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Effect of feeding supplementary diets containing soybeans and moringa leaves on growth and fillet quality of Nile tilapia (*Oreochromis* niloticus), and comparison of growth and fillet quality between Nile tilapia (*O. niloticus*) and Wami River tilapia (*O. urolepis hornorum*)

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TITLE PAGE

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DECLARATION

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Study programme and year of start

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ABSTRACT

The primary object of this study is to compare effects of various diets and tilapia species on growth performance and fillet quality. The secondary objective is to evaluate which diet could serve as a good alternative for usage in Tanzania´ backyard farming and perhaps in the planned breeding program of GIFT tilapia at Sokoine University of Agriculture (SUA).

The trial underlying this report was performed at SUA in Morogoro, Tanzania, comprising two experiments. Both experiments were implemented based on the following circumstances: Each of the 15 tanks contained 14 fishes in the beginning; however, mortality decreased the number of fish over time. The water was changed every two weeks and no addition of aeration, or other water improvement equipment has been used during the trial. The water temperature averaged 28.3 ± 0.3 °C for both experiments. The dissolved oxygen (DO) levels varied significantly between diets; the Tanzanian diet resulted in an overall higher DO level whereas the Norwegian diet resulted in the lowest DO levels observed. Note, however, once “cow manure” was added to the reference tanks, the overall DO levels lowered significantly. “Cow manure” was added twice a week.

The experiments were performed with triplicate parallels. The first experiment was based on three different diets. One diet consisted only of maize bran and served as the reference diet, whereas the Tanzanian diet contained 34.5% moringa meal as alternative protein source. The third diet, the Norwegian diet, contained 19.6% soybean meal as alternative protein source. The tilapias used in the first experiment were wild Nile tilapia (*Oreochromis niloticus*) caught from Lake Victoria. The second experiment that started 1 month after the first experiment and only fed on Tanzanian diet, was set to compare the growth performance and fillet quality of the wild Wami River tilapia (*Oreochromis urolepis hornorum*) against the Nile tilapia.

The mean thermal growth coefficient (TGC) was found to be 0.5% for the Norwegian diet and 0.4% for the reference and Tanzanian diets. The TGC for the Wami and Nile tilapia from the second experiment were 0.2%. The weight gains during 14 weeks of feeding for the diet experiment were, respectively: 27.1, 15.9 and 20.4g for the Norwegian, reference and

Tanzanian diets. The weight gain from the second experiment during 14 weeks of feeding was 6.5g for the Wami tilapia and 11.1g for the Nile tilapia. The tilapia fed on the different diets showed little differences in fillet quality. The condition factor (CF) for the different diets did not show a significant variation; in the Norwegian fed tilapia it was 1.7% while the other two diets had a CF of 1.6%. The fillet yield of the gutted weight was 41% for the fish fed on the Norwegian diet while 38% for the other two diets. The fillet yield gutted weight for the Nile tilapia and Wami tilapia in the second experiment were somewhat higher with 43% and 40%, respectively. The fat content in the fillets of the first experiment did varied according to the different diets: the Norwegian diet with 2.2% and the reference diet with 2.3% seemed to give fatter fish than the Tanzanian diet with 1.7%. For the second experiment with the Nile tilapia and Wami tilapia, the results were 1.7% and 2.2%, respectively. It seemed also that the tilapia were able to synthesise the available fatty acids in the dietary feed to the required long chained fatty acids 20:4 n-6, 20:5 n-3 (EPA) and 22:6 n-3 (DHA).

The colouration of the tilapia was very similar between diets and between species; the Nile tilapia fed with the reference diet and the Wami tilapia fed with the Tanzanian diet did show a higher value for yellow in the muscle. The fatty acids profile indicated that the tilapias had an ability of synthesising the linoleic acid to the long-chained arachidonic acid (20:4 c-6) and α-linolenic acid (18:3 n-3) to eicosapentaenoic acid (EPA 20:5 n-3) and docosahexaenoic acid (DHA 22:6 n-6). These fatty acids are relevant for tilapia in forms of healthiness.

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1. Introduction

The Nile tilapia (*Oreochromis niloticus*) is the tilapia species most commonly used for tilapia farming today and has been since the time of the Egyptians (Feidi 2010). Tilapia is the main farmed freshwater species worldwide, together with the carp. Tilapia farming is widely evolving around the tropical and sub-tropical countries, due to its rapid growth rate and beneficial nutritious values (Nations 2014; Xie et al. 1997). The tilapia is especially a popular fish in Tanzania and farmed in small back yard ponds. Therefore the Nile tilapia was chosen as the main species for the experiments underlying this report. This study was conducted to investigate what alternative protein source would be beneficial for the Tanzanian marked in terms of growth performance and fish fillet quality. The people living far from the bigger lakes and rivers rarely eat fresh fish. This could be changed if a community had the needed knowledge to farm their own fish. Tilapia is chosen because they are sturdy fish that tolerate very poor living conditions. If they are given the right conditions and a diet that satisfies the tilapia´s nutritional requirements, they will have a better growth rate and are sooner at the acquired size. The Wami tilapia (*Oreochromis urolepis hornorum*) is still an unexplored species with little research so far conducted on it. It would therefore also be interesting to know how Wami tilapia performs compared to the Nile tilapia.

This study examined the effects of different food sources on tilapia. The study had two specific objectives. The first objective was to study the growth performance and fillet quality (colouration, fat content and fatty acid composition) of the Nile tilapia fed on different diets. This experiment is called the “Diet experiment”. The second objective was to compare the growth performance and fillet quality of the Nile tilapia and Wami tilapia. This was done in the so-called “Species experiment”. While the gross national income (GNI) per capita of Tanzania was $540 in 2011 (IndexMundi 2013), the majority of the population has to live from far less. The study is therefore based on diets where the ingredients can be obtained even in smaller cities without the costs exceeding 2,500 TZS ($1.7) per kg diet. The experimental diets were based on two main ingredients, one diet is a soybean diet and the other one has moringa leaves as its main component. These diets are called the Norwegian diet and Tanzanian diet, respectively. The two diets are compared to a diet normally used in Tanzania, consisting only of maize bran. This diet is called the reference diet. The tilapias

used in the study were Nile tilapia brought from Lake Victoria in January 2013. The Wami tilapia was caught in the beginning of March 2013 in a river 50 kilometres away from Morogoro, the Wami River.

The approach of the experiments was to measure the specific growth rate and condition factor of the tilapia in the diet and species experiment during 7 periods with 2 weeks duration per period. Furthermore the nutritional differences in the two experimental diets were compared with the reference feed. This was done to determine the nutritional values of reference diet, Tanzanian diet and Norwegian diet and the main ingredient of the Tanzanian diet, the moringa leaves. The amount of feed given was based on an anticipated feed conversion rate (FCR) of 1.8 for the reference diet and 1.6 for both the experimental diets. After both of the experiments, the sampled fish were brought back to Norway, and tested for fat content, fillet colouration and "uptake" of fatty acids from the different diets. The water quality was studied by measuring the dissolved oxygen and water temperature after every morning and afternoon feeding. Before emptying the tanks after each weighing period and after refilling water for a new period, five different parameters in the water were measured; nitrite, total hardness, total alkalinity, pH and nitrate. This was done to observe how the unchanged water behaves in a two weeks period. In the 14th week the ammonium levels from the second experiment were measured in order to characterize the tank environment.

The trial was performed at the Magadu fish farm at Sokoine University of Agriculture in Morogoro, Tanzania and the chemical composition and fillet quality were tested in Sunndalsøra and Ås in Norway. For this study a total of 188 Nile tilapias and 42 Wami tilapias were used.

2. Theoretical background

Aquaculture has, yet, a much unexplored potential in Tanzania and comprises mainly of freshwater fish farming. There are, at present, only small-scale farmers practicing either extensive or semi-intensive fish farming. So far, approximately 14,100 freshwater farms are spread over Tanzania with an average size of 150m^2 (FAO 2004). Often these farms are combined with other agricultural activities, such as land based animal production and gardening. For the fish farming activities, economic use of fishponds in Tanzania is limited by certain factors such as available water, suitable land areas, and community awareness of potential economic gain of fish farming (Nations 2014). Shrimp farming in Tanzania is still in experimental trials but internationally already very profitable. In addition to shrimp farming, seaweed farming has also become popular in coastal regions in the last two decades. Several indigenous and introduced species are being farmed in Tanzania but the most dominating cultured species are the tilapias, followed by the African catfish. Tilapia and catfish are mostly cultured in earthen ponds and tanks. The contribution of aquaculture was still so negligible in 2004 and therefore did not have any significant impact on the national economy. However, it contributes to a better life-style with improvement of animal protein for the farmers family and as a source of income and employment (FAO 2004).

The Nile tilapia (see figure 1) has a blue-grey colour with darker stripes from its back to belly, called fingerprints. It has a fast growth and is in addition quite resistant to pathogens and parasites. The reproduction capabilities are high (Meyer & Meyer 2007). The Wami tilapia is still a rather unexploited tilapia species yet, though its characteristics are similar to the Nile tilapia, only a bit darker in colour. The Wami tilapia is also a species that can grow and thrive in brackish waters, which a Nile tilapia is not capable of (Community 2008). The reason to compare the Wami tilapias with the Nile tilapias was to find out which of these two species would perform better on an overall basis for growth rate and fillet quality.

Fig. 1. Nile tilapia, with colouration of sexual maturity on male tilapia

The tilapia belongs to the tilapiine cichlid group, which consists of almost a hundred different species. The Nile tilapia has its origins in Africa and is mostly farmed in tropical and sub-tropical countries. The Nile tilapia is one of the most important of the tilapia species due to its rapid growth, good survival rate in high-density populations and its disease tolerance. The tilapia species is both an omnivorous and herbivorous species, which means that it can digest both plant and animal materials. Tilapia can live in most freshwater sources, such as shallow streams, lakes, rivers and are even found living in brackish waters. The tilapia are very tolerant to poor water conditions and can endure periods with low oxygen (Francois et al. 1996).

In the Tanzanian diet, the most important ingredient was moringa (*Moringa oleifera*) leaf meal. The tree is around 5 to 10 metres high. It grows in most terrains, and thrives best in the subtropical and tropical climate. The moringa tree has its origins in the western and sub-Himalayan tracts (Anwar et al. 2006). Firstly it became popular in the Eastern world, and since it is "the miracle tree" due to it rich and healthy qualities. Today, it becomes more visible in the Western world as well where it is used for body products, medicine and nutrition. Moringa provides high protein concentrations, vitamins, β-carotene, amino acids and different phenolics (Anwar et al. 2006). Most of the tree is useable: the fruit, flowers, immature pods and the leaves are used for different purposes. The leaves (see figure 2), for instance, are used in fish feed pellets for nutritional purposes (Richter et al. 2003). Richter et al. (2003) performed a study based on freeze-dried moringa leaf meal. The objective of the study was to evaluate whether this was an alternative protein source or not. The conclusion was that the moringa leaf meal could be used as a 10% substitute of dietary protein. Anything used above this level would lead to growth reduction of the fish.

Fig. 2. Leaves of the Moringe (Moringa oleifera) tree used in the Tanzanian diet (Shak 2013).

Feed storage and preparation are important factors when it comes to feed conservation. There are many factors influencing the quality of the feed, such as storage time and conditions, the quality of raw materials, and how the production of the pellets is done. The feeds nutritive value depends on the nutrient composition of the raw materials. An animal's ability to digest and absorb these nutrients depends on the species and age of the individual (Moreau 1996; Ng & Romano 2012). Research has proven that floating pellets result in higher growth performance than sinking pellets (Ng & Romano 2012). Soybean meal can either be extruded or non-extruded. Ammar et al. (2008) found that feeding tilapia fingerlings with extruded soybean meal increases the growth performance compared to non-extruded soybean meal.

Fig. 3. Manufacturing of the pellets manually performed with a meat grinder

The colouration of the tilapia fillets is measured on a tristimulus meter chart (Kestin & Warriss 2001). The colours in the muscle of the fillets can be observed as white-black, red-green or yellow-blue. When combining these so-called "primaries" colours and the tritimulus sphere one can plot any colour at any point. In the case of measuring muscle colour with a tristimulus meter (Minolta), the L* value is measured as lightness, a* determines the red-greenness and b* determines the yellow-blueness. From this, the Hue

and Chroma can be calculated. The Hue is what is normally described as the colour tone while the Chroma defines more the saturation of the colour (Waagbø et al. 2001).
To determine the growth performance of fish one can calculate the thermal growth coefficient (TGC). The TGC measures the growth rate from the weight gain of the fish. This coefficient takes the temperature in consideration by means of day degrees (DGR), since the temperature is one of the most important abiotic factors affecting the growth rate, feed conversion and feed intake of fish (Azaza et al. 2007).

The proximate analysis is a method used to determine the macronutrients in feeds. This method partitions compounds of the feed in moisture content, ash, crude protein, crude fibre, crude lipids and nitrogen-free extracts (NFE) (AOAC 1984).

Herbivorous animals normally have 10% fat in their diet, but they can degrade and absorb much more than this (Sjaastad et al. 2003). Most fat in the diets are in form of triglycerides, which mostly are long-chained acids made of 16 or 18 carbon atoms. Animal tissue contains cholesterol esters and phospholipids while plant feed contains galacto-glycerides and phospholipids. The end product in warm blooded animals of non-microbial digestion of two fatty acids splitting of the triglyceride molecule are free fatty acids (FFA) and monoglycerides. These monoglycerides and FFA are after digestion absorbed in the intestinal tract. Lipase are lipolytic enzymes coming from the pancreas, the lipase is water-soluble while the triglycerides are lipid-soluble, therefore, the degradation is only possible in an interface between water and fat. Since the fat droplets coming into the stomach are large, they need to emulsify through bile salts, into smaller droplets (micelles) in order to increase the interface between water and fat and thus quickening the degradation. Inside the epithelial cells the monoglycerides are re-esterified with the fatty acids to form new triglycerides. Saturated fatty acids contains only single bonds and cannot bind more hydrogen atoms to the skeleton. Unsaturated fatty acids contain one or more double bonds. Polyunsaturated fatty acids are dominating in some plants and fishes; triglycerides contain almost double as much chemical energy as carbohydrates or proteins (Justi et al. 2003; Sjaastad et al. 2003; Waagbø et al. 2001).

The lipid content in diets must meet the required energy level and essential fatty acids (EFA) in order for a good growth rate. Salmonids are shown to be able to produce EPA and DHA if 18:3 n-3 is present in diet. But this production is limited. To give high levels EPA and DHA in salmon, it is required to have these in the diet. HUFA is also a required compound in the diet for freshwater fish. Since tilapias are herbivorous tropical freshwater species, satisfying the HUFA requirement in the diet can be easily done by adding rapeseed oil (Lie 2008). When correct requirement of HUFA is achieved in the diet of the specific fish, the growth level will probably increase.

3. Materials and methods

For both experiments the following parameters were tested: Growth rate, water parameters, apparent feed conversion rate, fillet quality, fillet colour quality and fatty acids composition.

3.1 Fish origin

The Nile tilapia was caught from Lake Victoria in January 2013. When the fish arrived at Sokoine University of Agriculture (SUA) it was placed in an introductory tank and fed on maize meal twice a day as appetite feeding. The Nile tilapia stayed in this tank for 23 days and was, thereafter, transferred to the experimental tanks. For three days they were undisturbed to adjust to the tank and commit to a social order within each tank. The Wami River tilapia was caught in the Wami River and brought to the SUA site in March 2013. The fish was placed in the same type of introductory tank as the Nile tilapia and was fed the same way. The fish stayed in these tanks for 12 days before being placed in the second row of experimental tanks.

3.2 Experimental fish

Fish size data was obtained by bi-weekly sampling and at harvest as described in section 3.4.2 “final weighing procedures”. Table 1, illustrates the average weight of the fish from the diet experiment and the average weight of the fish from the species experiment.

Table 1. Average initial weight of the tilapia in each diet with standard error; the first three rows show the mean weights from the diet experiment. The “Nile tilapia” and “Wami tilapia” are measurements of the mean weights from the species experiment.

Group	Weight (g)	S.E.M.
Reference diet	5.9	0.31
Tanzanian diet	5.6	0.19
Norwegian diet	5.6	0.28
Nile tilapia	5.7	0.45
Wami tilapia	6.1	0.20

A total of 146 Nile tilapias with an average weight of 5.7g were randomly selected for the feeding experiment. At the start of the experiment the Nile tilapias ranged in weight from 3.3g to 7.9g. The fish was divided into 3 groups in a completely randomized manner. Fourteen fish were set out per tank with a total of nine tanks. Each group of fish was fed

Fig. 4. Experimental tanks with inlet and outlet

either with the reference diet, the Tanzanian diet or the Norwegian diet. The three tanks fed with the reference diet got 200 grams manure every 5th day added to the water in order to produce an algae culture in these tanks.

The tanks were newly built ahead of each experiment as armed concrete tanks (see figure 4). Each tank was 1.5 meters wide, 3 meters long and 1 meter deep.

The daily natural photoperiod was 12 hours light and 12 hours dark with ± 20 minutes difference in the duration of the experiment.

In the species experiment a total of 42 Nile tilapias and 42 Wami tilapias were used. These were distributed in a completely randomized manner in three tanks per species with 14 fish in each tank. The initial weight of the Nile tilapia ranged between 4.0g and 8.8g with an average of 6.1g. For the Wami tilapia the initial weight ranged between 4.2g and 7.6g with an average of 5.7g.

3.3 Experimental feed

3.3.1 Feed production

There were two diets chosen for the first experiment, a Tanzanian and a Norwegian composition. These diets were being compared to a diet regularly fed to tilapia in Tanzania, which is called the reference diet (see Table 2). To produce the diets, a balance Kern DS model (Kern & Sohn, Germany) was used to weigh the main ingredients of the diets. A balance Precisa 180 model (Precisa Gravimetrics, Swizerland) was used to weigh the finer amounts of the diet ingredients. The dry ingredients were mixed thoroughly in a bowl, after which sunflower oil and water were added until it obtained the right consistency for making wet pellets. The mixture was then manually pelleted through a meat-grinder (see figure 3) and spread out on canvas bags to shade-dry for two days before being brought to the research site. The gathered moringa leaves were shade-dried for 2 days on concrete floor, thereafter the leaves were grinded until a fine powder was achieved.

3.3.2 Chemical composition of the diets

Table 2, presents the ingredients for the two experimental diets and the reference diet by name and percentage for 1kg diet.

Table 2. Ingredients for each used diet in the diet experiment. The Tanzanian diet was also used in the species experiment.

Composition %	Reference diet	Tanzanian diet	Norwegian diet
Maize bran	100	-	-
Moringa leaves meal	-	34,5	-
Sunflower meal	-	34,5	20.4
Maize flower	-	12,0	8.0
Fish meal	-	13,0	7.0
Sunflower oil	-	3,0	7.7
Wheat flower	-	2,0	30.0
Min & vit. mix*	-	1,0	-
Soybean meal	-	-	19.6
Pea meal	-	-	5.0
Di-calcium phosphate	-	-	1.5
L-Lysine	-	-	0.2
D-Methionine	-	-	0.6
Vitamin C**	-	-	0.03
Total %	100	100	100

* The mineral and vitamin mixture contained: Vitamin A, D3, E, K, B2, B6, B12, C, Biotin, Calcium Phantothenate, Nicotinamide, Iron Sulphate, Manganese Sulphate, Copper Sulphate, Potassium Chloride, Zinc Sulphate, Magnesium Sulphate, Sodium Sulphate, Sodium Chloride, Lysine and Methionine.

** Vitamin C used in the Norwegian diet was Vitamin C produced for human dietary.

3.3.3 Apparent feed conversion rate

The feed conversion rate (FCR) explains how the fish utilizes the diets. The biological feed conversion rate (bFCR) includes the dead fish during this period in the quantity of fish produced, while the economical feed conversion rate (eFCR) does not include the dead fish. For calculating eFCR and bFCR, the following formulas were used:

$$eFCR = \frac{total\ feed\ used\ (g)}{final\ body\ weight - initial\ body\ weight\ (g)}$$

$$bFCR = \frac{total\ feed\ used\ (g)}{final\ body\ weight + (n\ dead\ fish \times average\ weight\ whole\ period) - initial\ body\ weight}$$

3.3.4 Feeding

The feeding procedures were performed on feed conversion rate (FCR) feeding with an estimated FCR of 1.8 for the reference diet and 1.6 for both experimental treatments. The available Boeco BEB 43 model balance, (Boeckel & Co, Germany) was used to weigh the diets. Each daily amount is measured from the weight value in the schedule (see attachment 1) and an iron pipe was used to crumble the pellets according to fish size as shown in Table 3. The fish were fed three times a day (i.e. 9 am, 1 pm and 5 pm) and every feeding took 30 minutes, so the fish would be able to utilize as much of the diets as they could. Before each feeding, the tanks were cleaned out to remove the floating leaves and other organic compounds.

Table 3. Pellet sizes according to fish size

Performance	Tilapia size (g)	Apr. pellet size (mm)
Crush with pipe	0-5	0-1
Crumble with pipe	5-10	1,5
Break into pieces	10-20	2
Break into pieces	20-40	3

3.4 Procedures

3.4.1 Weighing procedures of the fish

The water level in the tanks was reduced to a level of 5 cm. In doing so, it was critical to ensure that no escapees would occur. In the next step, the fish was gathered in a 20l bucket. When all the fish in a specific tank had been collected, the balance Boeco BEB 43 model was used to bulk-weight the fish. Bulk weighing instead of individual weighing was done in order to achieve the lowest possible stress level for the fish. The total biomass was recorded and the Specific Growth Rate (SGR) was calculated with the following formula

$$SGR = (\frac{Final\ weight}{Initial\ weight})\text{^}(days\ of\ feeding\ trial)^{-1}$$

During the weighing procedure the tank was cleaned and faeces and other organic compounds were removed. When all the fish had been weighed and returned to their tanks, the water level was restored to the original 60cm and the fish were fed the following day, so their appetite would be back upon feeding.

3.4.2 Final weighing procedures

The first steps are the same as during the regular weighing procedure explained in chapter 3.4.1 “weighing procedures of the fish”. After being collected from the tank, the fish was placed in a bucket containing metomidate hydrochloride powder – a strong anaesthetic – until it was fully anesthetized. The fish was individually weighed on the balance Boeco BEB 43 model and the length of each fish was measured in millimetres. After selecting the fish with average tank weight for further analysis, the fish was placed in a separate bucket, overdosed with metomidate hydrochloride powder and in this way killed. This fish was placed in Ziploc bags marked with experiment number 1 or 2, tank number from 1 to 9 or 1 to 6, depending on the experiment they had been used in, final weight in gram, length in cm, sex (male or female) and date. The Ziploc bags containing the selected fish were frozen at –20°C and stored for transportation. The fish that was not chosen for further measurements were kept alive for the sensory quality tests performed as a follow-up study on these experiments by master student Scontina Mgina (2014).

3.5 Study parameters

3.5.1 Water quality measurements

After the morning and afternoon feeding both the dissolved oxygen (DO) and water temperature were measured, for determining the water quality, with an YSI model 55, (Yellow Spring Instrument Co, USA). The probe was held 30cm under water, which is equal to the middle of the tanks’ water level. When the display showed a steady temperature and DO level, the values were recorded in the schedule (see attachment 1).

3.5.2 Measuring water quality parameters

Fig. 5. Mardel 5-1 test strips

For measuring the water quality in forms of chemical compounds found in the water both JBL – Joachim Böhme Ludwigshafen – and Mardel water quality 5 in 1 test strips (see attachment 2) were used. One test strip was used per measurement and tank, the tank number was written down at the bottom of the test strip, as shown in figure 5. The test strip was dipped in the water so that the 5 patches

on the strip were wetted. The test strip needed 30 seconds before reading. The colouration of the strip was compared to the chart on the box. When reading the strip, one start with reading nitrite levels and the nitrate level was measured last, as it needed 60 seconds before reading. The two different producers of the test strips used for water quality tests, each used different units. Mardel observing the measurement in parts per million (ppm) and JBL observes degrees of general hardness (dGH). For clarity these units were recalculated to mg/l, shown in Table 4 in the results section.

3.5.3 Ammonium level measurement

For measuring the ammonium level in the water the JBL (Joachim Böhme Ludwigshafen, Germany) was used. An ammonium test kit was used including a colour card and a table of intensity. Both test jars were rinsed several times with the water to be tested. A five-millilitre sample of water was added to both test jars. One was put aside as a reference jar while the three different reagents were added to the other jar. First, four drops of reagent A were added and the jar was mixed thoroughly. Secondly, four drops of reagent B were added and mixed and, thirdly, five drops of reagent C were added. The sampling water was then settled for 15 minutes. After 15 minutes both jars were placed in a comparator block and read of a colour card comparing the reference test jar with the mixture jar.

3.5.4 Fillet quality

After being transported from Tanzania to Norway the frozen fish was half thawed, one experiment at a time, in a refrigerated room at 4 °C. Six fish per round taken from the refrigerated room, so the fish did not thaw completely while measurements were performed, were weighed with a DeltaRange Mettler PM460 balance (Mettler Toledo International Inc., USA), and the length measured in millimetres. The fish was then gutted and reweighed before filleting. After weighing the fillets, the right side fillet was placed in a Ziploc bag and the left side fillet was kept on the plate for further measurement. The condition factor (CF), gutted yield (GY), fillet yield of body weight (FY BW) and fillet yield of gutted weight (FY GW) were calculated through the following formulas

$$CF = 100 * \left(\frac{whole\ body\ wet\ weight\ (g)}{length^3 (cm)} \right) \qquad GY = \frac{gutted\ weight\ (g)}{whole\ body\ wet\ weight\ (g)}$$

$$FY\ BW = \frac{fillet\ weight\ (g)}{body\ weight\ (g)} \qquad FY\ GW = \frac{fillet\ weight\ (g)}{gutted\ weight\ (g)}$$

3.5.5 Fillet colouration

A colour sphere as shown in figure 6 determines the colouration of the muscle.

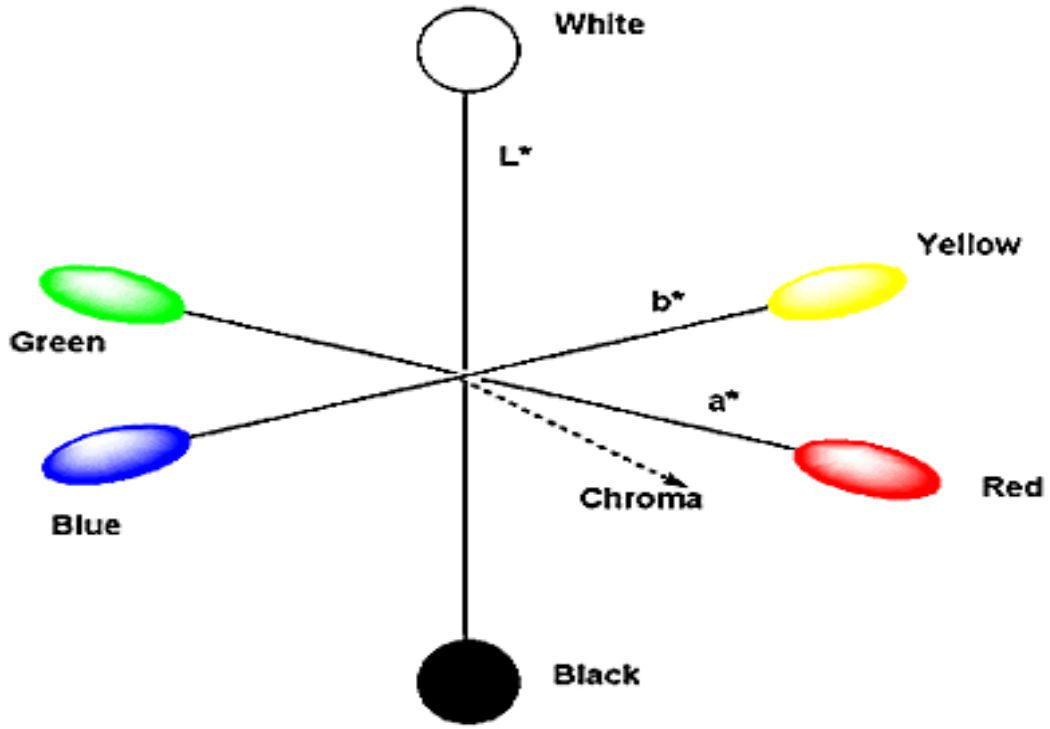

Fig. 6. The tristimulus sphere indicating L, a* and b* colour intensities.*

To measure the muscle colour of the tilapia, the left fillet was measured with a Minolta Chroma meter CR300 model (Konica Minolta Sensing Europe BV, Netherlands) on the centre of the back as shown in figure 7. The same spot was measured three times in order to get an approved average. L* describes the lightless (L* = 0 is black and L* = 100 is white), a* is the intensity in red (a* > 0) or intensity in green (a* < 0) and b* is the intensity in yellow (b* > 0) or intensity in blue (b* < 0). Values are presented as means ± SEM (n=3).

Fig. 7. Measuring spot for the Minolta to determine the colour of the fillet. From each tank per diet three sampling fish were used (n = 3)

Hue is calculated as $\frac{b*}{a*}$ and describes the colour tone.

Chroma is calculated as $\sqrt{(a^{*2} + b^{*2})}$ and describes the colour saturation.

3.5.6 Proximate analysis

The analysis methods mentioned in this chapter were all applied according to the methods described by the AOAC (1984). All three diets were analysed at Nofima Sunndalsøra in Norway for the dry matter content (DM), ash, crude protein (CP), crude fibre (CF), crude lipids (CL) and energy (MJ/kg).

Dry matter was determined by drying the samples at 105°C using a Termaks heater until a constant weight was obtained. The determination of ashes was done by combustion of the samples. The samples were burned to ash in a 550°C hot oven until stable weight was obtained. The samples were then cooled in a desiccator, and weighed immediately afterwards. This was important in order to avoid moisture pollution of the samples.

To determine crude protein content in the samples, the Kjeldahl method (AOAC 1984) was used with a Kjeltec Auto 2300 Analyser unit and a Tecator™ Digestion Auto.

The crude fibre was determined by breaking down the starch with heat stable amylase, and in a next step, the starch polysaccharides were degraded by the enzyme amyloglucosidase. With a glucoseoxydase-peroxidase method (GODPOD) the glucose was determined on a Thermo spectrophotometer.

The crude lipids were obtained by the three-way-analysis. Firstly, the feed was extracted with petroleum ether, secondly the bonds were broken in a hydrolysis with 4M HCl in a SoxCap system 2047 hydrolysing unit and, finally, the samples were extracted one more time with petroleum ether. The mixtures for all the samples were then evaporated and weighed on a 2055 Sotex Avanti analyse-balance.

For the calculation of energy per kg of diet, a standard amount of feed was incinerated in oxygen, the increase of temperature was registered and the amount of energy produced was calculated using known thermic charts to compare with the samples chart.

3.5.7 Muscle homogenisation and lipid extraction

The lipid extraction is performed according to the commonly used Folch procedure (Folch et al. 1957). Two grams of each fillet was homogenized with dry ice for 60 seconds. The samples were then placed in the freezer for one day with open bags so the dry ice could evaporate. The next day the samples were homogenized in a solution of 0.9 % sodium chloride and chloroform:methanol (2:1) combined with the antioxidant butyl hydroxyl toluene. The samples were filtered through into the flasks making use of a funnel with cotton filter. After two hours, the solution separated into two phases. The lower phase as a chloroform:methanol:water mixture with a ratio of (86:14:1) containing almost all the lipids, methanol and water making the total volume of this solution to 75ml. The upper phase as a choloform:methanol:water mixture with a ratio of (3:48:47). This phase contained most of the water-soluble components. The upper phase was removed with a water-vacuum pump. Later on, 20ml of the lower phase was transferred by pipette to a 25ml beaker. Hereby, it was important to have the exact weight of the empty beaker. The beakers were then placed on a heating plate to evaporate the chloroform from the samples, leaving just the moisture lipids behind. Furthermore, the samples were transferred to a preheated incubator (105°C) for approximately 20 minutes. When the samples were completely dry they had to be measured on the same balance (Mettler Toledo model XS603S DeltaRange) used during the whole procedure. The results are presented as a mean of two parallel samples as % fat of the weighed sample.

3.5.8 Fatty acids composition

The chloroform phase of the samples that was not used to measure the fat content was evaporated at 60°C with a nitrogen overflow. This was done so that no oxygen could interfere with the samples. The next step was to methylate the samples in order to trans-esterify the fatty acids by heating in excess of methanol and an acid functioning as a catalyst. To perform this, 2ml benzene, 2ml metanolic-HCL, and 0.2ml dimetoxypropan were added. The tubes were mixed and incubated in room temperature for one day. Afterwards 2ml hexane and 3ml 6% $NaHCO_3$ were added for neutralization of the samples. Separating the sample into two phases, the upper phase was removed and the samples were evaporated at 60°C with nitrogen overflow. The samples were then dissolved in hexane, before being transferred to a gas chromatograph (Hewlett Packard 6890). They were run

through a 60 meters long and 0.25 millimetres thick column (SGE) (see figure 8). The different molecules in the samples all have an own retention time. Meaning, the progress of the samples from the start of the column to the end measured in time. A detector was used to monitor the outlet stream and determine the amount of constituents and how long it takes for the constituents of a sample to reach the end of the column.

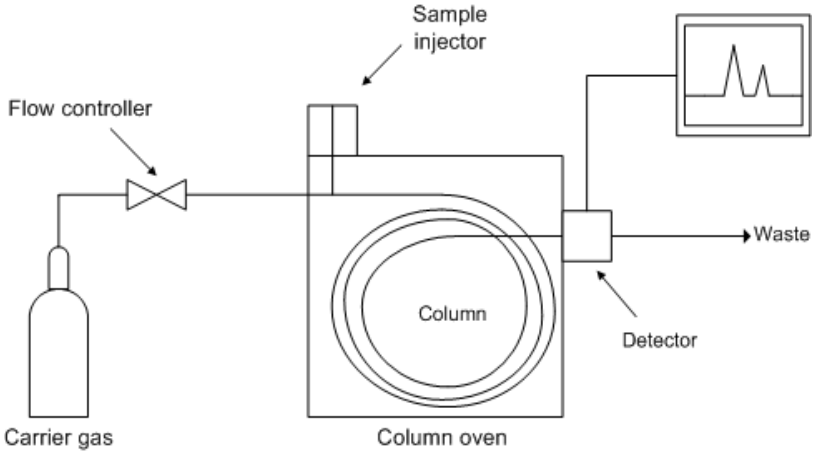

Fig. 8. Gas chromatograph illustrating how the fatty acids are analysed (Welsh 2005)

3.6 Statistical analysis

The results of the diet experiment were subjected to One-Way Analysis of Variance (ANOVA) at a significant difference level of 5%. Thereafter, the tests were subjected to Duncan multiple range test in order to indicate significant differences between the diets (using SAS 9.2 statistics software (produced by SAS Institute, North Carolina, USA)). For the species experiment we performed a T-test to determine significance between the samples (using Microsoft Office Excel 2010 (Microsoft Corporation, Albuquerque, New Mexico)).

4. Results

4.1 Background results

After the morning and afternoon feeding both the dissolved oxygen (DO) and water temperature were measured with an YSI model 55, (Yellow Spring Instrument Co, USA). The results for the feeding and species experiment are shown in figure 9 and 10, respectively.

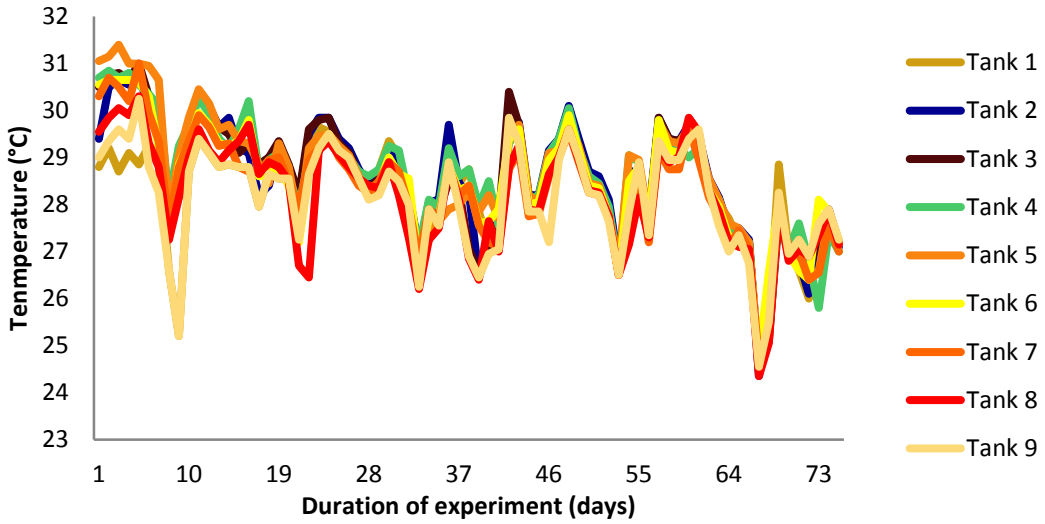

Fig. 9. Temperatures from January to May in the feeding experiment. The daily average temperatures of the tanks. A total mean temperature of 28.4°C ± 0.3 for the whole experiment.

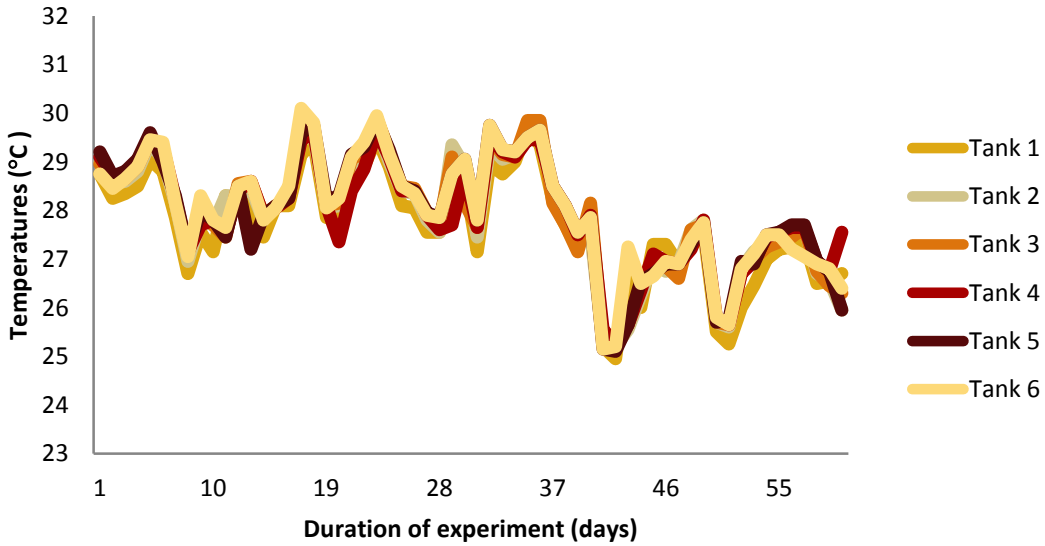

Fig. 10. Daily average temperatures measured from February to June for the species experiment for all the tanks. A total mean temperature of 27.9°C ± 0.2 for the whole experiment.

In figure 11 the observed average dissolved oxygen levels with the including standard errors of the mean (SEM) are given. The minimum DO level was observed in tank 8 with 1.5mg/l. The maximum DO level was observed in tank 6 with 16.9mg/l. The tanks fed with the Tanzanian diet seem to have had an overall higher DO level than the tanks fed with the Norwegian diet.

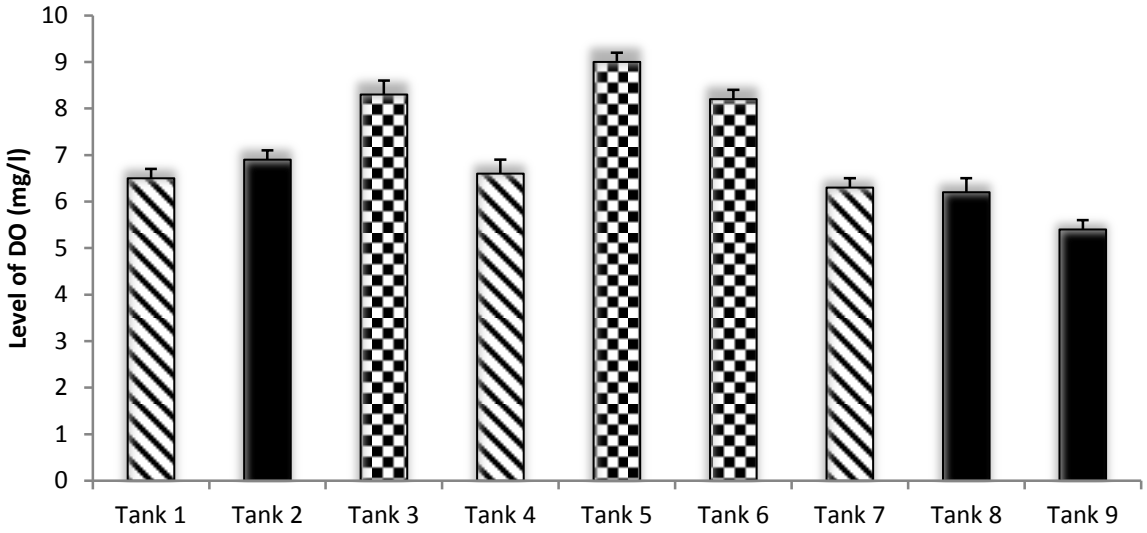

Fig. 11. Observed average with SEM for dissolved oxygen in different tanks over the whole period of January to May for the feeding experiment. Different diets show different DO levels. The striped marked columns were fed with the reference diet, the solid black columns were fed with the Norwegian diet and the blocked marked columns were the Tanzanian diet fed tanks.

The average observed DO levels including standard errors of the mean in the species experiment are presented within figure 12. All tanks were fed with the Tanzanian diet in this experiment. The minimum DO level was found in tank 6 with 4.0mg/l and the highest DO level was observed in tank 1 with 13.8mg/l.

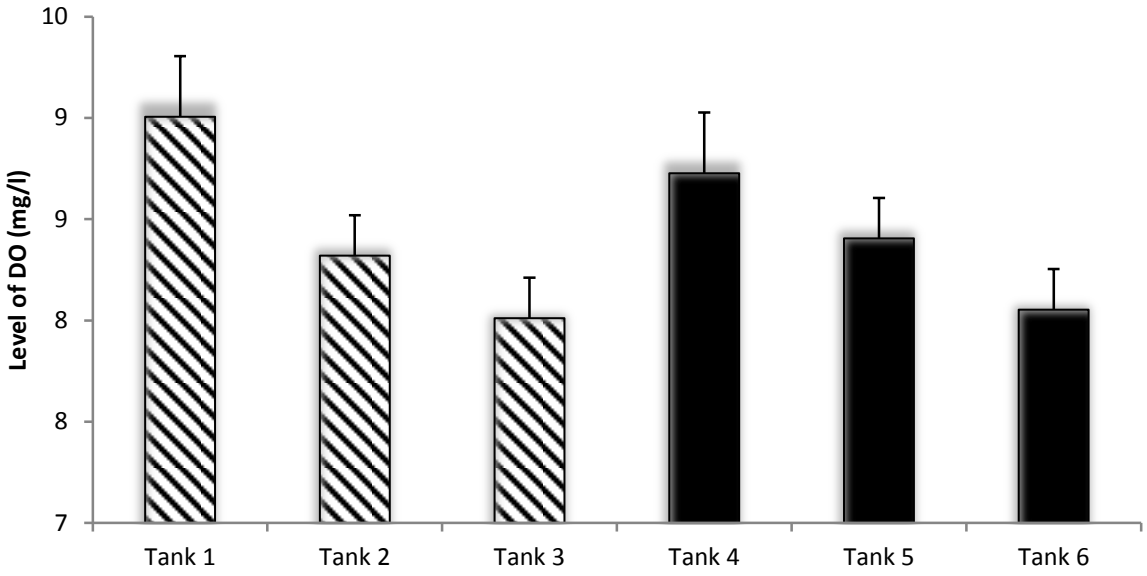

Fig. 12. Observed average with SEM DO for different tanks in the species experiment. Values observed between February and June. The striped marked columns were the tanks containing Wami River tilapia and the solid black columns contained the Nile tilapia.

Table 4 shows that the water quality parameters tested every second week were within the proper limits, and some parameters were not measurable due to the small amounts. The pH ranged within the neutral zone, which is preferred by tilapia (Meyer & Meyer 2007).

Table 4. Water quality measurement table: Highest values observed in tank 2 for the total hardness of the water. No critical values were observed and the water showed stable conditions throughout the period.

Water quality	1	2	3	4	5	6	7	8	9
Nitrite (mg/l)	0	0.5	0	0	0	0	0	0	0
Total Hardness (mg/l)	119.9	249.7	25	25	25	25	25	25	25
Total Alkalinity / buff. capacity (mg/l)	119.9	179.8	0	0	0	0	0	0	0
pH	6.8	7.6	7.2	7.2	7.2	7.2	7.2	7.2	7.2
Nitrate (mg/l)	0	20	0	0	0	0	0	0	0

Table 5 presents the ammonium levels measured within the tanks. Measurements indicate a good environment in the tanks (Meyer & Meyer 2007). The NH_4^+ values never exceeded 0.05mg/l and pH values were never higher than 7.6 (JBL 2014).

Table 5. Ammonium intensity chart based on pH per tank. The table is enhanced to the understanding of what NH_4^+ levels are dangerous for the different stages of the fish. The crosses indicating the observed values in the current study.

pH	NH_4^+ mg/l	0,1	0,2	0,4	0,8	1,2	2,0	3,0	5,0
	7,0	X							
	7,5	X							
	8,0								
	8,2								
	8,4								
	8,6								
	8,8								
	9,0								

	May be harmful for fry
	May be harmful for adult fish
	Lethal for fry and very harmful for adult fish
	Lethal for all fish

Table 6 presents the proximate compositions of the diets. The moringa leaves meal indicates high crude protein values, contributing to the observed high crude protein level in Tanzanian diet. In addition, the moringa leaves meal is low in crude fibre and crude lipid.

Table 6. Proximate composition of the three diets and the main ingredient in the Tanzanian feed, moringa leaves.

Proximate composition (%)	Reference diet	Tanzanian diet	Norwegian diet	Moringa leaves meal
Water content	10.3	10.5	11.2	9.0
Ash	3.4	10.0	5.9	10.3
Crude protein (*N x 6.25*)	10.4	24.1	18.3	33.0
Crude fibre	27.8	9.2	27.2	0.4
Crude lipid	11.1	9.5	10.8	4.5
Rest*	37.0	36.7	26.6	42.8
Energy (MJ/kg)	18.2	17.8	18.0	17.1

* Indicating Nitrogen Free Extract (NFE) and starch, etc. This is calculated as 100% - (water content + ash + crude protein + crude fibre + crude lipid).

4.2 Diet experiment

4.2.1 Performance on diets

The calculated initial weight, final weight, weight gain, average specific growth rate (SGR), thermal growth coefficient (TGC), economic feed conversion rate (eFCR), biological feed conversion rate (bFCR) and mortality for the reference, Tanzanian and Norwegian diets, respectively, are presented in Table 7. Table 7 shows a significant difference between Norwegian diet on one hand and the Tanzanian and reference diet on the other for TGC. There were significant differences with a high R^2 in the final weight between the diets, and the Norwegian diet performed better. The Norwegian diet performed also better for the weight gain and SGR compared to the other two diets. The eFCR is higher than the estimated FCR. The Tanzanian diet did not show any significant difference between the Norwegian diet and reference diet in the weight gain. Observed mortality for the reference diet was 2.4% while for the two experimental diets was 11.9%. The values varied a lot between tanks within each diet, which is explained by a low R^2 for the initial weight and mortality with 13% and 14%, respectively

Table 7. Performance parameters of the fish fed reference, Tanzanian and Norwegian diets. Values are presented as means ± SEM (n = 3). Values with different superscripts were significantly different at $P < 0.05$ with an accuracy provided by R^2.

Performance parameters	Norwegian diet	Reference diet	Tanzanian diet	*P value*
Initial weight (g)	5.6 ± 0.28	5.9 ± 0.31	5.6 ± 0.18	*0.65*
Final weight (g)	32.7^{a} ± 2.17	21.8^{b} ± 1.87	26.0^{b} ± 0.81	*0.01*
Weight gain (g)	27.1^{a}	15.9^{b}	20.4^{ab}	*0.07*
SGR (%)	1.8 ± 0.03^{a}	1.3 ± 0.07^{c}	1.6 ± 0.06^{b}	*0.00*
TGC (%)	0.5 ± 0.02^{a}	0.4 ± 0.03^{b}	0.4 ± 0.02^{b}	*0.01*
eFCR (%)	2.8 ± 0.31	3.2 ± 0.21	3.5 ± 0.14	*0.22*
bFCR* (%)	3.1 ± 0.47	3.2 ± 0.22	4.0 ± 0.22	*0.18*
Mortality	11.9 %	2.4 %	11.9 %	*0.64*

*bFCR is probably overestimated due to no feed collection after every feeding period.

4.2.2 Growth performance

The SGR and TGC of the tilapia per diet during the duration of the experiment are presented in figure 13 and 14, respectively. During the first feeding period high growth rates were observed for all dietary treatments. The growth rate decreased with the duration of the experiment and this is illustrated in the figures.

The Tanzanian diet, resulted in negative values in the last two periods while the Norwegian diet, as expected, performed better through the duration of the experiment. With the exclusion of the last period where the Tanzanian and Norwegian diets performed surprisingly less than the reference diet (see figure 13).

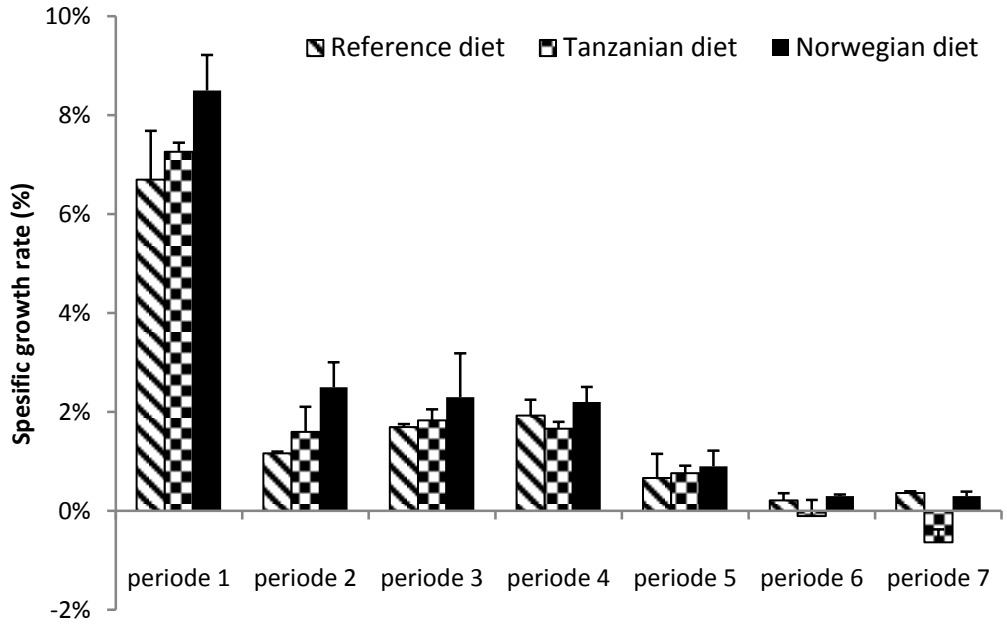

Fig. 13. Specific growth rate of the experimental fish during all periods. The values are presented as means ± SEM (n = 3).

The TGC in figure 14 presents a more consistent growth pattern. The tilapia had an increasing growth rate towards period 3, then decreasing until the end of the duration of the experiment. In period 1 the Norwegian diet is significantly different from the reference diet, but the Tanzanian diet was not significantly different from either. Again, the Tanzanian diet resulted in low values for the last two periods with significant difference in period 7. During period 4 the fish showed low growth rates in all tanks for all diets.

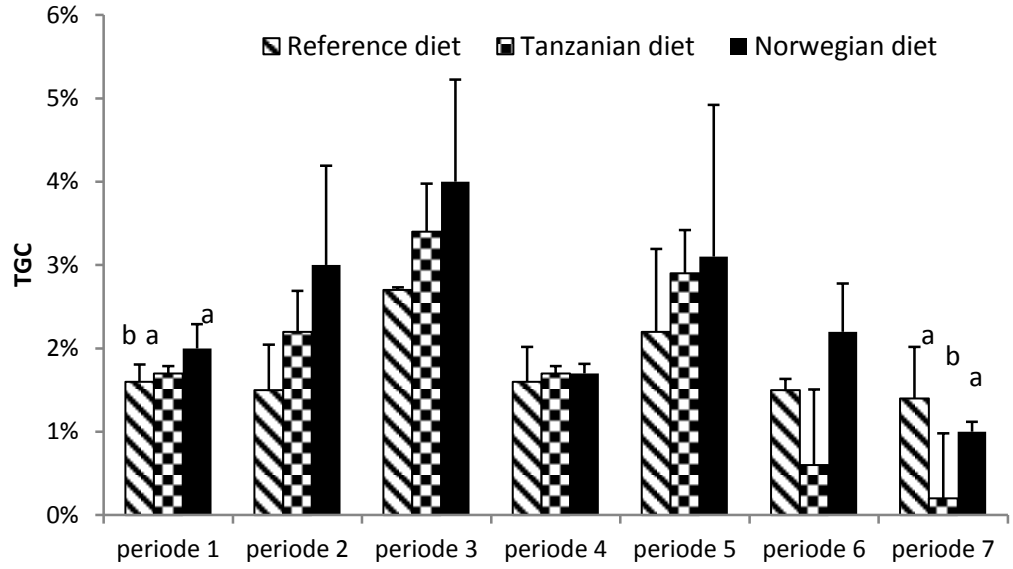

Fig. 14. Thermal growth coefficient (TGC) of the experimental fish values is presented as means ± SEM (n = 3). Superscripts are significantly different at p < 0.05%. For period 2 R^2 was 56% and for period 7 R^2 measured 69% certainty.

4.2.3 Fillet quality

The sampled fish brought from Tanzania to Norway was weighed, gutted and filleted. The body weights, lengths, gutted weights and fillet weights all showed significant differences in favour to the tilapia fed on the Norwegian diet as presented in Table 8. The fat content of the fillets was measured among the diets. The tilapia fed on the Tanzanian diet seemed to be the leanest.

Table 8. Body weight, length, gutted and fillet weight had a significant difference per diet. Values are presented as means ± SEM (n = 3). Values with different superscripts were significantly different at *P < 0.05*. The gutted yield had a lot variation between the samples with R^2 of 8%.

Fillet quality	Reference diet	Tanzanian diet	Norwegian diet
Body Weight (g)	22.8^{b} ± 1.25	22.6^{b} ± 0.70	30.7^{a} ± 1.72
Length (cm)	11.2^{b} ± 0.12	11.1^{b} ± 0.10	11.9^{a} ± 0.10
CF (100*(g/cm^3))	1.6 ± 0.07	1.6 ± 0.03	1.7 ± 0.09
Gutted Weight (g)	20.3^{b} ± 1.04	20.0^{b} ± 0.82	26.7^{a} ± 1.62
Gutted Yield (%)	89 ± 0.01	89 ± 0.01	88 ± 0.00
Fillet Weight (g)	7.7^{b} ± 0.54	7.8^{b} ± 0.64	11.0^{a} ± 0.70
Fillet Yield BW (%)	34 ± 0.01	34 ± 0.02	36 ± 0.00
Fillet Yield GW (%)	38 ± 0.01	38 ± 0.02	41 ± 0.01
Fat Content (%)	2.3 ± 0.50	1.7 ± 0.20	2.2 ± 0.37

4.2.4 Fillet colouration

There was no significant difference found between the diets in whiteness, redness, or yellowness. There was some variation in the fillets between diets and b* varied among the diets in favour of the reference diet (see Table 9).

Table 9. Colouration of the fillets per diet. Values are presented as means ± SEM (n = 9). No significant differences (*P < 0.05*) were observed.

Fillet colouration	Reference diet	Tanzanian diet	Norwegian diet	*P value*
Minolta White (L*)	44.3 ± 1.44	44.5 ± 2.27	44.4 ± 3.00	*1.00*
Minolta Red (a*)	8.3 ± 1.00	8.1 ± 1.75	8.6 ± 0.95	*0.96*
Minolta Yellow (b*)	4.6 ± 0.49	3.9 ± 0.25	3.5 ± 0.35	*0.21*
Hue (b*/a*)	0.6	0.5	0.4	*0.66*
Chroma	9.5	9.0	9.3	*0.97*

4.2.5 Fatty acid analysis

The fatty acid compositions in the diets are presented in Table 10. The reference diet did not contain any of the very long chain n-3 fatty acid, while the Tanzanian and Norwegian diets did, although minimally.

Table 10. Fatty acid compositions in the diets. The most important fatty acids are illustrated so that each diet represents a mean of two parallels ± standard error.

Fatty acid	Reference diet	Tanzanian diet	Norwegian diet
C 16:0	13.6 ± 0.01	9.9 ± 0.01	8.2 ± 0.04
C 18:0	2.6 ± 0.01	4.7 ± 0.01	4.1 ± 0.01
C 24:0	0.3 ± 0.02	0.8 ± 0.01	0.5 ± 0.02
C 16:1 n-7	0.2 ± 0.01	1.3 ± 0.07	0.6 ± 0.00
C 18:1 n-9	34.5 ± 0.05	26.8 ± 0.06	30.4 ± 0.05
C 18:1 n-7	-	-	0.3 ± 0.06
C 18:2 n-6	45.4 ± 0.15	44.6 ± 0.08	48.7 ± 0.01
C 18:3 n-3	0.9 ± 0.01	2.3 ± 0.02	0.7 ± 0.71
C 20:1 n-9	0.04 ± 0.06	0.2 ± 0.00	0.2 ± 0.00
C 20:4 n-6	-	0.3 ± 0.01	-
C 20:5 n-3	-	0.5 ± 0.01	0.1 ± 0.00
C 22:4 n-6	-	-	-
C 22:5 n-3	-	0.2 ± 0.02	0.1 ± 0.03
C 22:6 n-3	-	0.6 ± 0.67	0.3 ± 0.00

- means that these fatty acids were not detected in the respective diet (<0.01).

In Table 11, the fatty acid composition of the tilapia's muscle shows no significant difference between the diets. The total n-6 PUFA was high, as expected in a freshwater fish (Waagbø et

al. 2001), while the very long chain n-3 PUFA presented higher values than perhaps assumed (Waagbø et al. 2001). The omega 6 / omega 3 ratio was lowest in the fish that had been fed the reference diet.

Table 11. Muscle fatty acid composition (%) of the Nile tilapia fed with the three diets for 14 weeks. The presented values are as means ± SEM (n = 3).

FA Composition (%)	Reference diet	Tanzanian diet	Norwegian diet
C 16:0	17.0 ± 0.61	16.2 ± 1.66	15.8 ± 1.53
C 18:0	8.9 ± 0.76	7.8 ± 0.44	8.0 ± 1.30
C 24:0	3.4 ± 0.49	2.6 ± 0.09	2.6 ± 0.68
C 16:1 n-7	3.2 ± 0.17	3.3 ± 0.19	3.4 ± 0.29
C 18:1 n-9	17.6 ± 3.71	21.0 ± 1.94	20.1 ± 5.94
C 18:1 n-7	2.7 ± 0.31	2.5 ± 0.28	2.5 ± 0.43
C 18:2 n-6	18.1 ± 2.97	21.1 ± 4.33	22.0 ± 4.81
C 18:3 n-3[a]	0.9 ± 0.06	0.9 ± 0.15	0.9 ± 0.07
C 20:1 n-9	0.8 ± 0.26	0.9 ± 0.23	0.7 ± 0.20
C 20:4 n-6	5.3 ± 2.07	3.6 ± 0.37	3.9 ± 1.89
C 20:5 n-3[b]	0.4 ± 0.15	0.4 ± 0.17	0.4 ± 0.33
C 22:4 n-6	1.5 ± 0.21	1.3 ± 0.14	1.3 ± 0.03
C 22:5 n-3[c]	1.6 ± 0.25	1.6 ± 0.40	1.4 ± 0.68
C 22:6 n-3[d]	6.9 ± 1.56	5.5 ± 1.08	5.5 ± 3.44
Total SFA	32.4 ± 1.01	29.7 ± 1.32	29.5 ± 1.97
Total MUFA	24.3 ± 2.02	27.7 ± 0.99	26.7 ± 3.36
Total n-3 PUFA	10.2 ± 1.10	8.8 ± 1.02	8.63 ± 2.66
Total n-6 PUFA	28.2 ± 0.78	29.5 ± 2.35	30.6 ± 2.03
n-6/n-3	2.8 ± 0.30	5.0 ± 0.73	4.3 ± 1.18

[a] α-linolenic acid

[b] Eicosapentaenoic acid (EPA)

[c] Docosapentaenoic acid (DPA)

[d] Docosahexaenoic acid (DHA)

The fatty acids with main relevance to the experiments are illustrated in figure 15, 16 and 17 for the reference, Tanzanian and Norwegian diets, respectively. The tilapias capacity to elongate and desaturate α-linolenic fatty acids to eicosapentaenoic and docosahexaenoic fatty acids is indicated from these results. The highest index of n-3 PUFA was observed in the tilapia fed on the reference diet while the highest values for n-6 were found in tilapia fed with the Norwegian diet.

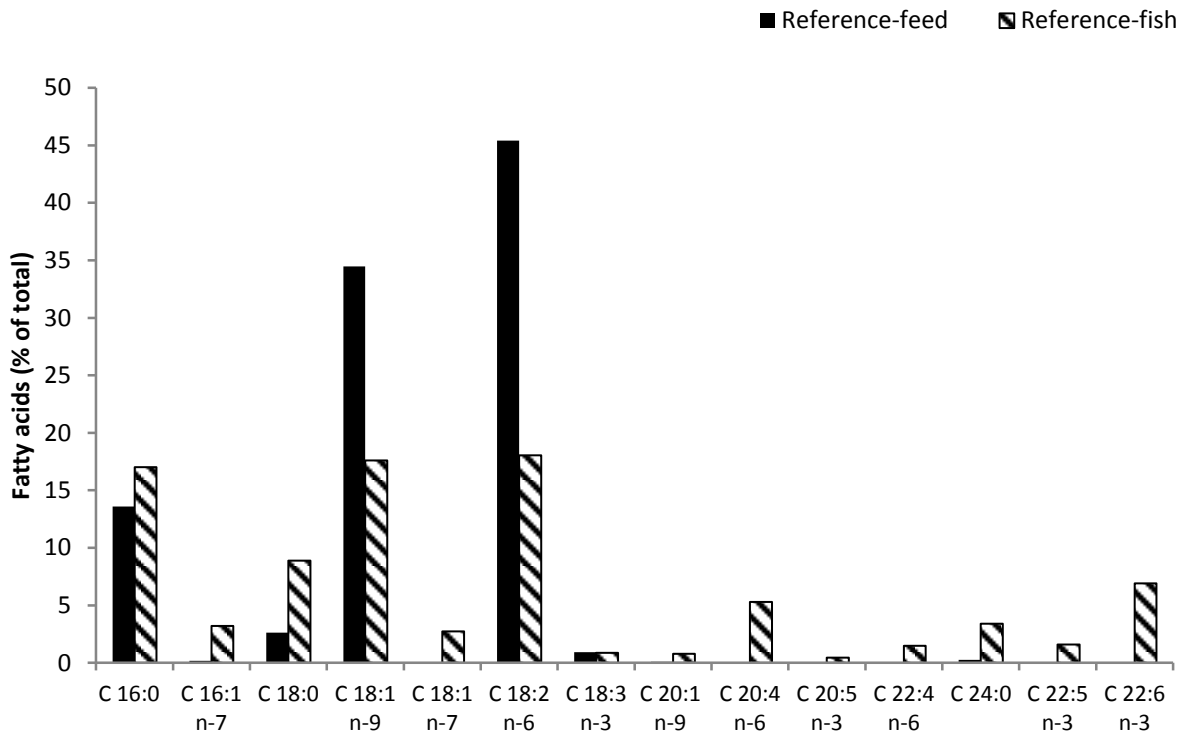

Fig. 15. The fatty acids of the reference diet compared to the fatty acids composition in the fish fed the reference diet.

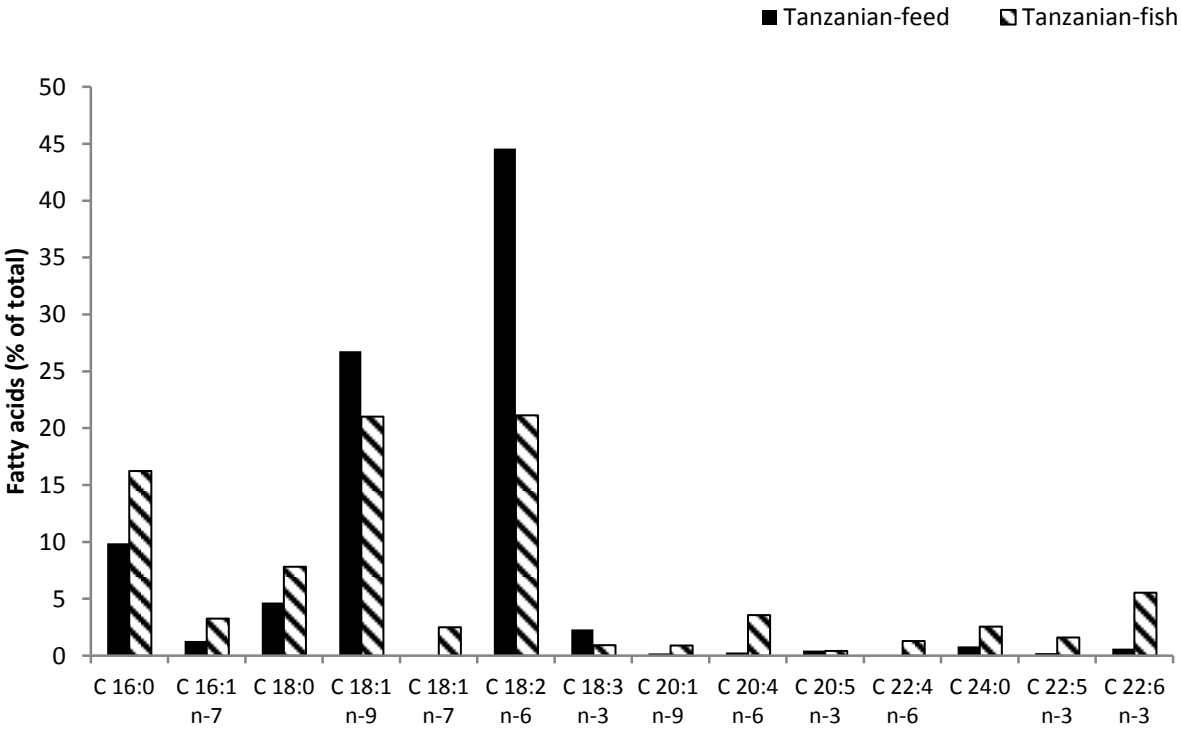

Fig. 16. The fatty acids of the Tanzanian diet compared to the fatty acids composition in the fish fed the Tanzanian diet.

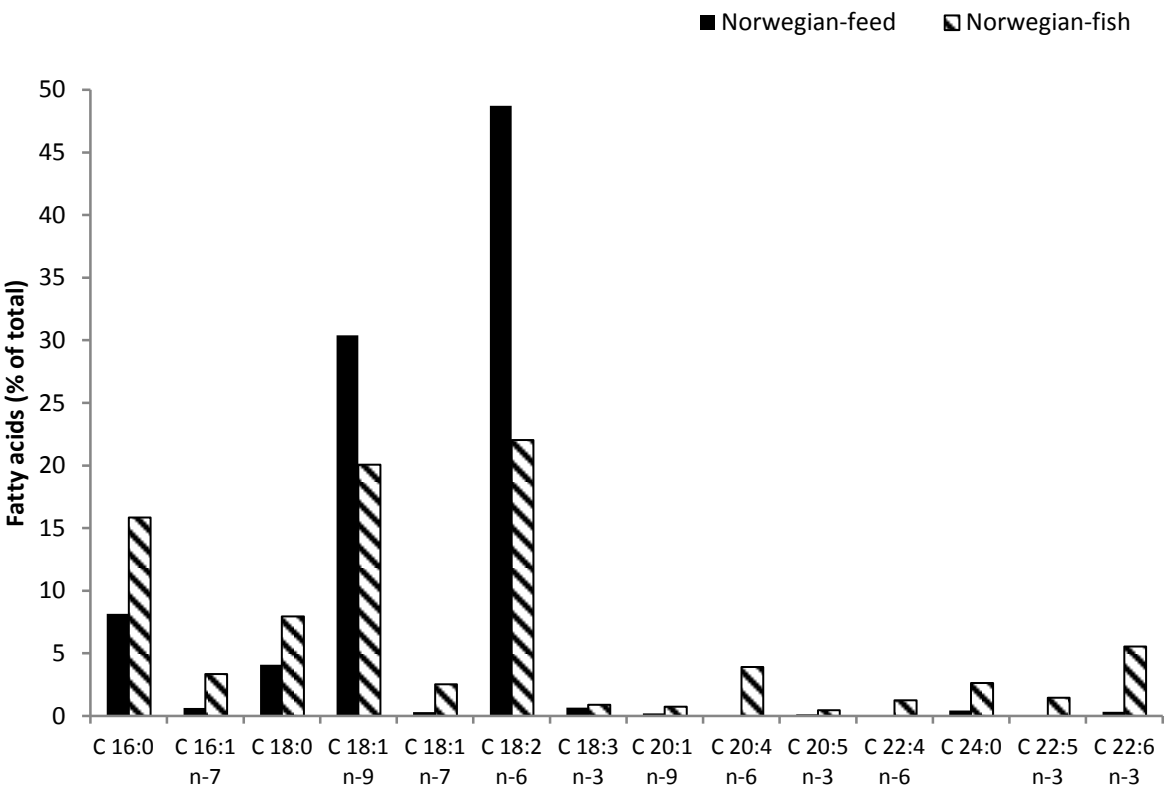

Fig. 17. The fatty acids of the Norwegian diet compared to the fatty acids composition in the fish fed the Norwegian diet.

4.3 Species experiment

4.3.1 Performance on diets

The calculated initial weight, final weight, weight gain, average SGR, TGC, eFCR, bFCR and mortality are presented in Table 12 for the Wami tilapia and Nile tilapia, respectively. Significant differences were found between the final weights of the two species and it can be stated that the Nile tilapias' performance was better than the Wami tilapia. Also the SGR, TGC and eFCR showed significant differences.

Table 12. Performance parameters of the Wami and Nile tilapia. Values are presented as means ± SEM of all three tanks (n = 3). Values with different superscripts were significantly different at $P < 0.05$.

Performance parameters	Wami tilapia	Nile tilapia	*T-test***
Initial weight (g)	5.7 ± 0.15	5.8 ± 0.31	*0.85*
Final weight (g)	12.2^{b} ± 0.13	14.6^{a} ± 0.56	*0.01*
Weight gain (g)	6.5 ± 0.79	8.8 ± 1.44	*0.16*
SGR (%)	0.8^{b} ± 0.03	0.9^{a} ± 0.05	*0.05*
TGC (%)	0.19^{b} ± 0.01	0.24^{a} ± 0.01	*0.02*
eFCR (%)	5.4^{a} ± 0.13	3.8^{b} ± 0.25	*0.00*
bFCR* (%)	11.5 ± 4.44	5.9 ± 1.24	*0.29*
Mortality	19.0 %	19.0 %	*1.00*

*bFCR is probably overestimated due to no feed collection after every feeding period.
***T-test* calculated in Microsoft Excel 2010 Mac version

4.3.2 Growth performance

The SGR and TGC of the different tilapia species are presented in Figure 18 and 19, respectively, for the whole duration of the experiment. During the first diet week, high growth rates were observed for both species. The growth rate decreased with the duration of the experiment as illustrated in figure 18 and 19. Figure 18 shows that the SGR decreased steadily during each period. The Wami tilapia had a slightly higher SGR the first two periods but received negative values for period 4, whereas the Nile tilapia performed well, but also had a decline in SGR for period 4.

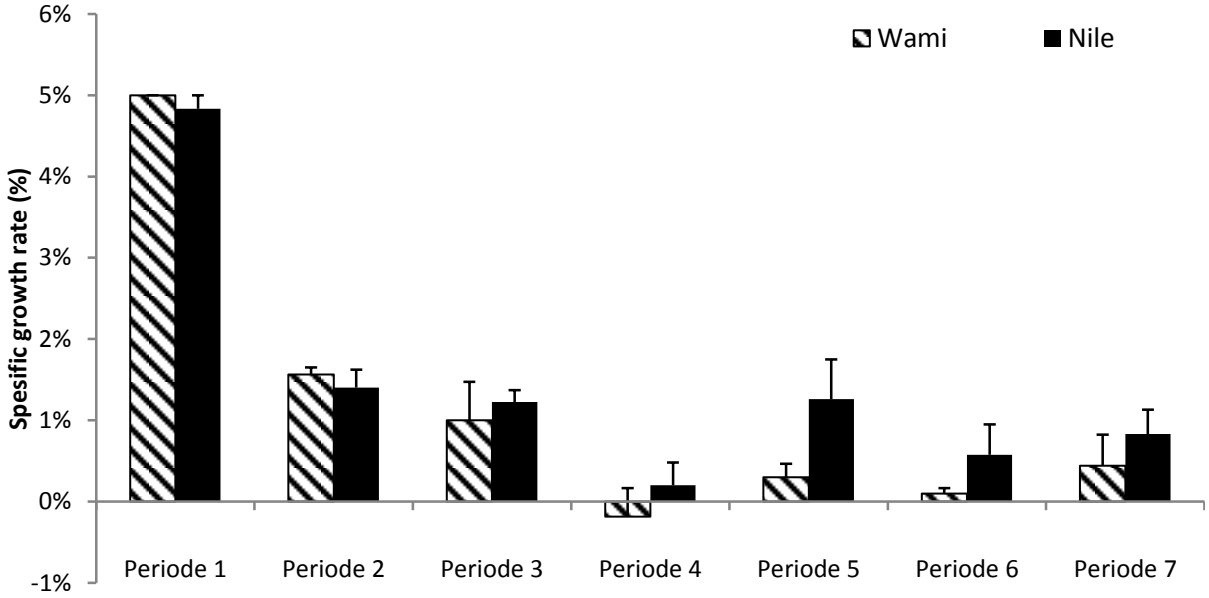

Fig. 18. Specific growth rate of the experimental fish over all periods. The values are presented as means ± SEM (n = 3).

The TGC in figure 19 shows a steady decrease of growth rate and gives a negative value for the Wami tilapia in period 4 and even a growth reduction in the Nile tilapia. The Wami tilapia only tended to performe better than the Nile tilapia in the two first periods and had almost no growth increases in period 6. No significant differences were found within the periods between the two species. Overall, the Nile tilapia showed significant difference in TGC compared to the Wami tilapia: *P = 0.02*.

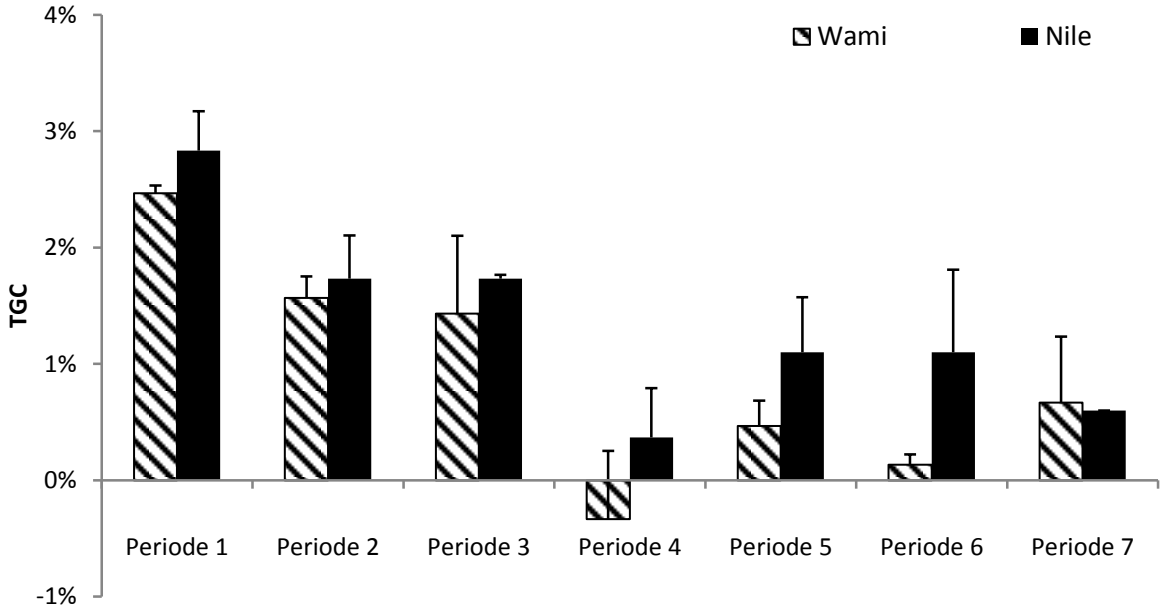

Fig. 19. The thermal growth coefficient (TGC) of the experimental species is presented as means ± SEM (n = 3).

4.3.3 Fillet quality

The fillet quality parameters are presented in Table 13. The condition factor, fillet yield of body weight and fillet yield of gutted weight were significantly different at $P < 0.05$ with higher values for the Nile tilapia. Higher condition factor and fillet yields were observed for the Nile tilapia.

Table 13. Condition factor, fillet yield of BW and of GW had significant differences per species. Values are presented as means ± SEM (n = 3).

Fillet quality	Wami tilapia	Nile tilapia	*T-test**
Body Weight (g)	12.1 ± 0.30	13.7 ± 0.59	*0.07*
Length (cm)	9.3 ± 0.18	9.3 ± 0.10	*0.84*
CF (100*(g/cm^3))	1.5^b ± 0.06	1.7^a ± 0.02	*0.05*
Gutted Weight (g)	11.0 ± 0.32	12.7 ± 0.62	*0.08*
Gutted Yield (%)	91 ± 0.01	92 ± 0.01	*0.16*
Fillet Weight (g)	4.4 ± 0.12	5.5 ± 0.38	*0.06*
Fillet Yield BW (%)	37^b ± 0.00	40^a ± 0.01	*0.04*
Fillet Yield GW (%)	40^b ± 0.00	43^a ± 0.01	*0.03*
Fat Content (%)	2.2 ± 0.57	1.7 ± 0.08	*0.43*

* *T-test* calculated in Microsoft Excel 2010 Mac version

4.3.4 Fillet colouration

Figure 20 indicates a slightly stronger intensity in the redness and a significant higher yellowness of the Wami tilapia muscle, compared to the Nile tilapia. Moreover, there was no significance for whiteness between Nile tilapia and Wami tilapia. In Hue the Nile tilapia had a slightly higher (more yellowish) colour tone than the Wami tilapia, but the Chroma indicates that the Wami tilapia had higher colour saturation (more colour). Chroma and b* were significantly different ($P < 0.05$) in the Wami tilapia compared to the Nile tilapia.

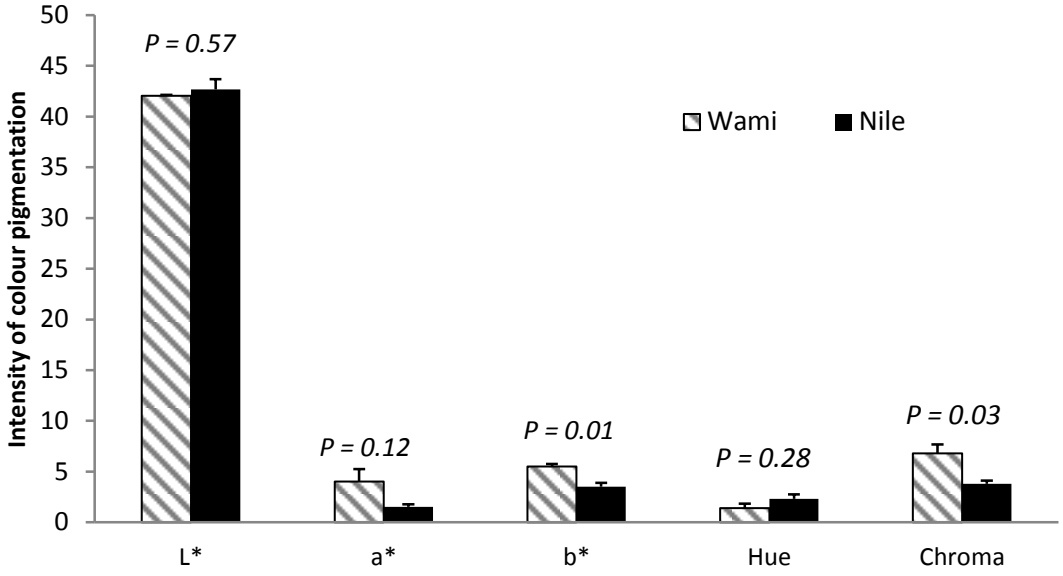

Fig. 20. Measurements of the Minolta taken in form of colour intensity. L describes lightless (L* = 0 for black, L* = 100 for white), a* intensity in red (a* > 0) and b* intensity in yellow (b* > 0).*

4.4 Fatty acid analysis

The fatty acid composition of the muscle showed no significant difference between the two species. The PUFA n-6 is 1.5% higher in the Wami tilapia and the *T-test* indicates a close to significant difference at *0.11*. The SFA was 1.3% higher in the Nile tilapia. However, the n-6/n-3 ratio is low in both species (see Table 14).

Table 14. Muscle fatty acid composition (%) of the Nile tilapia and Wami tilapia. The presented values are as means ± SEM (n = 3). No significant differences were found between the species.

FA Composition (%)	Wami tilapia	Nile tilapia	*T-test**
C 16:0	16.8 ± 0.51	17.4 ± 0.54	*0.26*
C 18:0	8.1 ± 0.61	8.0 ± 0.28	*0.79*
C 24:0	3.0 ± 0.90	3.0 ± 0.38	*0.99*
C 16:1 n-7	2.3 ± 0.27	2.5 ± 0.31	*0.43*
C 18:1 n-9	17.9 ± 2.47	17.3 ± 1.55	*0.75*
C 18:1 n-7	2.9 ± 0.29	2.8 ± 0.25	*0.62*
C 18:2 n-6	21.4 ± 2.51	18.4 ± 0.79	*0.11*
C 18:3 n-3[a]	1.6 ± 0.37	1.6 ± 0.17	*0.87*
C 20:1 n-9	0.7 ± 0.03	0.7 ± 0.06	*0.42*
C 20:4 n-6	3.8 ± 1.19	5.0 ± 0.41	*0.18*
C 20:5 n-3[b]	0.7 ± 0.22	0.8 ± 0.04	*0.81*
C 22:4 n-6	1.2 ± 0.27	1.4 ± 0.17	*0.20*
C 22:5 n-3[c]	1.8 ± 0.37	1.7 ± 0.23	*0.66*
C 22:6 n-3[d]	6.2 ± 1.68	6.5 ± 0.94	*0.82*
Total SFA	30.6 ± 0.40	31.2 ± 0.59	*0.44*
Total MUFA	23.8 ± 1.36	23.3 ± 1.02	*0.78*
Total n-3 PUFA	10.9 ± 1.09	11.0 ± 0.71	*0.94*
Total n-6 PUFA	29.5 ± 0.62	28.0 ± 0.59	*0.16*
(n-6/n-3)	2.71 ± 0.57	2.55 ± 0.83	*0.33*

* *T-test* calculated in Microsoft Excel 2010 Mac version

[a] α-linolenic acid
[b] Eicosapentaenoic acid (EPA)
[c] Docosapentaenoic acid (DPA)
[d] Docosahexaenoic acid (DHA)

The ratios in Table 15 show a comparison between Nile tilapia from the species experiment and Nile tilapia from the diet experiment fed with the same Tanzanian diet. The size of the fish from the last experiment was smaller. There are some minor differences between the two samples, however these are not statistically significant. The percentage of saturated fatty acids tended to be lower in the larger fish while the percentages of monounsaturated fatty acids increased proportionally to the size. The oleic acid differed the most with 3.7% difference in favour to the largest fish. Among the PUFAs n-6 fatty acids seemed to increase in the largest fish, while the sum of n-3 tended to be lower.

Table 15. Muscle fatty acid composition (%) from the Tanzanian diet on Nile tilapia in the diet experiment compared to the Nile tilapia fed on the same diet from the species experiment.

FA Composition (%)	Nile tilapia Exp I	Nile tilapia Exp II	Exp I / Exp II
C 16:0	16.2 ± 1.66	17.4 ± 0.54	0.9
C 18:0	7.8 ± 0.44	8.0 ± 0.28	1.0
C 24:0	2.6 ± 0.09	3.0 ± 0.38	0.9
C 16:1 n-7	3.3 ± 0.19	2.5 ± 0.31	1.3
C 18:1 n-9	21.0 ± 1.94	17.3 ± 1.55	1.2
C 18:1 n-7	2.5 ± 0.28	2.8 ± 0.25	0.9
C 18:2 n-6	21.1 ± 4.33	18.4 ± 0.79	1.1
C 18:3 n-3[a]	0.9 ± 0.15	1.6 ± 0.17	0.6
C 20:1 n-9	0.9 ± 0.23	0.7 ± 0.06	1.3
C 20:4 n-6	3.6 ± 0.37	5.0 ± 0.41	0.7
C 20:5 n-3[b]	0.4 ± 0.17	0.8 ± 0.04	0.5
C 22:4 n-6	1.3 ± 0.14	1.4 ± 0.17	0.9
C 22:5 n-3[c]	1.6 ± 0.40	1.7 ± 0.23	0.9
C 22:6 n-3[d]	5.5 ± 1.08	6.5 ± 0.94	0.8
Total SFA	29.7 ± 1.32	31.2 ± 0.59	1.0
Total MUFA	27.7 ± 0.99	23.3 ± 1.02	1.2
Total n-3 PUFA	8.8 ± 1.02	11.0 ± 0.71	0.8
Total n-6 PUFA	29.5 ± 2.35	28.0 ± 0.59	1.1
(n-6/n-3)	5.0 ± 0.73	2.6 ± 0.83	1.9

[a] α-linolenic acid

[b] Eicosapentaenoic acid (EPA)

[c] Docosapentaenoic acid (DPA)

[d] Docosahexaenoic acid (DHA)

5. Discussion

5.1 Environment and experimental fish

The tanks were built just a shortly before the start of each new experiment. If the tanks were not carefully washed and soaked with water, there could still have been traces of concrete causing concrete poisoning. This could have been one of the causes for the observed mortality. In addition, stress due to moving and creating new social circles within each tank might have affected the fish.

The tanks fed with the Tanzanian diet were full of algae after approximately four days. The tanks fed with the reference diet did surprisingly not produce any algae cultures, even though fresh "cow manure" was added as a fertilizer every 5th day. The reasons for this are unclear since it should have been a good fertilizer for the water (Hussain 2004). A reason could, however, have been that the algae was introduced to the tanks by the moringa leaf meal.

A stocking density of three fingerlings per m^3 was used, even though the recommendation is between 15 and 45 fingerlings per m^3 (El-Sayed 2006). The reason for this was the high variety between weights, in addition to low supply of fish.

The transportation of the fish is problematic in Tanzania, especially since the fish had to travel 1,000 km from Mwanza close to Lake Victoria to Morogoro where the research site was located. With the lack of the right equipment for transportation, such as e.g. aeration of fish transport tanks, the mortality during transportation was high. The fish that survived the trip needed some days to recover.

The Nile tilapia was 23 days in the introductory tanks before the first experiment started. The Wami tilapia was 12 days in the introductory tanks before the second experiment started. This does not affect the performance of the tilapia, however, it indicates how long they have been fed on the maize bran before they were transferred to the experimental tanks.

One of the controversies in aquaculture is the way of killing fish. According to Waagbø, (2001), this should be done in a humane way where the fish is killed quickly and with the lack of conscious pain (Waagbø et al. 2001). The method performed in these experiments was

according to ethic principles, i.e. metomidate hydrochloride powder was used in order to sedate the fish before killing.

5.2 Experiments

The diet experiment was conducted from February 19th until May 28th 2013. The species experiment started a month later, March 18th to June 26th 2013. The temperature varied in this period but the difference was small with an average temperature right above 28°C for the diet experiment. For the species experiment the average temperature was observed to be just below 28°C. This probably did not have any significant effect on the growth performance of the tilapia. Their preferred temperature range is between 20 and 35°C (El-Sayed 2006; Hussain 2004).

The dissolved oxygen levels in the diet experiment varied strongly, this mainly due to poor accessible material and equipment. In the first periods of the diet experiment, difficulties with the water supply occurred. Occasionally no water was available in critical periods. The dissolved oxygen in the tanks sank to levels as low as <1.0mg/l, and this is a problem in all freshwater fish, as oxygen is needed for survival. The tilapia however is a hardy fish (Hussain 2004) that tolerates low oxygen levels (hypoxia) even at rates of 0.0mg/l dissolved oxygen. As long as they are able to "pipe" at the water surface they will not die of suffocation, but rather of stress. The tolerance area of tilapia for dissolved oxygen is down to 0.0mg/l or 0.5mg/l and up to 400% supersaturation (El-Sayed 2006; Hussain 2004), although the preferred level of dissolved oxygen for good growth are between 3.0mg/l and 8.0mg/l (Hussain 2004). The tilapia in most tanks in the feeding experiment had occasionally DO levels below 3.0mg/l except for tank 5 and 6 with the lowest DO level at 4.0mg/l.

The Tanzanian diet contained 34.5% moringa leaves meal. Since the tanks only got cleaned once every two weeks, all the uneaten feed and faeces settled at the bottom of the tank and resulted in a small zone with low oxygen content with anaerobic decomposition. This resulted in relieve of hydrogen sulphide gas (H_2S) which could have been toxic for such small tilapia (Lekang 2007).

The DO measurements during the first 14 days period resulted to DO levels of 5.8, 5.2 and 6.7mg/l for the reference diet, Norwegian diet and the Tanzanian diet, respectively. During

this first period the fish was still small and had a large amount of water compared to biomass. The final period in the diet experiment revealed higher DO levels with values of 6.5, 6.2 and 8.5mg/l for the reference, Norwegian and Tanzanian diets, respectively. It can be stated that the increase of organic compounds and excessive feeds in the tanks fed with Tanzanian diet are the main reasons for the high DO levels. Another reason might be that the water parameters in the tank are stable due to the tank environment, giving a balanced environment between the phytoplankton and the fish (Feidi 2010).

The fish in both experiments had surprisingly a growth reduction during period 4, which was observed simultaneously with changed behaviour of the fish within the tanks. This is the first indication of tilapia becoming sexual mature, resulting in smaller appetite and thus smaller growth. For tilapia to mature, a constant water temperatures has to be above the necessary 23°C (Meyer & Meyer 2007). To prevent sexual maturation, Ng and Romano (2013) stated that when tilapia juveniles are fed with a diet containing between 10% and 17% protein the fish never reaches sexual maturity. While protein levels between 30% and 40% fed to sexual mature tilapia conducts to optimization of fecundity and seed production, in addition to decreased maturity age (Ng & Romano 2013). Another method is to feed a synthetic male steroid – methyltestosterone (MT) – to tilapia in fry stages (Hiott & Phelps 1993). This is, however, a more doubtable method to use due to the ethic questions connected to it. Sex determination is an often used method (University & Environments 1992) since the farmer is able to determine sexes of tilapia by inspecting their genital papilla when they reach approximately 10cm or 20g. Tilapia should be minimum 25g to separate sexes successfully (Popma & Lovshin 1995). PHD de Graaf (2004) indicate that higher feeding levels or polyculture in form of stunting – where the piscivorous / omnivorous fish will reduce the fingerlings and hatched fry of the tilapia – is a good solution to the problem of unwanted breeding and spawning. The polyculture would for example consist of a tilapia species and either the African catfish (*Clarias gariepinus*) or the African snakehead murrel (*Parachanna obscura*). This form of polyculture where both an herbivorous and an omnivorous species are introduced to the same pond is very effective and could be used in broodstock breeding where the product-fish are not allowed to reproduce. The catfish is not a threat to the adult tilapia, and does not influence their growth rate (Graaf 2004). However, if the catfish is too small, the recruitment will exceed the capability of the catfish to eat all the fry and thus, it is

more effective to have bigger catfish, 6.8g to 130g, in the ponds (Graaf 2004). Tilapias are mouthbreeders, meaning the female tilapia gathers the fertilized eggs in her mouth for brooding and during the yolk-sac stage. After period 4 in the experiments, several eggs were observed during weighing procedures followed by low growth performance.

Aggression was observed in the tanks fed with the Norwegian diet. This could be explained by competition for nutrition between the fish in the tank, which happened mostly during the first two periods. Another reason for aggressive behaviour is to find reproductive partners (Waagbø et al. 2001), which is assumed to be the case in period 4 when the growth rate dropped.

The water quality was tested in forms of testing strips where detection of nitrite, total hardness of the water, alkalinity in the water, the waters pH and nitrate levels were measured. The highest levels observed were 250mg/l hardness and 180mg/l alkalinity. The pH ranged from 6.8 to 7.6 and the maximum nitrate levels was 20mg/l in one tank. Ammonium levels were also observed for the water. These never reached a level above 0.1mg/l. The water quality was therefore acknowledged as a good water quality for tilapia farming.

The diet composition of the Tanzanian diet is interesting since it contains moringa leaves. Moringa leaves are very rich in protein. The proximate analysis performed on the moringa leaves of this study indicated a level of 33% crude protein, while in the study performed by Richter et al. (2003) they measured a crude protein content of only 25% only. Richter et al (2003) indicated from their study that a maximum of 10% moringa leave meal should be added as alternative protein source, anything above this level would only lead to growth reduction. The Tanzanian diet contained 34.5% moringa leaves meal, this might have been one of the reasons why the tilapia fed on this diet did not perform as good as the tilapia fed on the Norwegian diet and even got negative growth during the last two periods in the feeding experiment.

5.3 Performance on diets

The initial weight of the tilapia in the diet experiment was ranging from 2.7g to 8.6g. This is a large variation between sampling fish, but these were the closest in weight to each other since the total amount, 250 fish, of wild caught tilapia was not sufficient to choose a lesser variety of weights. The final weight indicated significant difference in favour of the tilapia that was fed the Norwegian diet. The mean final weights of the experimental fish were 21.8g in the reference diet, 26.0g in the Tanzanian diet and 32.7g in the Norwegian diet. This resulted to a daily weight gain of 0.16g, 0.21g and 0.28g for the reference diet, Tanzanian diet and Norwegian diet, respectively. For a wild fish, this is still not impressive, the weight gain observed in the study of Garduño-Lugo et al. (2007) had a daily weight gain of 2.8g for their adult fish, though, not completely comparative due to the difference in weight from the current study. The water quality, the negative influence of the different compounds in the Tanzanian and the missing nutritional compounds in the reference diet can explain the low growth performance. The growth of the Wami tilapia is not as strong as the growth of Nile tilapia as was observed in the species experiment. In addition the Wami tilapia was more reddish and yellowish in meat colour. Whether this is a preferred quality or not will be investigated and discussed in the follow-up study, performed by Scontina Mgina in her Msc. thesis "Perception and sensoric quality differences of tilapia fish species in Morogoro region, Tanzania".

It was observed that the reference diet, even though it contained a large amount of energy and seems to be healthy enough, is not the ultimate feed source. The important factor that influences the healthiness of the maize bran is the low content of the amino acid, lysine. A suggested daily requirement of the amino acid lysine was around 3% (Lievert & Benkendorff 2006). The lysine levels are low in maize bran (Otubusin 1987), certainly not higher than 3% of the daily requirement, and therefore the fish gained less weight than the two experimental diets. If somehow the lysine content in maize bran would be increased it would probably be a far more interesting diet to fish feed than it is now.

Concrete poisoning and natural conditions such as stress, competition, and diseases could have caused the observed mortality in the tanks over the duration of both experiments. Most likely the mortality was not feed related. The observed mortality was quite high,

ranging from 2.4% to 19% in the diet and species experiments, respectively. The most probable reason, however, is the tank environment. Since the tanks were new they needed time to relieve all their toxicity to the water, so when the fish were inserted too early the mortality increased.

The reason for the economical FCR numbers differing from the biological FCR was the use of new tanks probably causing fish mortality during the first periods of the trial. The calculated economical FCR was much higher than the estimated FCR, with 3.2 ± 0.21, 3.5 ± 0.14 and 2.8 ± 0.31 for the reference, Tanzanian and Norwegian diets, respectively. Since there were no feed collections it was not possible to calculate the specific feeding rate (SFR) and thus there was assumed that the fish had a 100% feed intake.

The colouration of the tilapia in the diet experiment did not vary much between diets. The Hue in the reference diet had the highest value with 0.6, but the Tanzanian and Norwegian diets followed closely with 0.5 and 0.4, respectively. The Chroma indicates, however, that the reference diet had more saturation of the colours, closely followed by the Norwegian diet with 9.3 and the Tanzanian with 9.0. The Hue in the species experiment did not show much of a difference between the two species. The Chroma on the other hand did show significant differences in favour to the Wami tilapia, and significantly higher b* indicates a higher saturation of yellowness in the Wami tilapia.

The diets were produced when the possibility was there. The same ingredients were used during the whole trial, therefore, it was sometimes of unstable conditions, and the storage time could have been too long turning the diets old. Sometimes the newly produced pellets moulded under the drying conditions due too much moisture in the air and lack of cool storage. Economically seen, the Tanzanian feed is cheaper since the moringa leaves were gathered by own means, while the lysine, methionine and vitamin C in the Norwegian diet were expensive ingredients. As discussed below, the growth performance using the Tanzanian diet could perhaps have been better if the amount of moringa leaves had been lower.

5.4 Growth rate

The specific growth rates are parallel samples of the three different diets used in this experiment; therefore the curves in figure 12 should all follow a distinctive pattern to each diet. Environmental effects, sexual behaviour, sexual maturity and measuring errors can lead to the differences within the diets. The SGR for the species experiment are presented in figure 12. It shows clearly how the SGR decreases by each period, however, not the entire drop in growth rate can be explained by the normal growth pattern of tilapia. Similar as in the diet experiment, the stage of sexual maturation, behaviour and measuring errors might have been reasons. An explainable reason for the low growth rate can be decomposition of the raw materials while being stored during the experiments duration. The diets were made for each period, thus turning older through the duration of the experiments. The specific growth rate is a measurement based on the daily growth expressed as percentage of body mass of the fish not taking into account the temperature (Lekang 2007). The thermal growth coefficient, however is based on water temperature, duration and size of the fish (Jobling 2003). The SGR does provide for growth information, but TGC includes temperature and is normally used as growth parameter for different trials. Therefore, instead of SGR, TGC was used to calculate a consistent growth parameter for the mentioned diets and species. In figure 13, a clear increase in the growth pattern until the fourth period can be observed. However, period 4 shows a far lower growth than period 5 although it has a high standard error in the Norwegian diet with 1.8%. The Norwegian diet presents an overall higher TGC but also a higher standard error than the other two diets. The Tanzanian diet has an overall poor growth performance, compared to the Norwegian diet. The high percentage of moringa leaf meal can be causing this, since Richter et al. (2003) conducted a study of what is the optimum level of moringa leaf meal that can be used in diets for tilapia. Here they concluded that everything that is above the 10% level would cause decrease in growth performance. In this current study, levels of 30% of dietary protein, are high and most likely will the relatively high total phenolics and phytic acid, found in moringa leafs contributed to the poor growth performance (Richter et al. 2003).

The TGC for the species experiment is a bit different (see figure 19). It is shown that from period 1 to period 6 the Nile tilapia performed better than the Wami tilapia. The Nile tilapia had almost as high TGC in period 6 as in period 5 but the TGC dropped again in period 7. In

period 7 the Wami tilapia performed better compared to the previous 3 periods where period 6 was the period with the worst performance. The only period where the Wami tilapia performed better than the Nile tilapia was in period 7. The reason for this is unclear but assumed is the difference in age between the fish species. The total weight gain was lower than the weight gain achieved in the feeding experiment. The age of these tilapias was not known since they were caught in the wild. This might have been one of the factors resulting to low growth performance and the occurring sexual maturation.

The growth performance was not as high as expected; this could have been caused by several reasons, i.e. the wild fish, unknown age of the fish and sexual maturity are all reasons for decrease in growth. Would the fish have been a GIFT fish for instance, the growth rate would have been considerably higher.

5.5 Chemical composition

Nutrition quality is a term often discussed to be important for human consumption. With nutrition quality of fish, the levels of lipids, protein and carbohydrates, in addition to essential vitamins, are determined to certain levels (Waagbø et al. 2001). This indicates that the nutrition value of the fish has to meet the demand of the consumers. In the Western world it is of importance that the fish eaten contains omega-3 fatty acids for human needs. Product tailoring is actually controlling the nutrition levels in the fish by use of determined dosages within the diets. The water content of the three diets is calculated as $Water\ content = 100 - dry\ matter$.

The Norwegian diet contained slightly more moisture with 11.2%, than the reference and Tanzanian diet with 10.3% and 10.5% respectively. The Tanzanian diet has the highest ash content (10.0%) due to the moringa leaves with 10.3%. This is similarly observed in the study performed of different moringa meal levels (Richter et al. 2003) where diet 4 is most similar to the Tanzanian diet in this study. The crude protein levels were significantly higher in the Tanzanian diet with 24.1% due to the high content in the moringa leaves with 33.0% compared to the Norwegian and the reference diet with 18.3% and 10.4%, respectively. The crude protein level is however considerably lower than is observed by Richter et al. (2003) where they observed levels of 35.4%. When comparing the crude fibre content, the Norwegian and reference diet are very similar with 27.8% and 27.2% respectively. The

Tanzanian diet had significantly lower crude fibre content due to the moringa leaves with only 9.2% and 0.4%, respectively. Richter et al. (2003) observed crude fibre contents of 3.6% in the diet, but the higher level in our Tanzanian diet can be explained due to the use of sunflower meal and maize flower. For the crude lipid it is observed that the Norwegian and reference diets differ from the Tanzanian diet with 10.8% and 11.1% respectively compared to the 9.5% in the Tanzanian diet. Whereas Richter et al. (2003) observed 11.4% in their diet. The nitrogen free extracts and starch, which are not measured, account for 26.6% to 37.0% of the total contents in the diets. The most energy rich diet was the Reference diet with 18.2MJ/kg. Closely followed by the Norwegian and the Tanzanian diets with 18.0MJ/kg and 17.8MJ/kg respectively. The values in (Fall et al. 2011) for its soybean diet, containing 45.2% soybean per kilogram had different values for the conducted proximate analysis. The reason for this is different total quantities of ingredients within the diet compared to the current study.

5.6 Fillet quality

The sampled fish that were used to perform fillet quality tests on were tilapia chosen closest to the average weight from the respective tanks. When observing Table 8, these tilapias had significant differences in body weights between diets where the Norwegian diet had an overall better performance than the other two diets. In addition was the body length larger in the tilapia fed with the Norwegian diet giving these tilapias a larger condition factor, indicating a bigger fish compared to the same length, or the same weight compared to a shorter length. Normally the condition factor will indicate the relation between weight and length, and decrease when the length becomes more dominating than the weight (Kestin & Warriss 2001). Once the tilapias were gutted the tilapias fed Norwegian diet showed a significant difference from the Tanzanian diet and reference diet. Even though the tilapia fed with the Norwegian diet had a higher gutted weight, the gutted yield was more in favour to the Tanzanian diet and reference diet. The mean fillet weight of the tilapia fed on the Norwegian diet showed that they produce more fillet weight (11g) while the tilapias fed on Tanzanian diet and reference diet reached only 7.8g and 7.7g, respectively. The fillet yield was based on the gutted weight (GW) and whole body weight (BW), the calculated mean fillet yield BW indicated that the Norwegian diet fed tilapias obtained a higher percentage of fillet yield, which is confirmed by the fillet yield GW. The fillet yield BW obtained from the

different diets was 34%, 34% and 36% for the reference, Tanzanian and Norwegian diets, respectively. This is lower than Thodesen et al. (2012) observed in their study of tilapia selected for growth and fillet yield. Their observations of the fillet yield ranged between 40.7% and 46.8 %. Even when the fillet yield GW received higher values, 38%, 39% and 41% of the reference, Tanzanian and Norwegian diets, respectively, they did not become as high as Thodesen et al. (2012) observed. The reason for this is probably because their fish size is significantly larger, due to better genetic potential and possibly better environment, than in the current study. The tilapia is a lean fish and contains below 8% lipids (Clement & Lovell 1994). Lean fish will store the excessive lipids, inquired through the diet, in its liver without really making use of these, therefore it is unnecessary to oversaturate the diets with lipids (Waagbø et al. 2001). Garduño-Lugo (2007) found lipid contents of 2.3% in the fillets of Nile tilapia, the results of this current study observed lipid contents ranging between 1.7% and 2.3%. The tilapia fed on the Tanzanian diet had the lowest lipid value of 1.7%. This was observed for the Tanzanian diet in the diet experiment. The Nile tilapia fed on reference diet contained numerically the highest value of 2.3% lipids, while the Nile tilapia fed on the Norwegian diet contained 2.2%. The Wami tilapia fed on the Tanzanian diet contained a higher fat content, at 2.2%, than the Nile tilapia fed on the same diet. The lipid content in the 1 tank of the species experiment, containing Wami tilapia differs from the other two Wami tanks. There was measured a lipid content of 3.3% while in tank 2 and 3 the lipid content was 1.4% and 1.9%, respectively. When examined the sampled fish within tank 1, the explanation becomes clear, there was found an outlier observation with lipid content of 5.2%, while the other two fish in this tank's parallels contained 2.1% and 2.6%. This is still a higher value than is observed as average on the other two tanks within the Wami species. These tanks had exactly the same conditions, except one, the first tank was partly shaded during the day by an overhanging tree. So it might give an idea to shade the tanks from direct sunlight

5.7 Fatty acid analysis

Feed containing a large amount of proteins and carbohydrates will most likely produce an over satisfaction of energy within the fish and be converted to fatty acids and stored as lipids (lipogenesis). Fatty acids that are synthesised *de novo* through the fish are called endogenous fatty acids (Waagbø et al. 2001). The fish fed on the reference diet contained the

20:5 n-3 and 22:5 n-3 at similar levels as the other two diets. These results indicate that the tilapia manages to synthesise the longer chain essential fatty acids (EFA). One option is to desaturate and elongate α-linolenic acid (18:3 n-3) to end product docosahexaenoic acid (22:6 n-3). The other option is to desaturate linoleic acid (18:2 n-6) and to elongate to arachidonic acid (20:4 n-6). These fatty acids are called PUFA fatty acids. Freshwater fish, such as the tilapia, requires addition of α-linolenic and linoleic acid in their diets for maintaining their fatty acid balance (Waagbø et al. 2001). Waagbø et al. (2001) also indicates that if these balances of fatty acids are not maintained within the fish it will be noted in poor growth, expansion of the liver due to accumulation of neutral lipids and under-developed membranes needing PUFA fatty acids (such as the chloride cells in the gills). The fatty acids composition in the species experiment is not significantly different within the two species. Comparing the Nile tilapia fed the Tanzanian diet in both experiments (see Table 15) indicated some small differences in fatty acids content. The fatty acids 20:4 n-6, 20:5 n-3 and 22:6 n-3 are typically present in the phospholipids and higher values of these indicates a leaner fish. It is said that too high values of 20:4 n-6 should be avoided because of the increased risk of cardiovascular disease in humans.

The diets in the first experiment contained 45.4%, 44.6% and 48.7% of 18:2 n-6 for the reference, Tanzanian and Norwegian diets, respectively. The level of 18:2 n-6 in (Justi et al. 2003) contained 53.8% and 32.3% for a diet without flaxseed oil, and a diet with flaxseed oil, respectively. Justi et al. (2003) found levels of 18:2 n-6 in the fish fillet fed on the diet with flaxseed oil of 30.8%, 30.9%, 27.2% and 26.6% for 0, 10, 20 and 30 days treatments, respectively. Compared to the diet experiment, were levels of 18.1%, 21.1% and 22.0% were found in the fillet of the reference, Tanzanian and Norwegian diets, respectively. This indicates that there is a context of the 18:2 n-6 fatty acids between amount in diet and storage in fillet.

In table 10 the fatty acid compositions in the diets are given. There were no observed long-chained fatty acids in the reference diet, but there were traces of long-chained fatty acids in the Tanzanian and Norwegian diet due to the added fishmeal as including ingredient. The fish managed to store a large amount of 18:2 n-6 and a small amount of 18:3 n-3. The proportion of the n-3 and n-6 in the leaner fish will indicate higher concentrations of n-6 fatty acids in the phospholipids, and the higher phospholipids concentration is more

accountable for the total fatty acid composition than when observed in a fatter fish. The leanest fish contains mostly phospholipids, which are used in the cell membranes. Explicit examples of these are the 20:5 n-3, 22:5 n-3 and 22:6 n-3 fatty acids within the fish. The 18:3 n-3 levels in this study were 0.9%, 2.3% and 0.7% for the reference, Tanzanian and Norwegian respectively, while in (Justi et al. 2003) levels of 1.6% and 24.3% were measured for a diet without flaxseed oil, and a diet with flaxseed oil, respectively. When observing the fatty acids composition, the Wami tilapia contained slightly and not significantly more 18:2 n-6 fatty acids than the Nile tilapia from the same experiment. Which could possibly be explained by a higher storage of diet fat in the Wami tilapia since it seems to be a bit fatter than the Nile tilapia. Or, since 20:4 n-6 is higher in the Nile tilapia it might indicate that the capacity of desaturation and elongation of 18:2 n-6 is higher in the Nile tilapia.

5.8 Future tilapia farming in Tanzania

These two experiments were of a rather simple construction. The farming of tilapia in armed concrete tanks is not a problem, as long as the preparations before introducing the fish to the tanks are well performed. Given the dissolved oxygen differences in the diets, it is strongly advised to supply the tanks with some form of aeration, such as e.g. a water propeller that adds oxygen to the water. It might also be an idea to shade over the tanks, although this is not proven to increase the growth rate. Water quality is important; therefore it would be wise to have running-through water instead of leaving the water in the tank over a longer time. This because organic compounds accumulate over time, decreasing the fish growth rate. It is also advised to rather choose tilapia with known age, maybe even to choose a tilapia that is selected for better growth performance, such as the GIFT tilapia (Thodesen & Ponzoni 2004). The Tanzanian diet is certainly a diet that could be used, only the amount of the moringa leaves should be reduced to a ratio that can be used without reducing the growth of the tilapia. The fatty acids content in the tilapia indicates that it is a healthy fish, which is important for the human diet. Culturing tilapia as mono-sex males will most probably increase the total growth performance of the tanks/ponds and one way to have all males is selection or another way is thermo sex reversal at 36.8°C (Azaza et al. 2007).

6. Conclusion

As a conclusion the tilapia fed with the Norwegian diet had an overall better growth rate compared to the tilapia fed with the Tanzanian diet. However, considering the large weight variances between the tanks exposed to the same diet, it remains uncertain if the observed growth rate would be fully due to the supplied diet. From an economic perspective the Tanzanian diet could be considered a less expensive diet than the Norwegian due to the costs associated with the ingredients of the Norwegian diet, such as the added lysine, methionine and vitamin C.

The Wami tilapia had an overall lower growth rate than the Nile tilapia in the species experiment. Hence, we could carefully conclude that the Wami tilapia did not perform as well as the Nile tilapia. The colouration of the tilapia fed on the different diets is similar to each other as well. The only observed difference is in fact resulting from the reference diet where the yellow was more visible opposed to the red in the diet experiment. The Wami tilapia instead had more red and yellow in the muscles, in addition to a higher saturated colour than the Nile tilapia in the species experiment. The fatty acid contents in the two diets were very similar, both when the diets themselves are compared and when the fish fed on the diets were compared. Therefore we could conclude a relatively equal performance from this angle for the Tanzanian and the Norwegian diets.

Moreover, it appears that during the fourth period for both experiments, the sexual maturations are associated with the energy levels of the tilapia, no matter the specie or consumed diet. This will be a problem using wild caught tilapia, since the fish may be quite old and ready for maturing when enough energy is available.

This was the first nutritional experiment conducted on the Magadu site at SUA, Morogoro. Challenges occurred throughout the trial and were resolved in order to continue the experiments. Hopefully, the findings of this study can give some recommendations as a source for further studies. Also the planned breeding program based on the GIFT tilapia may give a good basis for future Tanzanian aquaculture.

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LIST OF FIGURES

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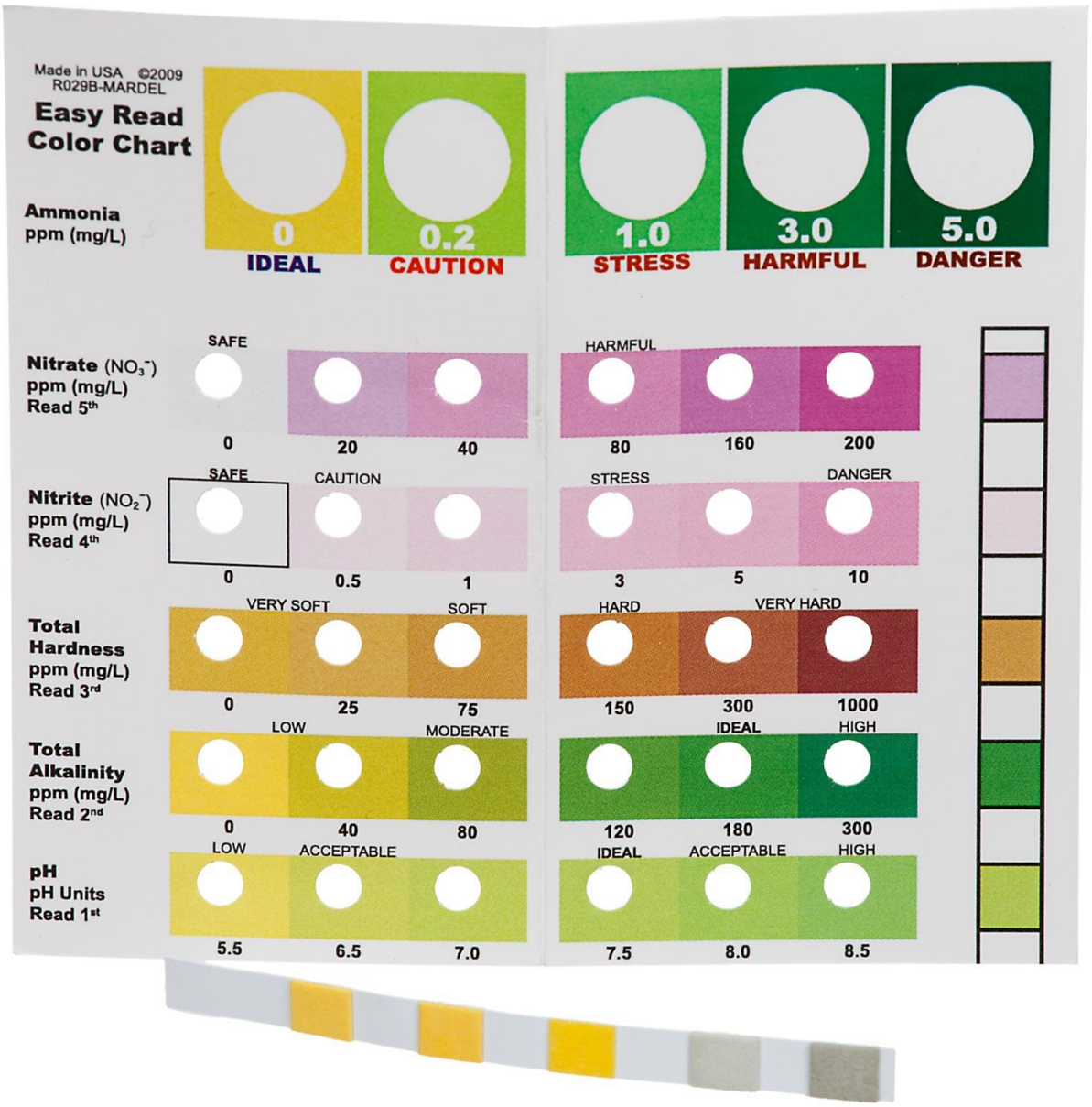
LIST OF ATTACHMENTS

Attachments 1: Schedule used per period for weight measurements and feeding regime

Tilapia experiments

						Feed gram										
	Date	Total biom.	No.fish	Ind.size	FCR	9:00 AM	1:00 PM	5:00 PM	Temp		Oxygen		Mort nr.	Mort Biom	Name/sign
Start	27-3-2013		n	0,0	1,80		0,0	0,0							
	28-3-2013		n	0,0	1,80	0,0	0,0	0,0							
	29-3-2013		n	0,0	1,80	0,0	0,0	0,0							
	30-3-2013		n	0,0	1,80	0,0	0,0	0,0							
	1-4-2013		n	0,0	1,80	0,0	0,0	0,0							
	2-4-2013		n	0,0	1,80	0,0	0,0	0,0							
	3-4-2013		n	0,0	1,80	0,0	0,0	0,0							
	4-4-2013		n	0,0	1,80	0,0	0,0	0,0							
	5-4-2013		n	0,0	1,80	0,0	0,0	0,0							
	6-4-2013		n	0,0	1,80	0,0	0,0	0,0							
	7-4-2013		n	0,0	1,80	0,0	0,0	0,0							
	8-4-2013		n	0,0	1,80	0,0	0,0	0,0							
	9-4-2013		n	0,0	1,80	0,0	0,0	0,0							
End	10-4-2013		n	0,0											
End	Measured		n	0,0		0,0	0,0	0,0							
	Expected SGR			1,5 %											
	Calculated SGR			(LN(E22)-LN(E8)/14)/100											

Attachments 2: Mardel 5 in 1 test strips used for water quality measurements



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