40	60
	30	70		30	70

3.1.3.3.2 Mixtures with Sweet Potato Leaves

For the mixtures with sweet potato leaves, 54 g of gelatine and agartine respectively, were dissolved in one litre of water. 200 ml of this solution and 200 ml of distilled water were added to each mixture. At the same quantity, agartine dissolved faster than gelatine. With gelatine, the upper limit was reached at 54 g per litre since it was not dissolved in the water any more.

For the produced mixtures see table 3.9. The numbers in table 3.9 represent the share of the various feed components in the mixture in %.

Table 3.9: Mixtures of brewery waste and sweet potato leaves

Gelatine			Agartine		
	BW [%]	SPL [%]		BW [%]	SPL [%]
Volumetric	50	50	volumetric	50	50
Gravimetric	70	30	gravimetric	70	30
	60	40		60	40
	50	50		50	50
	40	60		40	60
	30	70		30	70

3.1.3.3.3 Mixtures with Cassava Leaves

For the mixtures with cassava leaves, 36 g of gelatine or agartine each were dissolved in one litre of water. 200 ml of this solution and 200 ml of distilled water were added to each mixture.

The different mixtures are shown in table 3.10. The numbers in the following table represent the mixing ratio of the various feed components in %.

Table 3.10: Mixtures of brewery waste and cassava leaves

Gelatine			Agartine		
	BW [%]	CL [%]		BW [%]	CL [%]
volumetric	50	50	volumetric	50	50
gravimetric	70	30	gravimetric	70	30
	60	40		60	40
	50	50		50	50
	40	60		40	60
	30	70		30	70

3.1.4 Pelletising

By means of a mincer (see figure 3.9), pellets were produced from each mixture. In order to make pellets from the individual mixtures, the sun-dried feed components were mixed in the above mentioned proportions and the binders were added. To increase the cohesion of the pellets, the mixtures were pelletised twice and sun-dried on a black plastic film. The finished pellets had a diameter of 8 mm and were stored in labelled, transparent plastic bags until further use.



Figure 3.9: Masticator – Mastro FTS117, 2006, Germany



Figure 3.10: Sun-dried pellets on black plastic film



Figure 3.11: Storage of pellets in labelled, transparent plastic bags

3.2 Nutrient Analysis

In the course of the laboratory work, 18 mixtures each with the binder; gelatine and agartine were produced and analysed. In doing so, these 36 mixtures were subjected to 5 different analyses. Each analysis was carried out in triplicate and therefore a total of 540 samples were researched, in accordance with AOAC (Association of Official Agricultural Chemists [1990]) regulations.

Before the nutrient analyses, the pellets were ground with a mill of the brand GE Motors and kept in plastic sample containers.



Figure 3.12: Mill – GE Motors, 3383-L10, Thomas Scientific, USA

3.2.1 Dry Matter

The dry matter is what remains of a sample after all of the crude ammonia liquor is evaporated. In simple terms, dry matter is the difference between the total mass of the sample and its water content. For nutrient analysis, the dry matter is of great importance because all of the other quantitative analyses refer to it. The dry matter contains essential food ingredients like proteins, fats, carbohydrates, minerals, vitamins, and so on [WIKIPEDIA, 2012].

The dry matter was calculated with the following formula:

$$DM [\%] = 100 \% - M [\%]$$

$DM [\%]$	<i>Dry matter in percent</i>
$M [\%]$	<i>Moisture in percent</i>

To determine the dry matter of a sample about 5 g of the feed sample were put into a crucible and dried to constant weight at about 103 °C for 24 hours in the drying oven.

3.2.2 Ash Content

The ash content is the solid residue that remains after the incineration of organic matter and hence a measurement for the mineral and micronutrient contents of a sample. It is calculated by subtracting the remaining ash content from the original total mass after incineration.

$$AC = m_{tot} - m_o$$

AC	<i>Ash content</i>
m_{tot}	<i>Total mass</i>
m_o	<i>Organic mass</i>

The organic mass contains crude protein, crude fibre, crude lipid and NfE (Nitrogen-free Extract).

The sample from the dry matter determination was incinerated in a muffle furnace at 550 °C for 4 1/2 hours. After cooling in the drying oven at 103 °C for another hour, the crucible including the incinerated sample was weighed.



Figure 3.13: Muffle furnace – Barnstead/Thermolyne – 62700 Furnace, Model No F62730, 240V [source: HEIMBERGER, 2010]

The following formula was used to calculate the crude protein content:

$$AC [\% \text{ of } DM] = \frac{AC [g]}{DM [g]} * 100$$

<i>AC [% of DM]</i>	<i>Ash content referred to the dry matter in percent</i>
<i>AC [g]</i>	<i>Ash content in gramme</i>
<i>DM [g]</i>	<i>Dry matter in gramme</i>

3.2.3 Ether Extraction

Crude lipid is the part of the feed that can be dissolved in grease solvents such as petroleum ether. Crude fat is also known as the ether extract or the free lipid content. There are two methods most commonly used to determine crude fat contents:

- wet extraction and
- dry extraction.

For dry extraction, both the ether and the samples must be free of moisture. A common dry extraction method is Soxhlet, named after its inventor Franz von Soxhlet, where the crude fat is extracted into ether [FOOD SCIENCE, 2008]. Crude lipid is the predominant source of energy for fish [NATUREFOOD, 2012].

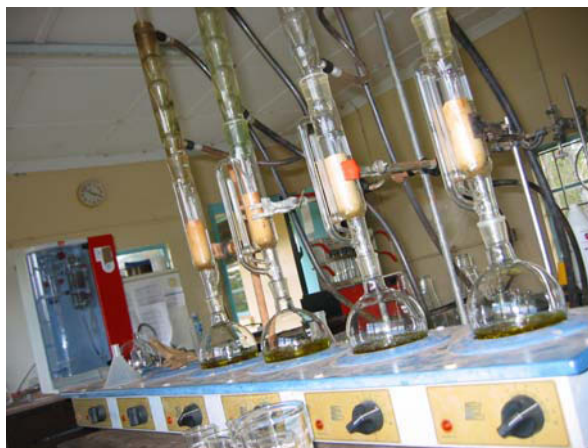


Figure 3.14: Ether extraction – Electrothermal, CAT No EME60250/CE, 220V

Two grammes of each individual sample were weighed into a thimble and slipped into a thimble holder. In the fat beaker beneath, petroleum ether was heated to 80 °C (boiling point of petroleum ether is 40-60 °C). The solvent evaporated, ascended towards the cooler and condensed there. Next, the solvent dripped into the thimble, where it extracted crude lipid from the sample. Past extraction, the thimble was dried to constant weight in the drying oven at 103 °C and the residue weighed. The crude lipid content amounts to the difference between the weight of the sample before and after the extraction.

The calculation of the ether extract was done according to the following formula:

$$EE [\% \text{ of } DM] = \frac{EE [g]}{DM [g]} * 100$$

<i>EE [% of DM]</i>	<i>Ether extract referred to the dry matter in percent</i>
<i>EE [g]</i>	<i>Ether extract in gramme</i>
<i>DM [g]</i>	<i>Dry matter in gramme</i>

3.2.4 Crude Fibre

Crude fibre is what remains of a sample after treating it with diluted acids and bases. This indigestible component of the feed represents the insoluble carbohydrates, such as cellulose, hemicellulose and lignin, and must not be confused with dietary fibre. Crude fibre has little or no nutritional value and hence can help increase weight loss in fish [EXOVA, 2012].



Figure 3.14: Vacuum pump – GE Motors & Industrial Systems, MOD 5KH36KN193HT, 220-250V [source: HEIMBERGER, 2010]

For the analysis of the crude fibre content, 0.5 g of the feed sample were sequentially digested with 0.312 N sulphuric acid (H_2SO_4) and 0.125 N sodium hydroxide solution (NaOH). The sample including 100 ml of sulphuric acid were brought to the boil in a piker for 10 minutes and afterwards filtrated with a vacuum pump. The residues of the sample were returned to the piker and brought to the boil in 100 ml of sodium hydroxide solution for 10 minutes. Once again the sample was filtrated, then put into the drying oven for 24 hours. After weighing the sample, it was incinerated in the muffle furnace at 550 °C for 4 1/2 hours, cooled in the drying oven at 103 °C and then reweighed.

The crude fibre was calculated with the following formula:

$$CF [\% \text{ of } DM] = \frac{CF [g]}{DM [g]} * 100$$

<i>CF [% of DM]</i>	<i>Crude fibre referred to the dry matter in percent</i>
<i>CF [g]</i>	<i>Crude fibre in gramme</i>
<i>DM [g]</i>	<i>Dry matter in gramme</i>

3.2.5 Crude Protein

Crude protein is the quantitative determination of all nitrogen compounds. On that account mostly the nitrogen content is determined by the Kjeldahl method, developed by Johan Kjeldahl in 1883, and from that, the amount of protein within the feed is estimated. The analysis consists of three worksteps [GERHARDT ANALYTICAL SYSTEMS, 2008-2011]:

- digestion of the sample with acid sulphur
- distillation of the digestion solution with water vapour
- titration of the distillate.

Crude protein is synonymous with growth in fish production, but it is of prime importance that all of the protein contained in the feed is ingested by the fish. Otherwise, ammonium is produced from proteolysis which transforms into ammonia at a high pH-value [NATUREFOOD, 2012].

To determine the crude protein content, 4 g of each feed sample were digested in the digestion block with one “Kjeldahl catalyst tablet”, 15 ml of highly concentrated sulphuric acid and two drops of silicone oil for half an hour at 80 % energy performance and for another 3 1/2 hours at 60 % energy performance. Past cooling the steam, distillation was carried out in the distillation system. The distillate was mixed with 50 ml boric acid (H_3BO_3) including 3 drops of indicator, which coloured the mixture purple and then titrated with 0.1 M hydrochloric acid (HCl) until the mixture coloured white again. The spent volume of hydrochloric acid can be converted into the nitrogen content of the sample.



Figure 3.15: Digestion block Behroset InKjel M (behr Labor-Technik GmbH, Düsseldorf, Germany)



Figure 3.16: Steam distillatory Behr S 1 (behr Labor-Technik GmbH, Düsseldorf, Germany)



Figure 3.17: Titration - Mixed indicator 5 for ammonia titrations (Merck, Darmstadt, Germany) [source: STÖGER, 2010]

The crude protein content was calculated with the following formula:

$$CP [\%] = \frac{HCl [ml] * 0.1 [M] * 14 * 100}{1000 * SW [g]} * 6.25$$

<i>CP [%]</i>	<i>Crude protein in percent</i>
<i>HCl [ml]</i>	<i>Hydrochloric acid in millilitre</i>
<i>0.1 [M]</i>	<i>Amount of substance concentration of HCl in mol per litre</i>
<i>14</i>	<i>Molecular weight of nitrogen</i>
<i>SW [g]</i>	<i>Sample weight in gramme</i>
<i>6.25</i>	<i>Reciprocal value of the nitrogen content of crude protein (herbal protein)</i>

$$CP [\% \text{ of } DM] = \frac{CP [g]}{DM [g]} * 100$$

<i>CP [% of DM]</i>	<i>Crude protein referred to the dry matter in percent</i>
<i>CP [g]</i>	<i>Crude protein in gramme</i>
<i>DM [g]</i>	<i>Dry matter in gramme</i>

3.2.6 Nitrogen-free Extract (NfE)

Nitrogen-free extracts represent the soluble carbohydrates of a feed such as starch and sugar. The content of nitrogen-free extracts is calculated in the following ways:

$$NfE [\% \text{ of } DM] = DM [\%] - AC [\% \text{ of } DM] - EE [\% \text{ of } DM] - CF [\% \text{ of } DM] - CP [\% \text{ of } DM]$$

<i>NfE [% of DM]</i>	<i>Nitrogen-free extracts referred to the dry matter in percent</i>
<i>DM [%]</i>	<i>Dry matter in percent</i>
<i>AC [% of DM]</i>	<i>Ash content referred to the dry matter in percent</i>
<i>EE [% of DM]</i>	<i>Ether extract referred to the dry matter in percent</i>
<i>CF [% of DM]</i>	<i>Crude fibre referred to the dry matter in percent</i>
<i>CP [% of DM]</i>	<i>Crude protein referred to the dry matter in percent</i>

3.3 Methodology of Data Analysis

The data analysis within the research of this thesis was driven by underlying hypotheses, which were sought to be verified. These hypotheses are reflected in the following concrete questions:

Question 1: Which single feed component is best suited as feed constituent, regarding its contents of ash, ether extracts, crude fibre, and crude protein? In the course of these analyses, all feed components were scaled against each other.

H 1.1: Mexican sunflower leaves prove to be by far the best feed component, this however solely with regard to its content of protein. Its mean protein content is much higher than the one in any other analysed feed component.

H 1.2: Cassava leaves show the second highest protein content; the mean protein contents of all other feed components were found to be in a similar, but lower range than the one of cassava leaves.

Although the nutrient contents of different feeds were analysed with regard to their ash, ether extracts, crude fibre and crude protein contents, special emphasis was given to their protein contents. This is due to the decisive role that crude protein plays in fish growth.

The two hypotheses above were arrived at by two different methods since the homogeneity of the two or more samples is of vital concern in this study: the one-way analysis of variance for a parametric perspective and the Kruskal-Wallis test method for a non-parametric perspective.

Question 2: Have the three parameters of individual feed component, binder and mixing ratio had a significant influence on the nutrient content of the different feeds regarding their ash, ether extracts, crude fibre and crude protein contents? Is there a significant interrelation between the individual feed component, binder and mixing ration?

H 2.1: The three parameters have a significant influence on the variation of ether extracts in the individual feed samples.

H 2.2: The three parameters have a significant influence on the variation of crude fibre contents in the individual feed samples.

H 2.3: The three parameters have a significant influence on the variation of crude protein contents in the individual feed samples.

H 2.4: All three nutrient contents (ether extracts, crude fibre, crude protein) are assumed to have no interactional effect of the first or second order on one another.

H 2.5: In all nutrient analyses, protein shows the highest mean manifestations (arithmetic mean / median).

All hypotheses (H 2.1 – H 2.5) have been verified by means of the one-way variance analysis (ANOVA) as well as the Kruskal-Wallis test.

4 Results

The main aim of this master thesis was to find out if the nutrient contents of the self-made mixtures containing three different locally grown, easily available and cost effective leaves, brewery waste and two different binders meet the nutrient requirements of Nile Tilapia (*Oreochromis niloticus*) in cage fishing setups. A total of 36 mixtures were produced out of the four feed components and then analysed. Three runs of each feed component and each mixture were carried out. Besides, it was of interest whether the binder and moreover the amount of binder have a bearing on the nutrient content, especially concerning crude protein, and the descent rate of the produced pellets. The question of descent rate and the actual acceptance of the produced mixtures by the fish were analysed in STÖGER's master thesis [2010].

The analysis of the data was carried out by means of SPSS (Version 15.0, 2006) and Microsoft Office Excel (2003). The results were presented in tables which contain the arithmetic mean and the standard deviation. Furthermore the arithmetic mean was illustrated in figures using Microsoft Office Excel. The graphical representation compares the means and mixtures regarding feed components. A one-way analysis of variance has been made to show whether the feed component, binder or mixing ratio is significant for the results and how strongly pronounced the influence is.

In this chapter the results of the nutrient analyses of the single feed components and the self-made mixtures are presented in terms of ash, ether extracts, crude fibre and crude protein. After an overview of the different mixtures (see table 4.1), the single nutrient results are shown by using tables and diagrams. The numbers in table 4.1 represent the share of the various feed components in the mixture in %.

Table 4.1: Overview of all produced and analysed mixtures

Binders	Mixtures		
	BW/MSF [%]	BW/SP [%]	BW/C [%]
Gelatine	50/50 volumetric	50/50 volumetric	50/50 volumetric
	50/50	50/50	50/50
	70/30	70/30	70/30
	30/70	30/70	30/70
	60/40	60/40	60/40
	40/60	40/60	40/60
Agartine	50/50 volumetric	50/50 volumetric	50/50 volumetric
	50/50	50/50	50/50
	70/30	70/30	70/30
	30/70	30/70	30/70
	60/40	60/40	60/40
	40/60	40/60	40/60

4.1 Feed Components

All feed components were tested separately for their nutrient composition. Only sun dried, crushed leaves without any additives were used. While the leaves of Mexican sunflower, sweet potato and cassava were collected in the surroundings of the Sagana Aquaculture Centre, brewery waste was purchased from the Kenya Breweries Ltd., located near Nairobi.

For each of the four feed components, brewery waste, Mexican sunflower, sweet potato and cassava, excel sheets were created. The results of each single feed component were presented in tables, containing the arithmetic mean and the standard deviation, and graphical representations. On the y-axis the nutrient analyses are shown, on the x-axis the arithmetic mean in % of dry matter can be seen.

4.1.1 Brewery waste

The results of the nutrient analysis of brewery waste are shown in table 4.2 and figure 4.1.

Table 4.2: Brewery waste - nutrient composition [arithmetic mean in % of DM \pm standard deviation]

Feed component	Nutrient analysis [% DM \pm SD]			
	Ash	Ether extracts	Crude fibre	Crude protein
Brewery waste	5.2 \pm 0.07	15.2 \pm 0.34	15.3 \pm 1.06	28.0 \pm 0.17

Brewery waste has the lowest ash content of all feed components, which highlights the desirable qualities of this product, given that the ash content represents the degree of contamination. The ash content accounts for 5.2 %. As a predominant energy source for fish, crude lipids are very important ingredients in a feed. Brewery waste comes in first with 15.2 %. Since crude fibre has little or no nutritional value, low crude fibre contents are desirable in a feed. With 15.3 % the value of crude fibre is in the upper range of all feed components. The crude protein content is 28.0 %.

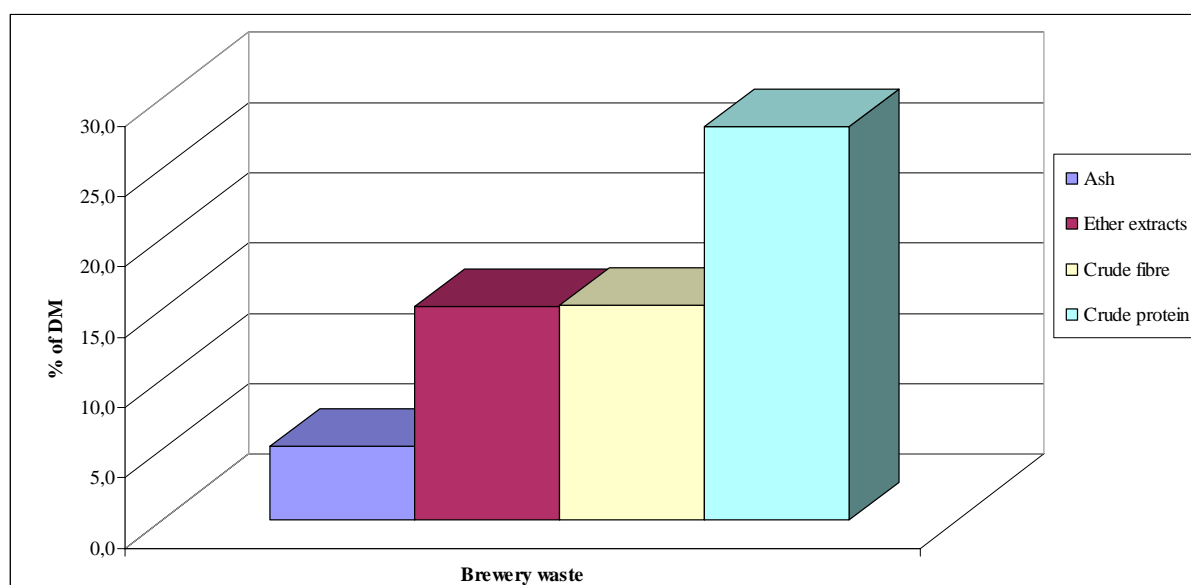


Figure 4.1: Brewery waste - graphical representation of the nutrient composition [arithmetic mean in % of DM]

4.1.2 Mexican Sunflower Leaves (*Tithonia rotundifolia*)

For the nutrient composition of Mexican sunflower leaves see table 4.3 and figure 4.2.

Table 4.3: Mexican sunflower leaves - nutrient composition [arithmetic mean in % of DM \pm standard deviation]

Feed component	Nutrient analysis [% DM \pm SD]			
	Ash	Ether extracts	Crude fibre	Crude protein
Mexican sunflower leaves	14.7 \pm 0.06	14.2 \pm 1.03	12.0 \pm 0.26	30.4 \pm 0.23

With 14.7 %, the ash content in Mexican sunflower leaves is the highest of all feed components. As the ash content is the measure for the mineral and micronutrient contents of a sample, it is assumed that this value can be explained by the place of collection. The Mexican sunflower leaves were collected next to unhitched streets and consequently the pollution from the traffic is likely to have caused this high ash content. The crude lipid content is 14.2 %. Concerning crude fibre, the leaves have the lowest value, namely 12.0 %. After collecting the leaves, they were dried in the sun, chopped to reduce the bitterness and separated from the coarse parts, like leaf stalks as well as possible. Mexican sunflower has the best results regarding of crude protein, with 30.4 %. This makes it highly suitable as fish feed.

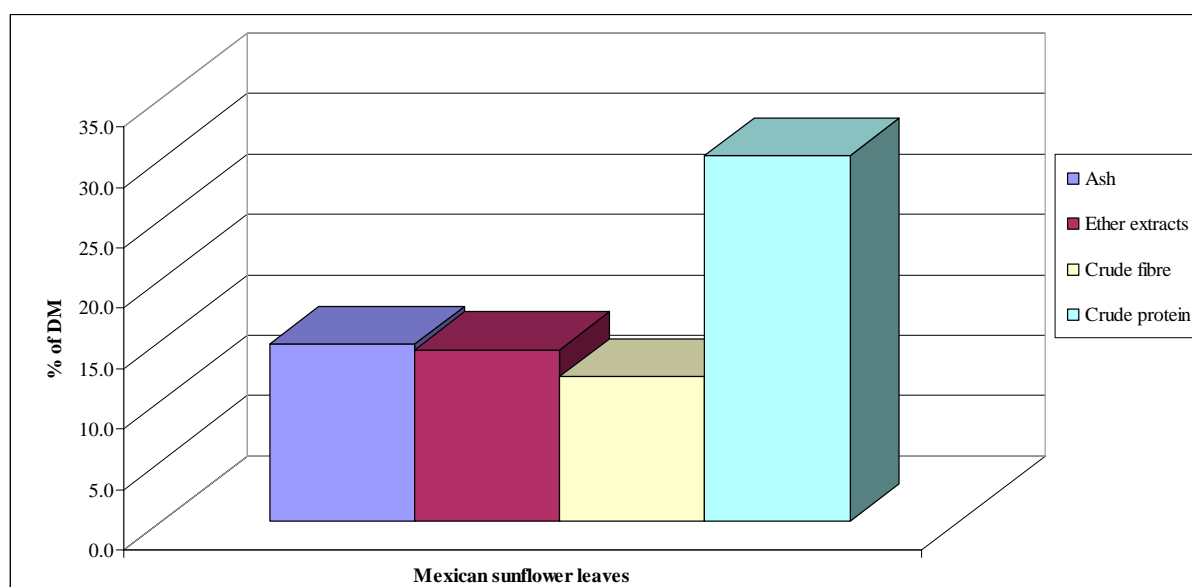


Figure 4.2: Mexican sunflower leaves - graphical representation of the nutrient composition [arithmetic mean in % of DM]

4.1.3 Sweet Potato Leaves (*Ipomoea batatas*)

The results of the nutrient analysis of sweet potato leaves are shown in table 4.4 and figure 4.3

Table 4.4: Sweet potato leaves - nutrient composition [arithmetic mean in % of DM \pm standard deviation]

Feed component	Nutrient analysis [% DM \pm SD]			
	Ash	Ether extracts	Crude fibre	Crude protein
Sweet potato leaves	13.1 \pm 0.15	10.7 \pm 0.55	15.1 \pm 0.07	28.1 \pm 0.47

Sweet potato leaves feature the second highest ash content, accounting for 13.7 %. Likely the results have to do with the fact that the leaves grow at ground level and therefore are exposed to pollution caused by wind, rain or agricultural cultivation, whereby they become polluted easier. With 10.7 %, sweet potato leaves show the lowest content measured by ether extracts. The crude fibre content accounts for 15.1 %. Due to the relatively coarse leaf stalks of the sweet potato, it was easy to separate them from the leaves, hence the good fibre contents result from. The crude protein content is 28.1 %.

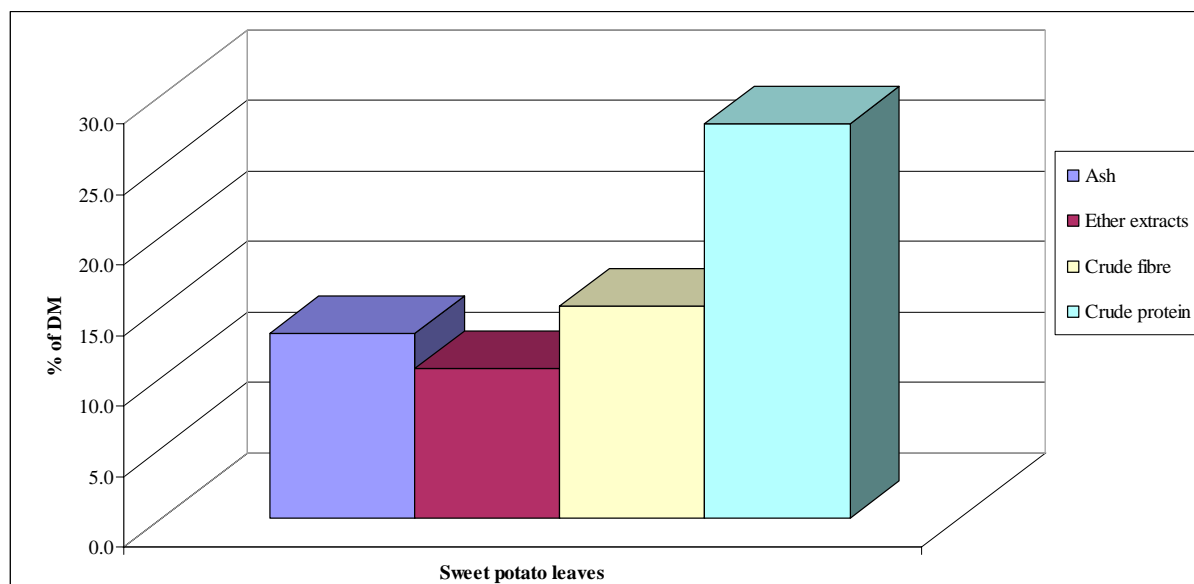


Figure 4.3: Sweet potato leaves - graphical representation of the nutrient composition [arithmetic mean in % of DM]

4.1.4 Cassava Leaves (*Manihot esculenta*)

For the nutrient composition of cassava leaves see table 4.5 and figure 4.4.

Table 4.5: Cassava leaves - nutrient composition [arithmetic mean in % of DM \pm standard deviation]

Feed component	Nutrient analysis [% DM \pm SD]			
	Ash	Ether extracts	Crude fibre	Crude protein
Cassava leaves	7.5 \pm 0.02	15.0 \pm 0.06	16.9 \pm 1.00	28.8 \pm 0.25

With 7.5 % cassava leaves have the second lowest ash content. The leaves of the woody shrub have been collected some distance from the ground and the place of collection was on a private field, offside the roads which may explain their low ash content. Concerning crude lipids, cassava finishes second with 15.0 %. Cassava has the highest content of crude fibre, namely 16.9 %. This can be attributed to the fact, that the leaves were chopped directly after harvesting to detoxify them and therefore it was not possible to completely separate the leaves from all coarse leaf stalks. The crude protein content is 28.8 % and thereby the second highest of all feed components.

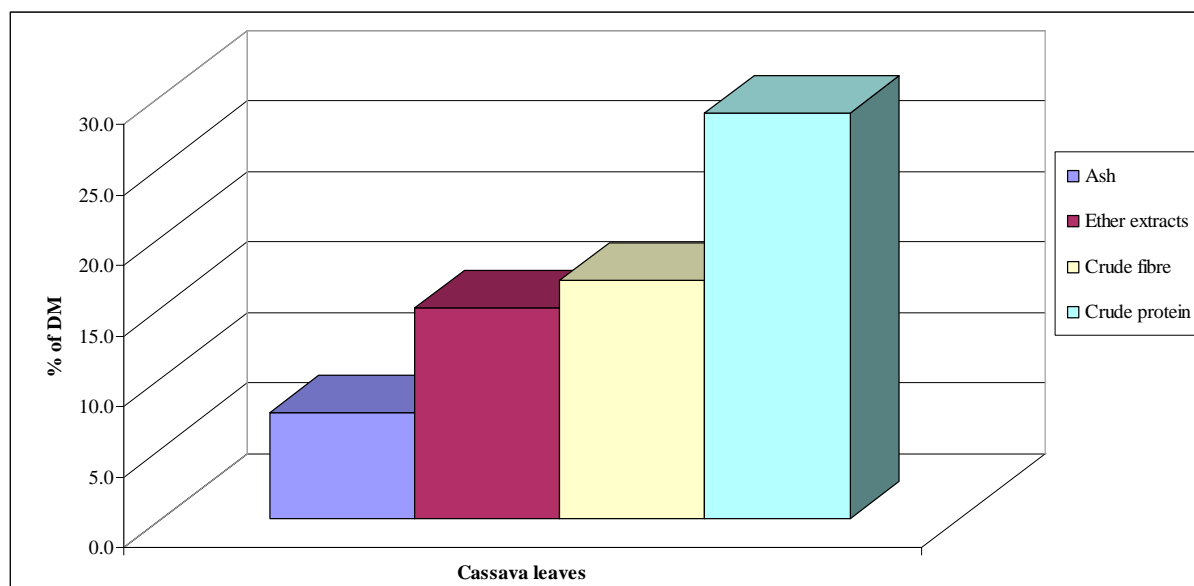


Figure 4.4: Cassava leaves - graphical representation of the nutrient composition [arithmetic mean in % of DM]

4.1.5 Comparison of Feed Components

The nutrient contents of crude lipid and crude protein are of particular importance in fish feeds. According to EL-SAYED [2006] the crude lipid content of fish feed should account for 15 % and the crude protein content should lie between 20-30 % to meet the demands of Nile Tilapia (*Oreochromis niloticus*). Table 4.6 shows that all feed components score the recommended values of crude protein. In contrast, sweet potato leaves is the only ingredient that does not reach the required 15 % of crude lipid. Based on these results, Mexican sunflower and cassava perform best followed by brewery waste. At the bottom of the list is sweet potato, failing the crude lipid requirements.

Table 4.6: Nutrient contents of all feed components compared to the recommendation of EL-SAYED [2006]

Feed components	Nutrient contents [% of DM]	
	Ether extracts	Crude protein
On the recommendation of EL-SAYED [2006]	15	20-30
Brewery waste	15.2	28.0
Mexican sunflower leaves	14.2	30.4
Sweet potato leaves	10.7	28.1
Cassava leaves	15.0	28.8

4.1.5.1 Nutrient Contents Details

Ash

The ash content is the solid residue that remains after the incineration of organic matter and hence a measurement for the mineral and micronutrient content of a sample, but also of the level of pollution. Referring to figure 4.5, brewery waste performs best with 5.2 %, closely

followed by cassava leaves (7.5 %), which were soaked for several hours in order to detoxify and therefore cleansed of all pollution. Far behind are sweet potato leaves (13.1 %) and Mexican sunflower leaves (14.7 %).

Ether Extracts

Given that ether extract (=crude lipid) is the predominant energy source for fish, a high crude lipid content is desirable. While brewery waste and cassava leaves perform similarly well (15-15.2 %), closely followed by Mexican sunflower leaves (14.2 %), sweet potato shows the weakest result with 10.7 %.

Crude Fibre

Since crude fibre can cause weight loss in fish due to its low nutritional value, the crude fibre content of fish feeds should be at the lowest possible level. Once again, Mexican sunflower leaves come out on top, accounting for 12 %. Brewery waste (15.3 %) and sweet potato leaves (15.1 %) almost come head to head. Cassava has the weakest results regarding crude fibre (16.9 %), which can be derived from the converting of the leaves. In order to detoxify the leaves they were chopped and most of the coarse leaf stalks could not be separated.

Crude Protein

Crude protein is synonymous with growth in fish production and therefore a crucial factor in every fish feed. As shown in figure 4.5, Mexican sunflower leaves have the highest protein content (30.4 %) and therefore meet the requirements as fish feed. Second is cassava (28.8 %) closely followed by sweet potato leaves (28.1 %) and brewery waste (28.0 %).

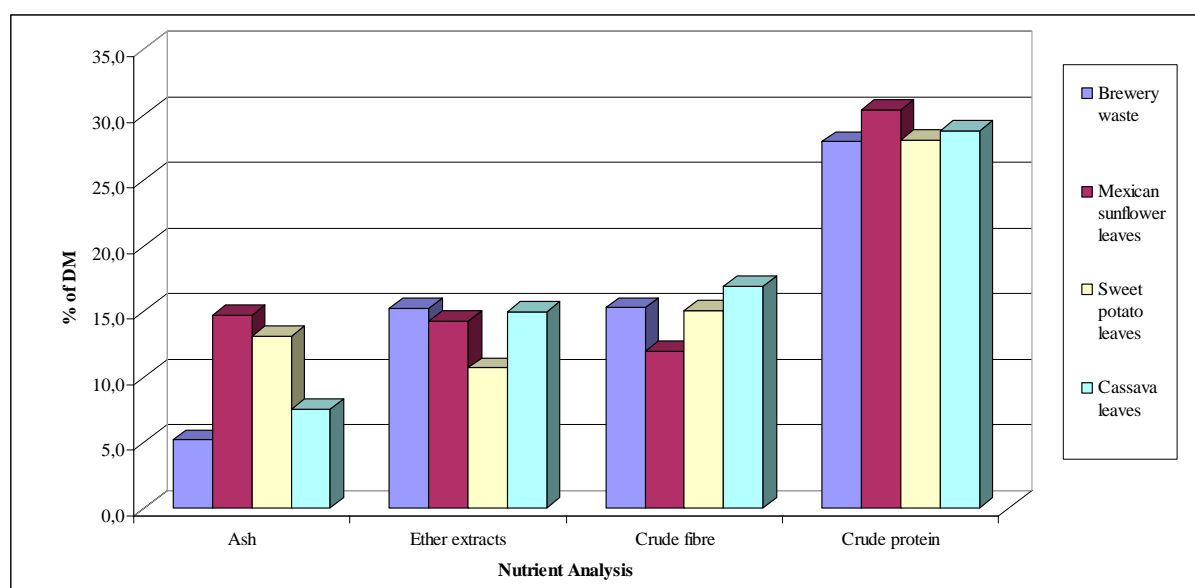


Figure 4.5: Feed components - graphical representation of the nutrient composition [arithmetic mean in % of DM]

Based on the deciding nutrient contents of fish feed, namely crude lipid and crude protein, Mexican sunflower can be favoured as ingredient for any feed. Brewery waste and cassava leaves meet the required standards of fish feed as well.

4.2 Gravimetric Mixtures

For the mixtures studied in this thesis, leaves of Mexican sunflower, sweet potato and cassava were mixed with the main feed component, brewery waste. As gravimetric mixtures, they were weighed and corresponding to the diverse proportions, mixed with each other. Afterwards, the mixtures were pelletised and each mixture was produced with two different binders; gelatine and agartine. In doing so, variable amounts of binders were used. The basic question was, whether the amount of binder in the feed has an effect on the descent rate of the pellets. Since the produced feed is intended for small-scale cage culture, the descent rate plays a decisive role. If the pellets sink too quickly, the availability by the fish is temporary and therefore negative for growth. Furthermore the amount of binder affects the rigidity of the feed and hence may be influential on feeding behaviour.

The data of the mixtures were split into the three species of plants and transferred from afore created excel sheets to the statistical program SPSS 15.0 for Windows. In the following tables, the results of the mixtures are presented containing the arithmetic mean and the standard deviation. The graphical representations show the results in terms of the arithmetic mean. On the y-axis the nutrient contents are shown, on the x-axis the arithmetic mean in % of dry matter can be seen. Furthermore, a one-way analysis of variance has been conducted in order to show its significance in influence and the degree of accuracy of the used feed component, binder or mixing ratio on the results.

4.2.1 Mixtures with Mexican Sunflower Leaves (*Tithonia rotundifolia*)

For the results of the nutrient analysis of the mixtures with Mexican sunflower leaves, see table 4.7. The graphical representation of the nutrient composition is differentiated by the two binders; gelatine and agartine, shown in figures 4.6 and 4.7.

Table 4.7: Mixtures with Mexican sunflower leaves - nutrient composition [arithmetic mean in % of DM \pm standard deviation]

Binders	Mixtures	Nutrient analysis [% DM \pm SD]			
	BW/MSF [%]	Ash	Ether extracts	Crude fibre	Crude protein
Gelatine	50/50	9.7 \pm 0.03	8.1 \pm 0.33	15.5 \pm 0.49	30.0 \pm 0.31
	60/40	8.8 \pm 0.24	9.0 \pm 0.12	17.3 \pm 0.29	29.2 \pm 0.18
	40/60	10.6 \pm 0.02	11.8 \pm 0.10	15.0 \pm 0.85	30.3 \pm 0.09
	70/30	7.5 \pm 0.03	13.3 \pm 0.16	15.5 \pm 0.59	28.9 \pm 0.18
	30/70	11.6 \pm 0.03	15.5 \pm 0.41	12.6 \pm 0.92	30.1 \pm 0.06
Agartine	50/50	9.5 \pm 0.05	11.0 \pm 0.38	15.8 \pm 0.70	28.9 \pm 0.29
	60/40	8.7 \pm 0.02	8.8 \pm 0.21	14.2 \pm 0.11	28.7 \pm 0.20
	40/60	10.4 \pm 0.09	12.2 \pm 0.16	16.7 \pm 1.29	27.3 \pm 0.29
	70/30	7.3 \pm 0.03	9.6 \pm 0.09	18.3 \pm 0.83	27.4 \pm 0.35
	30/70	11.7 \pm 0.06	17.9 \pm 0.42	12.1 \pm 0.59	31.0 \pm 0.14

Basically, mixtures with gelatine feature better results concerning crude protein than mixtures with agartine. The one exception is the mixture containing 30 % brewery waste and 70 % Mexican sunflower with agartine as binder. Due to the excellent protein values of Mexican sunflower the mixtures perform well also. The three mixtures with the highest content of

crude protein are highlighted in gray (see table 4.7), they are the ones with the highest percentage of Mexican sunflower in the mixture. It is clearly shown in figure 4.6 that the crude protein content increases with higher share in Mexican sunflower. It is not apparent from the figure that an increasing percentage of one feed component has an influence on the results of crude lipid. Figure 4.7 on the contrary, shows a correlation between good results in terms of crude lipid and a higher ratio of Mexican sunflower.

These mixtures have the lowest content of gelatine/agartine with only 18 g per mixture. As was to be expected, all mixtures with agartine show lower crude protein contents than the mixtures with gelatine, except for the 30/70 mixture.

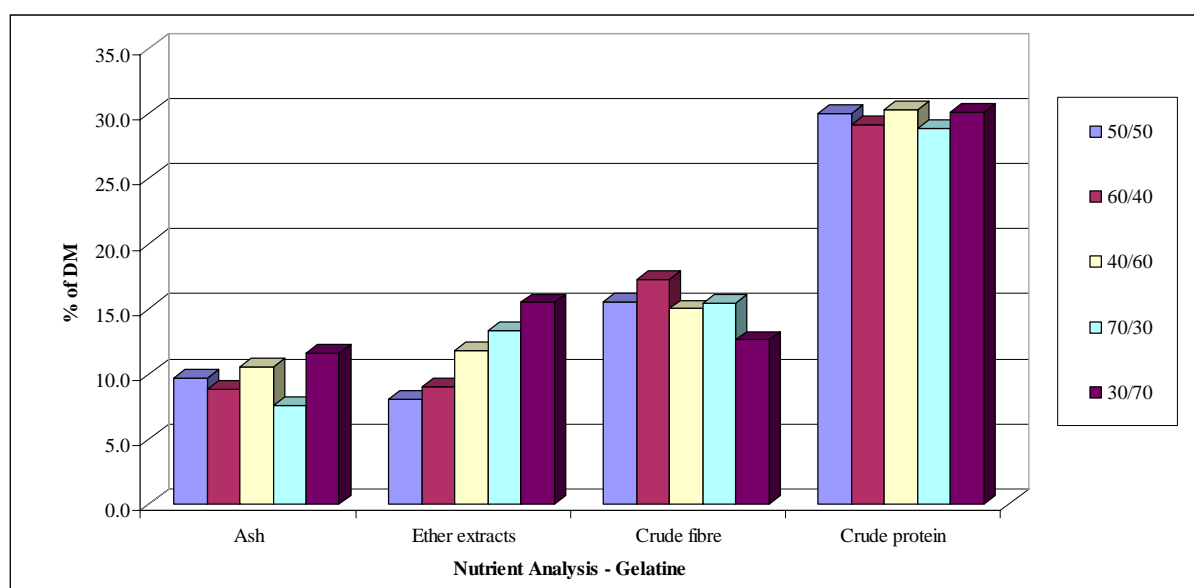


Figure 4.6: Mixtures with Mexican sunflower leaves and gelatine as binder - graphical representation of nutrient composition [arithmetic mean in % of DM]

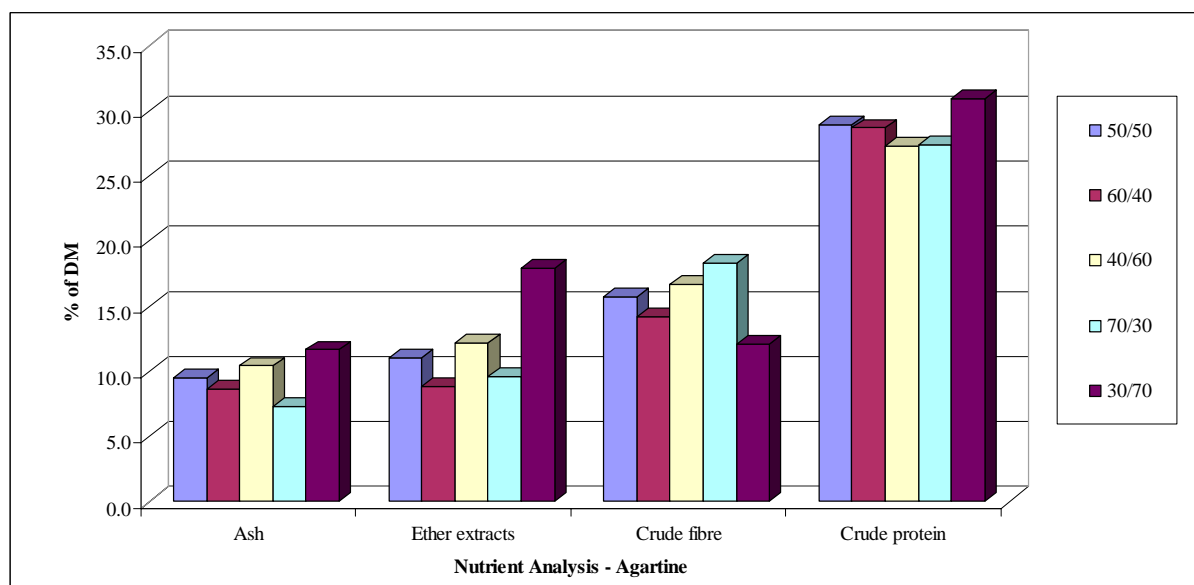


Figure 4.7: Mixtures with Mexican sunflower leaves and agartine as binder - graphical representation of nutrient composition [arithmetic mean in % of DM]

4.2.2 Mixtures with Sweet Potato Leaves (*Ipomoea batatas*)

For the nutrient composition of the mixtures with sweet potato leaves see table 4.8. The graphical representation of mixtures with sweet potato leaves and gelatine as binder is shown in figure 4.8 and the one with agartine as binder is shown in figure 4.9.

Table 4.8: Mixtures with sweet potato leaves - nutrient composition [arithmetic mean in % of DM \pm standard deviation]

Binders	Mixtures	Nutrient analysis [% DM \pm SD]			
	BW/SP [%]	Ash	Ether extracts	Crude fibre	Crude protein
Gelatine	50/50	8.7 \pm 0.02	10.8 \pm 0.15	15.4 \pm 0.60	26.8 \pm 0.02
	60/40	7.8 \pm 0.00	12.4 \pm 0.06	15.7 \pm 0.90	27.7 \pm 0.16
	40/60	9.4 \pm 0.04	12.4 \pm 0.25	14.7 \pm 0.11	26.2 \pm 0.12
	70/30	7.3 \pm 0.01	16.0 \pm 0.19	15.6 \pm 0.75	27.9 \pm 0.90
	30/70	10.2 \pm 0.08	15.6 \pm 0.01	13.6 \pm 0.06	24.9 \pm 0.54
Agartine	50/50	8.8 \pm 0.05	9.5 \pm 0.14	14.2 \pm 0.99	23.4 \pm 0.08
	60/40	7.9 \pm 0.05	10.5 \pm 0.14	15.6 \pm 0.61	22.5 \pm 0.17
	40/60	9.6 \pm 0.11	9.1 \pm 0.09	14.5 \pm 0.74	21.0 \pm 0.50
	70/30	7.2 \pm 0.02	13.0 \pm 0.81	15.0 \pm 0.42	24.1 \pm 0.22
	30/70	10.4 \pm 0.08	10.0 \pm 0.17	12.1 \pm 0.34	21.5 \pm 0.04

The greatest amount of gelatine/agartine was used with sweet potato leaves, namely 54 g per mixture. Despite this fact, these mixtures are not leading in the list of best results concerning crude protein content. Again, table 4.8 clearly shows that mixtures with agartine have a lower content of crude protein than the ones with gelatine. The three mixtures with the highest crude protein content, all of which are with gelatine as binder, are highlighted in gray. The mixture with the best result is the one with the mixing ratio 70/30 and gelatine as binder. It is evident in both figures that with an increasing percentage of brewery waste in the mixtures the content of crude protein is also increasing. This became apparent in the poor results of sweet potato with respect to crude protein as single component. Here, too, sweet potato does not reach the recommended 15 % of crude lipid.

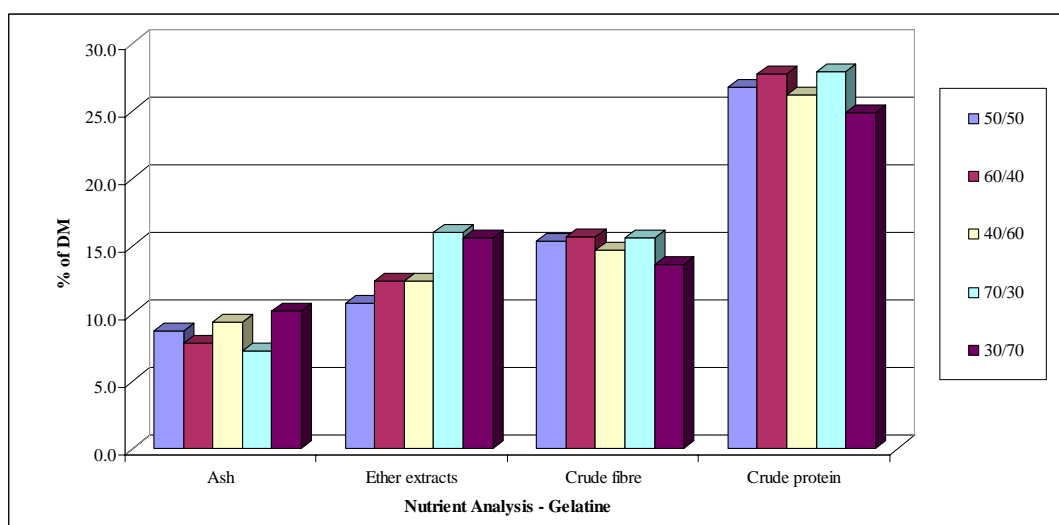


Figure 4.8: Mixtures with sweet potato leaves and gelatine as binder - graphical representation of nutrient composition [arithmetic mean in % of DM]

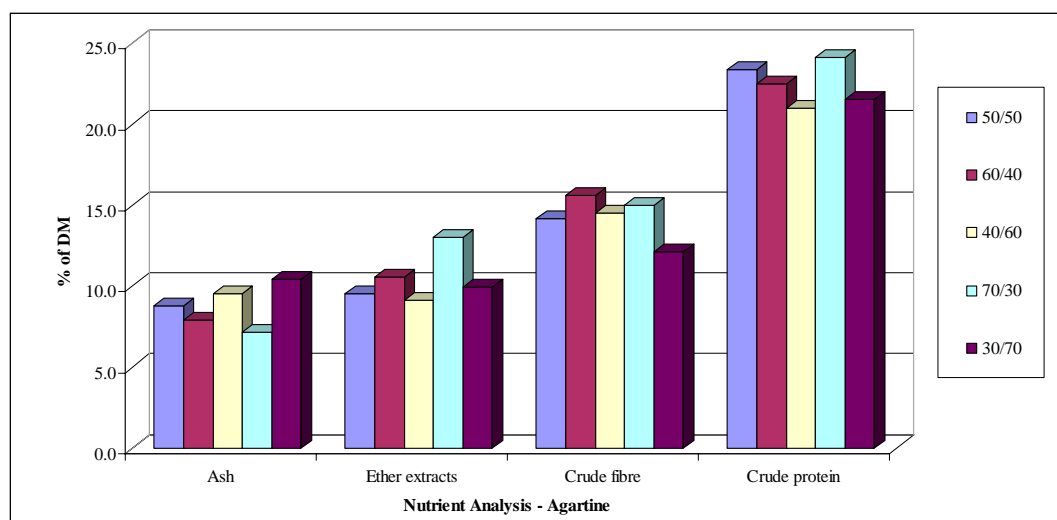


Figure 4.9: Mixtures with sweet potato leaves and agartine as binder - graphical representation of nutrient composition [arithmetic mean in % of DM]

4.2.3 Mixtures with Cassava Leaves (*Manihot esculenta*)

The result of the nutrient analysis of mixtures with cassava leaves are shown in table 4.9. The graphical representation is divided into gelatine and agartine and shown in figures 4.10 and 4.11.

Table 4.9: Mixtures with cassava leaves - nutrient composition [arithmetic mean in % of DM \pm standard deviation]

Binders	Mixtures	Nutrient analysis [% DM \pm SD]			
	BW/C [%]	Ash	Ether extracts	Crude fibre	Crude protein
Gelatine	50/50	6.2 \pm 0.06	16.5 \pm 0.10	17.8 \pm 0.46	31.1 \pm 0.15
	60/40	5.9 \pm 0.04	17.1 \pm 0.63	17.1 \pm 0.70	31.2 \pm 0.22
	40/60	6.4 \pm 0.02	17.3 \pm 0.78	17.8 \pm 0.56	31.1 \pm 0.21
	70/30	5.6 \pm 0.02	17.0 \pm 0.48	17.1 \pm 0.11	31.7 \pm 0.07
	30/70	6.6 \pm 0.08	15.9 \pm 0.18	15.8 \pm 0.26	32.4 \pm 0.11
Agartine	50/50	6.2 \pm 0.06	15.1 \pm 0.05	14.9 \pm 0.13	18.1 \pm 0.47
	60/40	5.9 \pm 0.04	14.6 \pm 0.44	16.7 \pm 0.32	27.7 \pm 0.39
	40/60	6.4 \pm 0.02	14.7 \pm 0.01	16.6 \pm 0.31	26.3 \pm 0.12
	70/30	5.6 \pm 0.02	14.8 \pm 0.30	16.3 \pm 0.53	27.8 \pm 0.21
	30/70	6.6 \pm 0.08	14.6 \pm 0.07	16.5 \pm 0.92	28.4 \pm 0.05

The mixtures with the highest crude protein contents are the ones with gelatine as binder. Therefore the mixture with 30 % brewery waste and 70 % cassava leaves comes in first. Cassava has good values as single feed component and the high protein content of gelatine amplifies this effect. It also has to be taken into consideration that 36 g of gelatine/agartine were used with cassava leaves, while with Mexican sunflower leaves for example, only 18 g were used. It is apparent that all mixtures have evenly spread results in relation to ether extracts, crude fibre and crude protein with gelatine. Only the results of mixtures with agartine deviate from this pattern.

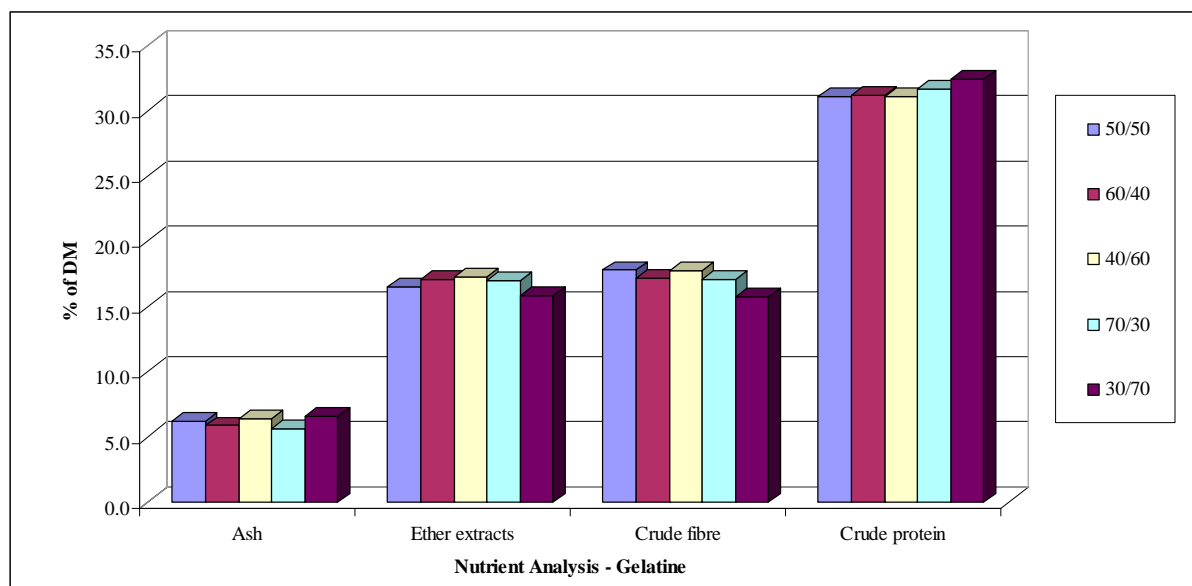


Figure 4.10: Mixtures with cassava leaves and gelatine as binder - graphical representation of nutrient composition [arithmetic mean in % of DM]

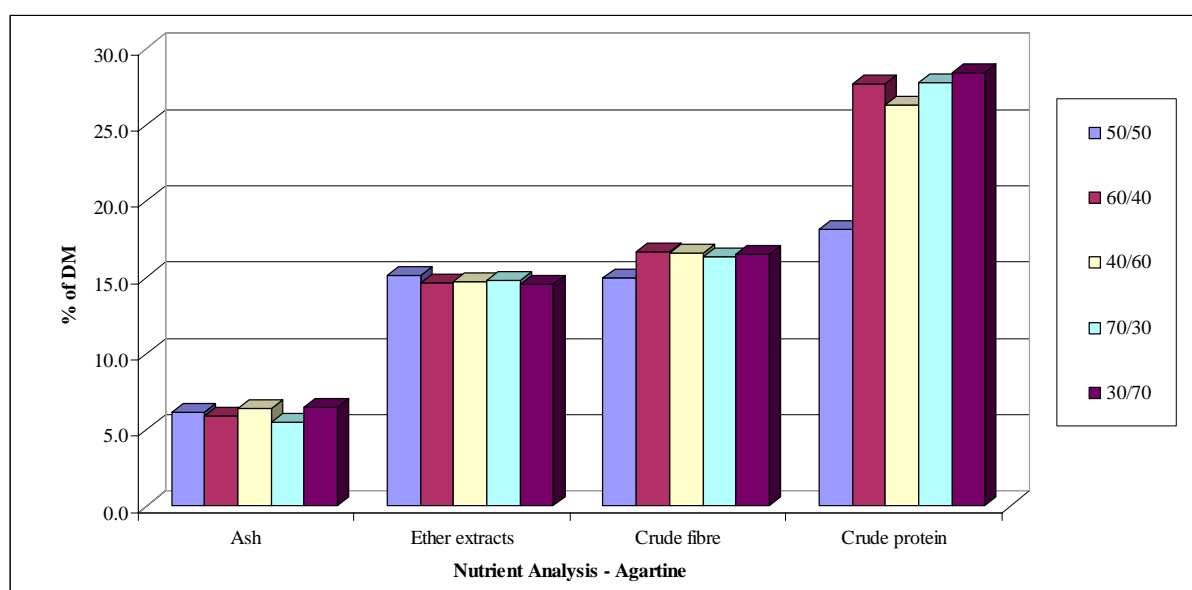


Figure 4.11: Mixtures with cassava leaves and agartine as binder - graphical representation of nutrient composition [arithmetic mean in % of DM]

4.2.4 Comparison of the Gravimetric Mixtures

Tables 4.10 and 4.11 give an overview of the results of the nutrient analysis of all mixtures, separated according to gelatine and agartine as binders. The feed components face each other in direct comparison and the ones with the best results concerning crude protein are highlighted in gray. As shown in table 4.10, mixtures with cassava are ahead of the other feed components. With respect to agartine, Mexican sunflower shows slightly better results with one exception, namely mixture 70/30 with cassava. As the values are all within the same range, both with gelatine and agartine respectively, it can be assumed that the higher protein content of gelatine accounts for this difference. Cassava as well as Mexican sunflower can be

recommended due to these results. The percentage of the mixture mentioned first is always brewery waste.

Table 4.10: Mixtures of all feed components analysed by mixing ratio with gelatine - nutrient composition [arithmetic mean in % of DM \pm standard deviation]

Mixtures	Feed components	Nutrient analysis [% DM \pm SD]			
		Ash	Ether extracts	Crude fibre	Crude protein
50/50	Mexican sunflower	9.7 \pm 0.03	8.1 \pm 0.33	15.5 \pm 0.49	30.0 \pm 0.31
	Sweet potato	8.7 \pm 0.02	10.8 \pm 0.15	15.4 \pm 0.60	26.8 \pm 0.02
	Cassava	6.2 \pm 0.06	16.5 \pm 0.10	17.8 \pm 0.46	31.1 \pm 0.15
60/40	Mexican sunflower	8.8 \pm 0.24	9.0 \pm 0.12	17.3 \pm 0.29	29.2 \pm 0.18
	Sweet potato	7.8 \pm 0.00	12.4 \pm 0.06	15.7 \pm 0.90	27.7 \pm 0.16
	Cassava	5.9 \pm 0.04	17.1 \pm 0.63	17.1 \pm 0.70	31.2 \pm 0.22
40/60	Mexican sunflower	10.6 \pm 0.02	11.8 \pm 0.10	15.0 \pm 0.85	30.3 \pm 0.09
	Sweet potato	9.4 \pm 0.04	12.4 \pm 0.25	14.7 \pm 0.11	26.2 \pm 0.12
	Cassava	6.4 \pm 0.02	17.3 \pm 0.78	17.8 \pm 0.56	31.1 \pm 0.21
70/30	Mexican sunflower	7.5 \pm 0.03	13.3 \pm 0.16	15.5 \pm 0.59	28.9 \pm 0.18
	Sweet potato	7.3 \pm 0.01	16.0 \pm 0.19	15.6 \pm 0.75	27.9 \pm 0.90
	Cassava	5.6 \pm 0.02	17.0 \pm 0.48	17.1 \pm 0.11	31.7 \pm 0.07
30/70	Mexican sunflower	11.6 \pm 0.03	15.5 \pm 0.41	12.6 \pm 0.92	30.1 \pm 0.06
	Sweet potato	10.2 \pm 0.08	15.6 \pm 0.01	13.6 \pm 0.06	24.9 \pm 0.54
	Cassava	6.6 \pm 0.08	15.9 \pm 0.18	15.8 \pm 0.26	32.4 \pm 0.11

Table 4.11: Mixtures of all feed components analysed by mixing ratio with agartine - nutrient composition [arithmetic mean in % of DM \pm standard deviation]

Mixtures	Feed components	Nutrient analysis [% DM \pm SD]			
		Ash	Ether extracts	Crude fibre	Crude protein
50/50	Mexican sunflower	9.5 \pm 0.05	11.0 \pm 0.38	15.8 \pm 0.70	28.9 \pm 0.29
	Sweet potato	8.8 \pm 0.05	9.5 \pm 0.14	14.2 \pm 0.99	23.4 \pm 0.08
	Cassava	6.2 \pm 0.06	15.1 \pm 0.05	14.9 \pm 0.13	18.1 \pm 0.47
60/40	Mexican sunflower	8.7 \pm 0.02	8.8 \pm 0.21	14.2 \pm 0.11	28.7 \pm 0.20
	Sweet potato	7.9 \pm 0.05	10.5 \pm 0.14	15.6 \pm 0.61	22.5 \pm 0.17
	Cassava	5.9 \pm 0.04	14.6 \pm 0.44	16.7 \pm 0.32	27.7 \pm 0.39
40/60	Mexican sunflower	10.4 \pm 0.09	12.2 \pm 0.16	16.7 \pm 1.29	27.3 \pm 0.29
	Sweet potato	9.6 \pm 0.11	9.1 \pm 0.09	14.5 \pm 0.74	21.0 \pm 0.50
	Cassava	6.4 \pm 0.02	14.7 \pm 0.01	16.6 \pm 0.31	26.3 \pm 0.12
70/30	Mexican sunflower	7.3 \pm 0.03	9.6 \pm 0.09	18.3 \pm 0.83	27.4 \pm 0.35
	Sweet potato	7.2 \pm 0.02	13.0 \pm 0.81	15.0 \pm 0.42	24.1 \pm 0.22
	Cassava	5.6 \pm 0.02	14.8 \pm 0.30	16.3 \pm 0.53	27.8 \pm 0.21
30/70	Mexican sunflower	11.7 \pm 0.06	17.9 \pm 0.42	12.1 \pm 0.59	31.0 \pm 0.14
	Sweet potato	10.4 \pm 0.08	10.0 \pm 0.17	12.1 \pm 0.34	21.5 \pm 0.04
	Cassava	6.6 \pm 0.08	14.6 \pm 0.07	16.5 \pm 0.92	28.4 \pm 0.05

In order to illustrate the above-mentioned results graphically two mixtures were chosen at random to compare the feed components with each other.

Mixing ratio 40/60

As previously mentioned, cassava and Mexican sunflower perform best in terms of crude protein (see figures 4.12 and 4.13). Sweet potato can not compete with these results. With regard to crude lipid and with agartine as binder, cassava comes off best, followed by Mexican sunflower. Again, the results of sweet potato do not meet the demands of Nile Tilapia (*Oreochromis niloticus*).

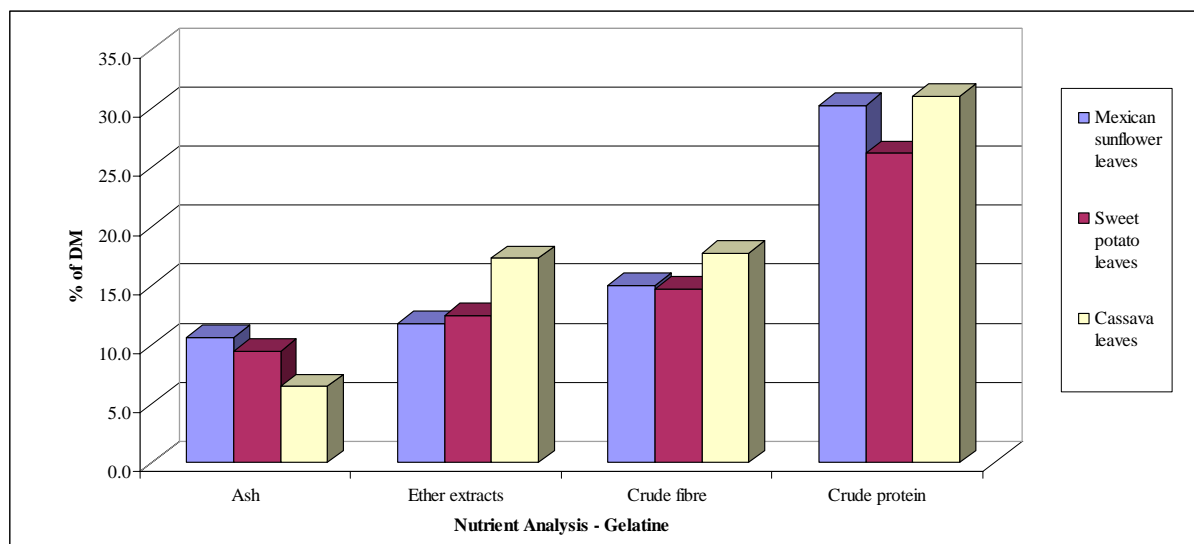


Figure 4.12: Mixture 40/60 with all feed components in comparison and gelatine as binder - graphical representation of nutrient composition [arithmetic mean in % of DM]

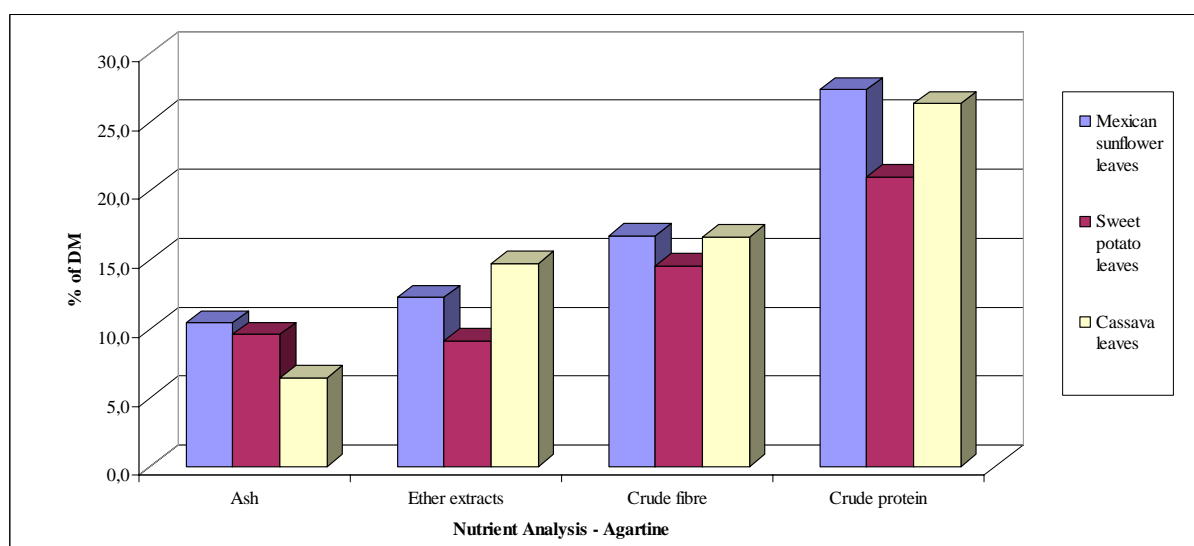


Figure 4.13: Mixture 40/60 with all feed components in comparison and agartine as binder - graphical representation of nutrient composition [arithmetic mean in % of DM]

Mixing ratio 70/30

It can clearly be seen in the graphical representations of figures 4.14 and 4.15 that cassava comes off best, followed by sweet potato concerning crude lipid. For crude protein cassava shows best values followed by Mexican sunflower. Sweet potato has better lipid results than Mexican sunflower but still can not reach the recommended 15 %.

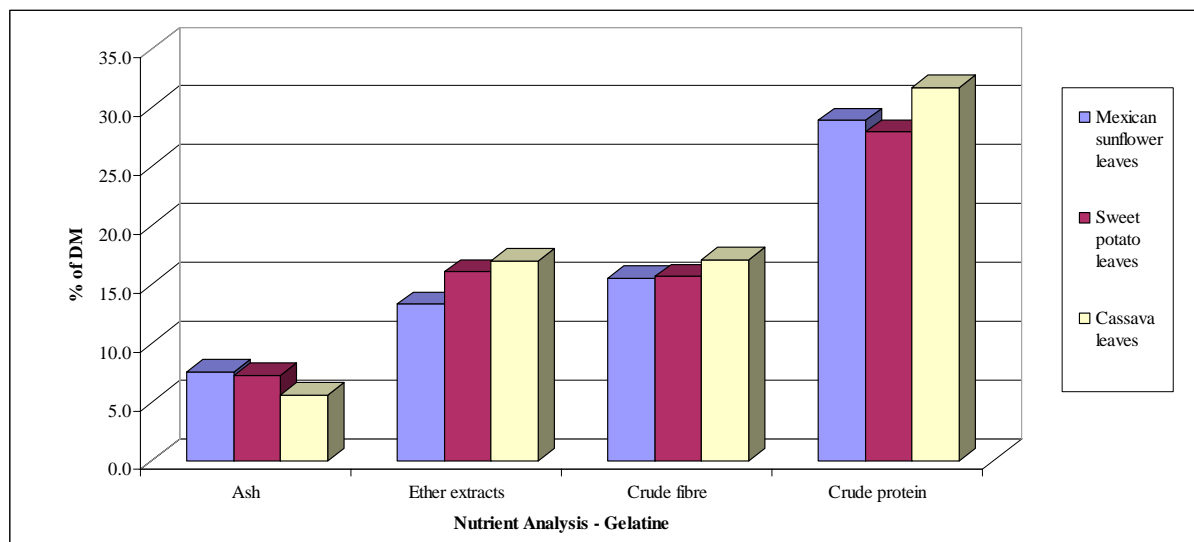


Figure 4.14: Mixture 70/30 with all feed components in comparison and gelatine as binder - graphical representation of nutrient composition [arithmetic mean in % of DM]

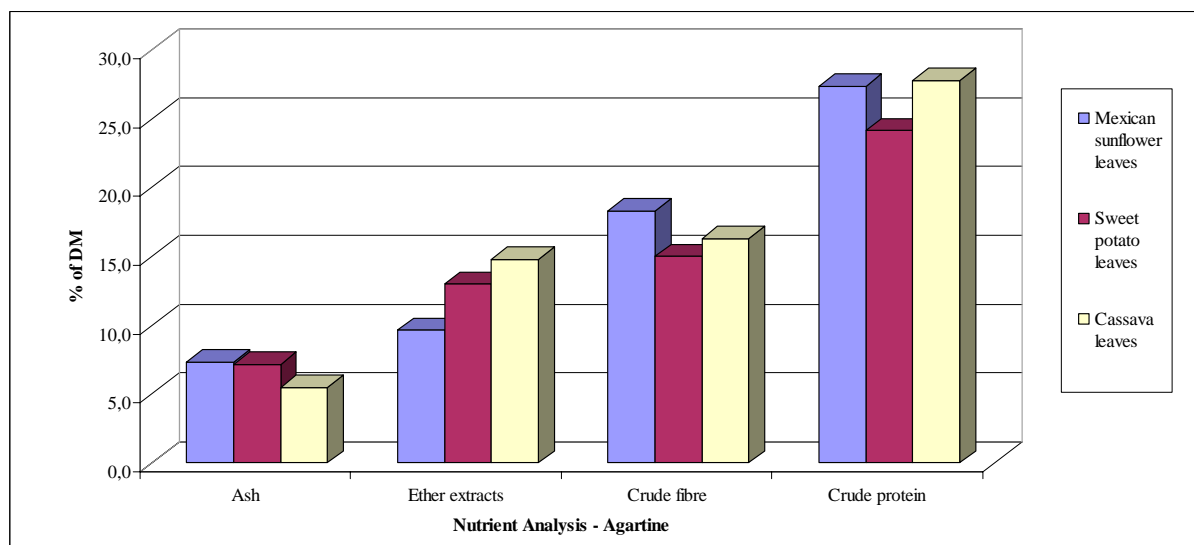


Figure 4.15: Mixture 70/30 with all feed components in comparison and agartine as binder - graphical representation of nutrient composition [arithmetic mean in % of DM]

Generally speaking, the mixtures with the highest crude protein contents are the ones with cassava leaves and gelatine as binder and therefore can be recommended as feed components for fish feed.

4.2.5 Confirmatory Factor Analysis (CFA)

So far the results of this thesis were merely analysed on an explorative level. However, it also has to be examined whether the results are statistically significant, i.e. whether they can be generalised (confirmatory factor analysis).

A one-way analysis of variance (ANOVA) gives information about the significance and the coefficient of determination of the individual nutrients relating to feed component, binder and mixture. This model of analysis is limited to main effects. A saturated model was completed but since no difference was assessed, interaction effects were turned down since main effects are easier to interpret.

In table 4.12 the results of the one-way analysis of variance are shown, distinguishing between feed component, binder and mixture. A p-value lower than or equal to 0.05 is referred to as statistically significant.

Table 4.12: Gravimetric mixtures - one-way analysis of variance (ANOVA)

Nutrients	Feed component		Binder		Mixture	
	p	ETA ²	p	ETA ²	p	ETA ²
Ash	0.000	0.653	0.933	0.000	0.000	0.282
Ether extracts	0.000	0.398	0.012	0.069	0.006	0.153
Crude fibre	0.000	0.258	0.181	0.020	0.000	0.304
Crude protein	0.000	0.348	0.000	0.288	0.544	0.035

The one-way analysis of variance has shown that feed components have a significant influence on all nutrients (p) and the coefficient of determination (ETA²) also shows a strong effect on these nutrients. The binder has no significant influence on ash and crude fibre, but with regard to ether extracts and crude protein, it has a significant, strongly pronounced influence. Relating to mixture, there is a significant influence on ash, ether extracts and crude fibre, but no significant influence on crude protein.

4.3 Volumetric Mixtures

In order to simplify the procedure of mixing the necessary feed components for fish farmers, a volumetric way of measurement has also been developed. The volumetric mixture (50/50) was composed with equal volume fractions of the individual feed components, by using a bucket. The nutrient compositions resulting from such volumetric mixtures are discussed in the following chapter.

By comparing the volumetric mixtures (same mixing ratio) with one another only the nutrient features of gelatine and agartine are compared. As the quantitative composition of the volumetric mixtures does not differ in anything but the binder within the particular species of plants, it should display an analogy to the greatest possible extent in matters of ash and crude fibre contents. It is clear that the crude protein content of the mixture with gelatine should be higher than the one with agartine. This is attributed to the fact that gelatine contains 83 g protein per 100 g, while agartine contains only 0.4 g protein per 100 g (see tables 3.6 and 3.7).

As both gelatine and agartine run clear from fat, there should be no difference between in lipid contents.

4.3.1 Volumetric Mixtures with Mexican Sunflower Leaves (*Tithonia rotundifolia*)

For the nutrient composition of volumetric mixtures with Mexican sunflower leaves see table 4.13 and figure 4.16.

Table 4.13: Volumetric mixtures with Mexican sunflower leaves - nutrient composition [arithmetic mean in % of DM \pm standard deviation]

Binders	Mixtures [%]	Nutrient analysis [% DM \pm SD]			
		Ash	Ether extracts	Crude fibre	Crude protein
Gelatine	50/50	9.1 \pm 0.03	12.8 \pm 0.65	17.0 \pm 1.08	29.2 \pm 0.24
Agartine	50/50	7.0 \pm 0.05	14.0 \pm 0.45	15.3 \pm 1.13	27.3 \pm 0.25

As was to be expected the crude protein content of the mixture with gelatine as binder is considerably higher than the one with agartine.

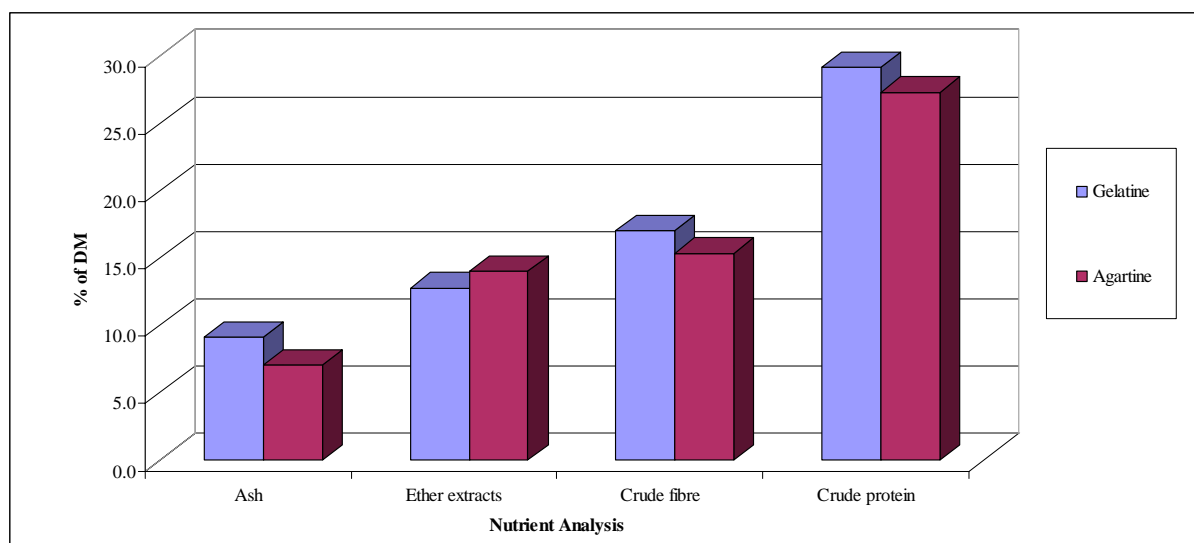


Figure 4.16: Volumetric mixtures with Mexican sunflower leaves - graphical representation of nutrient composition [arithmetic mean in % of DM]

4.3.2 Volumetric Mixtures with Sweet Potato Leaves (*Ipomoea batatas*)

The results of the nutrient analysis of volumetric mixtures with sweet potato leaves are shown in table 4.14 and figure 4.17.

Table 4.14: Volumetric mixtures with sweet potato leaves - nutrient composition [arithmetic mean in % of DM \pm standard deviation]

Binders	Mixtures [%]	Nutrient analysis [% DM \pm SD]			
		Ash	Ether extracts	Crude fibre	Crude protein
Gelatine	50/50	7.2 \pm 0.04	11.9 \pm 0.26	15.2 \pm 0.08	28.5 \pm 0.50
Agartine	50/50	6.6 \pm 0.03	10.8 \pm 0.13	15.9 \pm 0.69	23.5 \pm 0.25

Here, too, gelatine has significantly higher results in terms of crude protein.

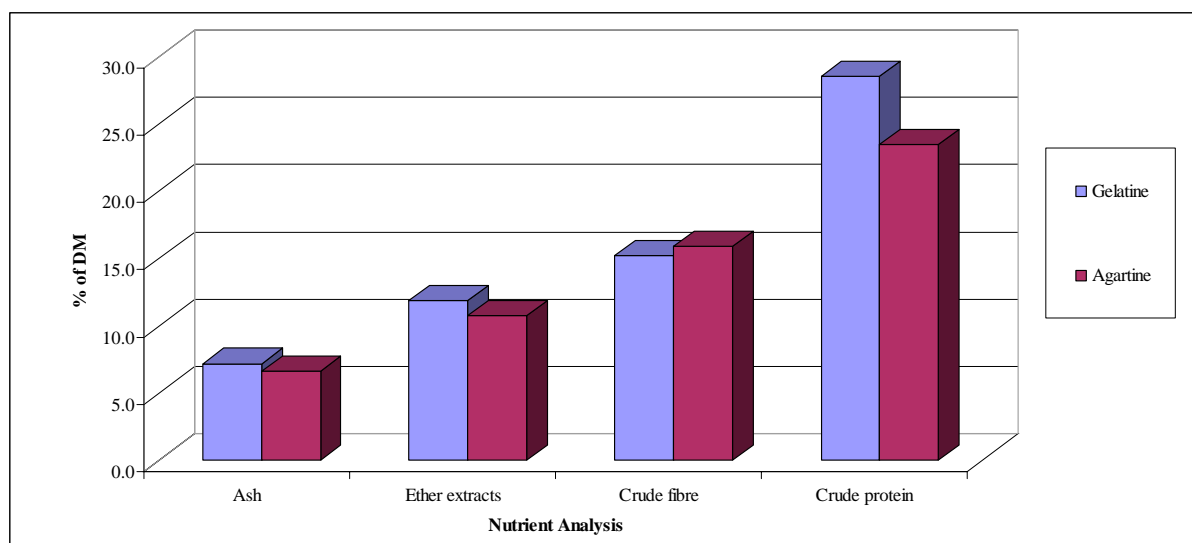


Figure 4.17: Volumetric mixtures with sweet potato leaves - graphical representation of nutrient composition [arithmetic mean in % of DM]

4.3.3 Volumetric Mixtures with Cassava Leaves (*Manihot esculenta*)

For the results of the nutrient analysis of volumetric mixtures with cassava leaves, see table 4.15 and figure 4.18.

Table 4.15: Volumetric mixtures with cassava leaves - nutrient composition [arithmetic mean in % of DM \pm standard deviation]

Binders	Mixtures [%]	Nutrient analysis [% DM \pm SD]			
		Ash	Ether extracts	Crude fibre	Crude protein
Gelatine	50/50	5.0 \pm 0.20	14.9 \pm 0.12	18.2 \pm 0.13	29.7 \pm 0.13
Agartine	50/50	5.2 \pm 0.04	14.1 \pm 0.33	17.4 \pm 0.40	27.1 \pm 0.08

The values of crude protein content are distinctly higher with gelatine as binder.

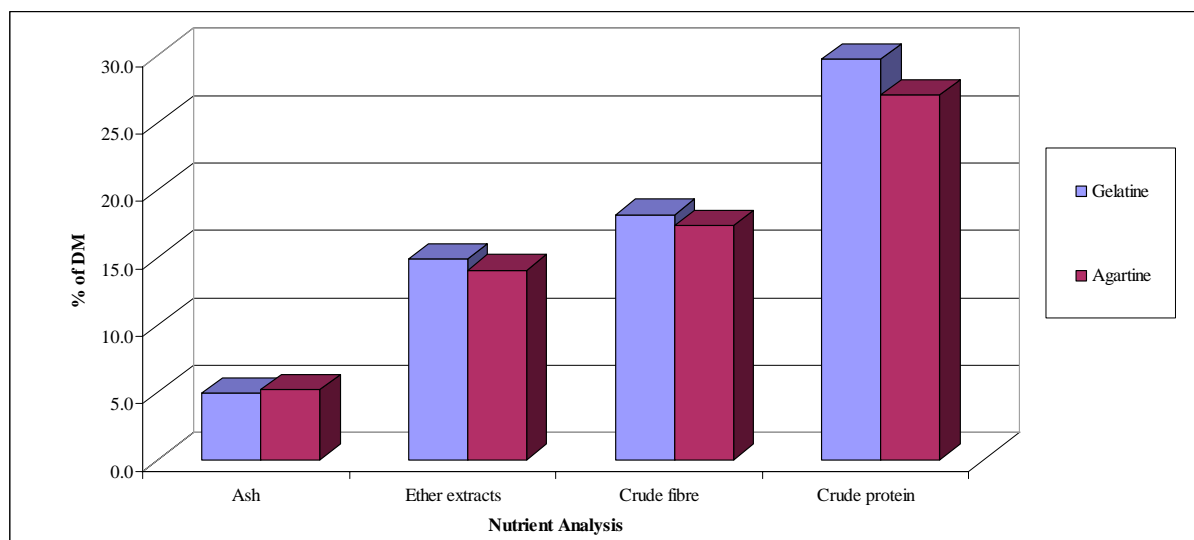


Figure 4.18: Volumetric mixtures with cassava leaves - graphical representation of nutrient composition [arithmetic mean in % of DM]

4.3.4 Comparison of the Volumetric Mixtures

Given that the volumetric mixtures of the various species of plants only differ in their binders, their distribution of nutrient analysis is almost identical, as was to be expected. In table 4.16 and figure 4.19, the results of the volumetric mixtures (mixing ratio 50/50) are displayed, comparing the feed components with each other. The best results are highlighted in gray.

Table 4.16: Volumetric mixtures, comparing all feed components - nutrient composition [arithmetic mean in % of DM \pm standard deviation]

Binders	Feed Components	Nutrient analysis [% DM \pm SD]			
		Ash	Ether extracts	Crude fibre	Crude protein
Gelatine	Mexican sunflower	9.1 \pm 0.03	12.8 \pm 0.65	17.0 \pm 1.08	29.2 \pm 0.24
	Sweet potato	7.2 \pm 0.04	11.9 \pm 0.26	15.2 \pm 0.08	28.5 \pm 0.50
	Cassava	5.0 \pm 0.20	14.9 \pm 0.12	18.2 \pm 0.13	29.7 \pm 0.13
Agartine	Mexican sunflower	7.0 \pm 0.05	14.0 \pm 0.45	15.3 \pm 1.13	27.3 \pm 0.25
	Sweet potato	6.6 \pm 0.03	10.8 \pm 0.13	15.9 \pm 0.69	23.5 \pm 0.25
	Cassava	5.2 \pm 0.04	14.1 \pm 0.33	17.4 \pm 0.40	27.1 \pm 0.08

Cassava lies ahead of the other feed components regarding crude lipid, crude fibre and - in combination with gelatine - also in terms of crude protein. Mexican sunflower only performs better than cassava with agartine as binder. As always, sweet potato mixtures lag far behind in every nutrient range. The results with gelatine are slightly better than the ones with agartine.

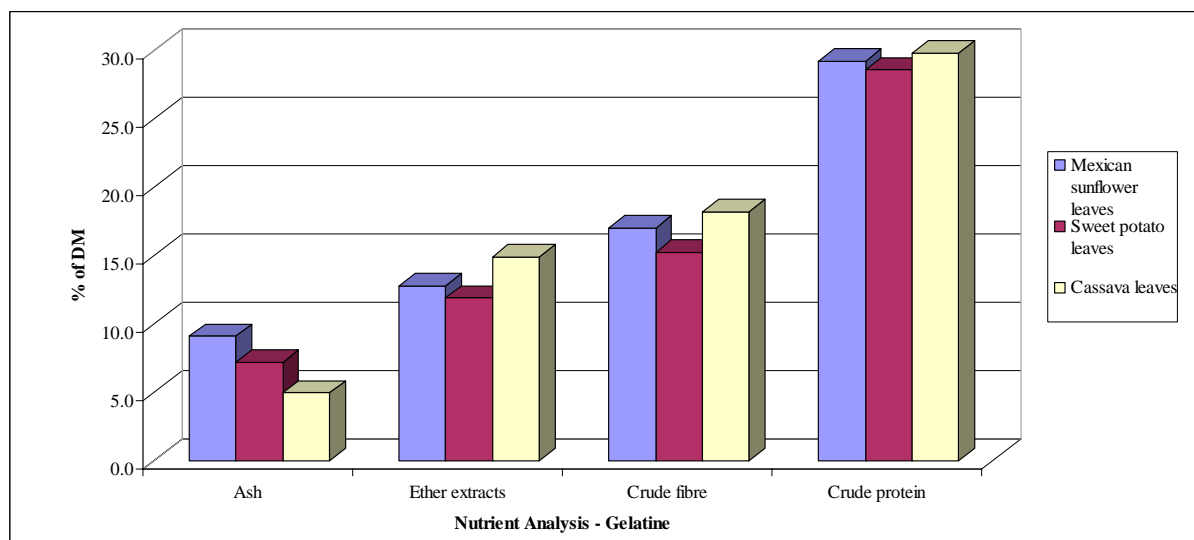


Figure 4.19: All volumetric mixtures by comparison with gelatine as binder [arithmetic mean in % of DM]

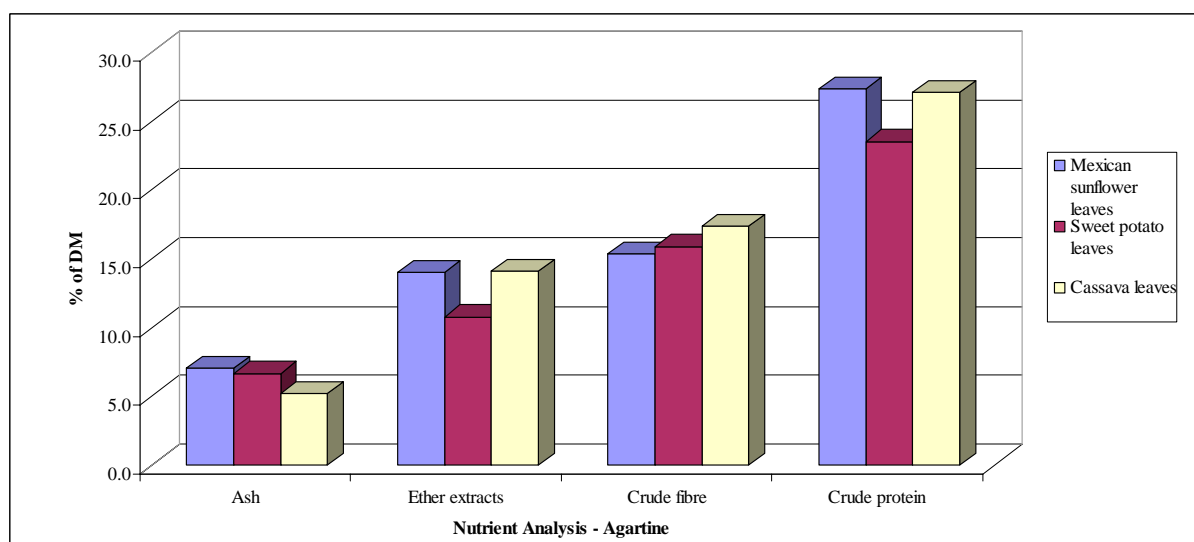


Figure 4.20: All volumetric mixtures by comparison with agartine as binder [arithmetic mean in % of DM]

4.3.5 Confirmatory Factor Analysis (CFA)

So far the results of this thesis were merely analysed on an explorative level. However, it also has to be examined whether the results are statistically significant, i.e. whether they can be generalised (confirmatory factor analysis).

A one-way analysis of variance has been made to give information about the significance and the coefficient of determination of the individual nutrients relating to feed component and binder. The model shown in table 4.17 is limited to main effects. A saturated model has been done but since no difference has been assessed, interaction effects have been turned down.

In table 4.17 the results of the one-way analysis of variance (ANOVA) are shown, distinguishing between feed component and binder. A p-value lower than or equal to 0,05 is referred to as statistically significant.

Table 4.17: Volumetric mixtures – one-way analysis of variance (ANOVA)

Nutrient Analysis	Feed component		Binder	
	P	ETA ²	p	ETA ²
Ash	0.000	0.881	0.252	0.081
Ether extracts	0.000	0.822	0.758	0.006
Crude fibre	0.002	0.565	0.335	0.058
Crude protein	0.084	0.282	0.000	0.598

The one-way analysis of variance shows that feed component has a significant influence on ash, ether extracts and crude fibre and the coefficient of determination also shows a strong effect on these nutrients. With regards to crude protein, the binder has a significant influence which is strongly pronounced. On the other hand, the binder has no significant influence on ash, ether extracts and crude fibre, but on the other hand there exists a strongly pronounced significance in terms of crude fibre.

4.4 Feeding Behaviour

Based on the excellent nutrient contents of Mexican sunflower, one basic question was, whether or not the fish accept it in the feed despite its bitterness. STÖGER [2010] responded to this question in her master thesis by analysing the feeding behaviour of Nile Tilapia (*Oreochromis niloticus*) concerning different feeds.

In the following chapter, the results concerning the acceptance of the mixtures by Nile Tilapia (*Oreochromis niloticus*) are briefly summarised. Table 4.18 gives an overview of 13 selected mixtures (highlighted in gray) out of altogether 36 produced mixtures. The numbers represent the share of the various feed components in the mixture in %.

Table 4.18: Overview of the 13 selected mixtures

Binders	Mixtures		
	BW/MSF [%]	BW/SP [%]	BW/C [%]
Gelatine	50/50 volumetric	50/50 volumetric	50/50 volumetric
	50/50	50/50	50/50
	70/30	70/30	70/30
	30/70	30/70	30/70
	60/40	60/40	60/40
	40/60	40/60	40/60
Agartine	50/50 volumetric	50/50 volumetric	50/50 volumetric
	50/50	50/50	50/50
	70/30	70/30	70/30
	30/70	30/70	30/70
	60/40	60/40	60/40
	40/60	40/60	40/60

The feeding behaviour was observed in two tanks stocked with five fish at a time. The fish were fed three times a day and at the same time filmed in sequences of eight minutes. The feeding behaviour was interpreted and statistically analysed. During the whole series of tests “Sagana diet” was used as reference feed, because it is a great favourite with the fish.

By observing feeding behaviour such as “snapping feed from the water column”, “spitting feed” and “scraping algae”, STÖGER [2010] analysed and interpreted whether or not particular behaviour was typical or atypical and hence not significant behaviour. Based on the statistical analysis of the feeding behaviour it was analysed which mixtures are accepted by the fish and therefore suitable as feed.

It is assumed that “spitting out the feed” is part of filtering favoured feed components, such as brewery waste out of the feed.

Algae intake reflects the acceptance for the feed and the outcome of this is the attempt to switch to another feed. This behaviour was observed commonly with Mexican sunflower leaf based feeds. Cassava and sweet potato showed a similar share in algae intake, which was relatively low compared to the feed intake. Consequently it can be assumed that they like the feed.

Furthermore it can be supposed that a high percentage of feed intake suggests a higher interest of the fish in the feed. Cassava had the highest number of feed intakes while the lowest number of feed intakes was observed with Mexican sunflower based feeds. It was even ignored after some time and finally the fish stopped feeding on it at all. Sweet potato lies between cassava and Mexican sunflower regarding acceptance of feeds.

4.5 Final Assessment

After the evaluation of all results, an order concerning ether extracts, crude fibre and crude protein was made. Subsequently, the results were distinguished between feed components, binders and mixtures. This assessment does not underlie any statistical analysis but is based upon the arithmetic mean of the empiric data of the three executed runs on the feed components.

4.5.1 Binders

Looking only at the binders, it is clear that the results of gelatine lie ahead of agartine in every single nutrient analysis. In figure 4.21 the assessment of the two binders is shown in which the binder coming off best is framed by a bold line.

Hence the application of gelatine basically has to be preferred especially given considerably higher protein contents, which are so important for the growth of the fish. Since agartine was mainly chosen in favour of the religious beliefs of Muslims, it still displays acceptable results and can be recommended for future feeds.

Ether extracts	Crude fibre	Crude protein
Gelatine	Gelatine	Gelatine
Agartine	Agartine	Agartine

Figure 4.21: Assessment of the two different binders, summing up crude lipid, crude fibre and crude protein

4.5.2 Feed Components

Feed Components only

Figure 4.22 shows an assessment of the used feed components according to ether extracts, crude fibre and crude protein. Here, the feed components were considered exclusively, ignoring the influence of binder and mixture ratio in the assessment. Since crude lipid and crude protein represent the most important nutrients of any fish feed, Mexican sunflower and cassava are the favoured ingredients of this listing. Sweet potato contains the lowest level of crude protein but the highest content of crude fibre. Mexican sunflower leaf contains the lowest level of crude lipid. The feed components are arranged according to significance in descending order. Consequently the feed component outlined with a bold line is the one of greatest importance. It has to be said that the lowest level of crude fibre is the best one, whereas the results of crude lipid and crude protein must be interpreted the other way round.

Ether extracts	Crude fibre	Crude protein
C	SP	MSF
SP	MSF	C
MSF	C	SP

Figure 4.22: Assessment of the feed components according to ether extracts, crude fibre and crude protein

Feed Components combined with Binders

In figure 4.23, the feed components are presented in combination with the binders, not including the influence of the mixture ratios. Cassava with gelatine shows the best results concerning crude lipid and crude protein. Sweet potato with agartine ranks last in this assessment in terms of crude lipid and crude protein but shows the best results with regard to crude fibre. Mexican sunflower mainly ranges in the middle. Once again it is apparent that gelatine achieves better results than agartine.

Ether extracts	Crude fibre	Crude protein
C - G	SP - A	C - G
C - A	SP - G	MSF - G
SP - G	MSF - G	MSF - A
MSF - A	MSF - A	SP - G
MSF - G	C - A	C - A
SP - A	C - G	SP - A

Figure 4.23: Assessment of the feed components combined with binders according to ether extracts, crude fibre and crude protein

4.5.3 Mixtures

Mixtures only

Figure 4.24 shows an assessment of the produced mixtures according to their ether extract, crude fibre and crude protein contents. The influence of the binders and the feed components were disregarded.

Feeds with the mixing ratio of 30/70 were assessed to be best suitable as fish feed, followed by a ratio of 70/30. The mixing ratio of 50/50 shows the weakest results in terms of crude lipid and crude protein. Based on this assessment it can not be definitely stated whether a high percentage of leaves is preferential or not.

The best results of each nutrient component are arranged on top of each column with the bold line.

Ether extracts	Crude fibre	Crude protein
30/70	30/70	30/70
70/30	50/50	70/30
40/60	40/60	60/40
60/40	60/40	40/60
50/50	70/30	50/50

Figure 4.24: Assessment of the mixtures according to ether extracts, crude fibre and crude protein

Mixtures combined with Binders

Mixtures and their binders are shown in figure 4.25. Here, the influence of the feed components on the results of the nutrient contents is not included. This rating shows again, that mixtures containing gelatine show best results with regard to their quality as fish feed. An unambiguous analysis whether the share of leaves within a mixture affects the results in a good or bad way, is not possible. At least the first two places are occupied by mixtures which have a high percentage of leaves. It should be noted that the figure below only features the five best results of each nutrient analysis. Therefore it does not represent the weakest results.

Ether extracts	Crude fibre	Crude protein
50/50 - G	30/70 - A	70/30 - G
60/40 - G	30/70 - G	60/40 - G
30/70 - A	50/50 - A	50/50 - G
40/60 - G	60/40 - A	40/60 - G
70/30 - G	40/60 - G	30/70 - G

Figure 4.25: Assessment of the mixtures combined with binders according to ether extracts, crude fibre and crude protein

Mixtures combined with Feed Components

Figure 4.26 shows the differentiation of the mixtures after feed components: ether extracts, crude fibre and crude protein. In principle Mexican sunflower contains the highest levels of all three components, followed by cassava for ether extracts and crude protein, and sweet potato regarding crude fibre. Since the Mexican sunflower leaf fails to be considered a suitable fish feed, due to its bad acceptance by fish, this leaves the cassava leaf in the highest position of best recommended feed components. Sweet potato does not even appear in this top ranking with regard to crude lipid and crude protein. The mixing ratio of 30/70 comes off best in this assessment. Mixtures with high leaf contents are in the fore of the listing. Since the assessment only shows the five best results of each nutrient analysis, it does not contain information about the weakest results.

Ether extracts	Crude fibre	Crude protein
30/70 - MSF	30/70 - MSF	30/70 - MSF
40/60 - C	30/70 - SP	30/70 - C
60/40 - C	40/60 - SP	70/30 - C
70/30 - C	50/50 - SP	60/40 - C
50/50 - C	70/30 - SP	50/50 - MSF

Figure 4.26: Assessment of the mixtures combined with feed components according to ether extracts, crude fibre and crude protein

Mixtures combined with Binders and Feed Components

In figure 4.27 all influencing factors were brought together. The binders as well as the feed components and the mixing ratios were considered. As a result, it turns out that mixtures with gelatine are leading the listing in terms of crude protein. Furthermore, feeds with the mixing ratio of 30/70 are in the highest position of best recommended feed components. When combining all factors, cassava with gelatine leads the list of crude protein levels. Concerning ether extract levels cassava with gelatine contains the highest levels, with one sole exception, namely the mixture of Mexican sunflower with agartine comes off highest.

Once again, Mexican sunflower and cassava are leading the list regarding all three nutrient contents.

Generally the application of gelatine shows higher levels of crude protein but agartine represents a good alternative. It should be noted the assessment stated below only specifies the five best results of each nutrient analysis. Therefore it does not contain information about the weakest results.

Ether extracts	Crude fibre	Crude protein
30/70 MSF- A	30/70 MSF- A	30/70 C - G
40/60 C - G	30/70 SP - A	70/30 C - G
60/40 C - G	30/70 MSF - G	60/40 C - G
70/30 C - G	30/70 SP - G	50/50 C - G
50/50 C - G	50/50 SP - A	40/60 C - G

Figure 4.27: Assessment of the mixtures combined with binders and feed components according to ether extracts, crude fibre and crude protein

Conclusion

The main objectives of this master thesis were to produce cost-effective fish feeds for small-scale Kenyan aquaculture, using locally available and inexpensive agricultural by-products and to analyse the nutrient composition of these feeds. In total 36 mixtures were produced and analysed on their content of ash, ether extracts, crude fibre and crude protein. For the production of feed, leaves of Mexican sunflower (*Tithonia rotundifolia*), sweet potato (*Ipomoea batatas*) and cassava (*Manihot esculenta*) were collected in the surroundings of the experimental site, desiccated and ground. Thereafter each of the leaves was mixed with brewery waste in different proportions, binders were added and pelletised. Two binders; gelatine and agartine were used for producing fish feed pellets. Agartine was chosen out of consideration for the religious beliefs of the many Muslims living in Africa. Given that gelatine is made of animal raw materials such as pigs, whose consumption is classified as impure to the Islam, an alternative was needed. Various amounts of binders were added to the mixtures to find out whether or not the binders have a bearing on the descent rate of the pelleted feeds.

The results of the nutrient analyses of the single feed components clearly show that all feeds reach the 20-30 % of crude protein required in fish feeds. Concerning the crude lipid content, all feed components (except sweet potato leaf) have shown values around the recommended 15 % level [EL-SAYED, 2006]. Mexican sunflower and cassava leaves performed best regarding high crude protein contents and also regarding crude lipid contents (after brewery waste) and have a strong potential to replace “Sagana diet”, the standard feed at the Sagana Aquaculture Centre.

The feed mixtures containing Mexican sunflower and cassava leaves deserve much attention. The results of Mexican sunflower leaf combined with agartine show that it contains the highest protein levels, but based on the high protein content of gelatine, the inherently good protein values of cassava are strengthened. It is important to note that to mixtures containing cassava leaves a higher amount of binders were added (36 g) than to the mixtures containing Mexican sunflower leaves (18 g). Therefore cassava leaf and gelatine feeds show the best results of all feed mixtures. The mixture with the highest crude protein content (32.4 %) is the one with 30 % brewery waste and 70 % cassava leaves.

In terms of the difference between gelatine and agartine feed mixtures, a considerably higher crude protein content was observable in feeds containing gelatine.

In the course of STÖGER's study [2010] the acceptance of the produced feeds by Nile Tilapia (*Oreochromis niloticus*) was observed. The tests show that feed mixtures containing Mexican sunflower leaf were least accepted. This fact is potentially related to the bitterness contained in the plant. Even though the leaves were sun-dried and finely chopped to reduce the bitterness, the procedure was apparently unsuccessful. It was observed that the fish ignored the feed and finally stopped feeding on this type of feed after several feedings. If the bitter or the bitter taste cannot be removed, the leaves of the Mexican sunflower are not suitable as feed due to the lack of acceptance by the fish. The mixtures with cassava leaves were better accepted, especially with increasing proportions of brewery waste. A similar feeding behaviour was observed for the mixtures with sweet potato leaves. All feed mixtures, except

the ones containing Mexican sunflower, are well accepted by the fish and qualify as feed in small-scale aquaculture [STÖGER, 2010].

The used binders, gelatine and agartine, had no bearing on the descent rate of the produced mixtures, which is essential in cage aquaculture. However, the various amounts of binders certainly had considerable influence on the descent rate. Small amounts of binder resulted in fast disintegration, whereas high amounts of binders resulted in rapid sinking of the pellets, due to their higher weight. Besides, the pellets with a high amount of binder had a hard consistency and therefore were difficult for the fish to chew.

Considering all parameters, the recommendation of this master thesis is to use fish feed mixtures containing brewery waste and cassava leaves. Due to the fact that the fish liked mixtures with a higher share in brewery waste better and the nutrient analysis cannot determine a preferable mixing ratio with respect to crude protein, mixtures with a higher percentage of brewery waste are recommended. Generally the application of gelatine results in a higher level of crude protein, which is highly desirable in fish feeds, but agartine represents a suitable alternative should there be religious reservations against gelatine.

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