Evaluating silage oil from rainbow trout (*Oncorhynchus mykiss*) viscera as a substitute for dietary fish oil on production parameters of juvenile African catfish (*Clarias gariepinus*)

by

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Abstract

Fresh rainbow trout (*Oncorhynchus mykiss*) viscera was used to extract fish silage oil by acid and fermentation ensiling methods. Organic acid mixtures (lactic acid x formic acid, lactic acid x propionic acid, and a commercial bacterial inoculum x molasses) were added to about 900 L of minced viscera in three airtight 1000 L, respectively. The silage oils were assessed for their volume, quality and fatty acid composition with the view to being used as feed oil in African catfish (*Clarias gariepinus*) diets.

After 30 days, across the treatments, an average volume of 291 L of silage oil was produced, with a quality deemed favourable by food grade standards. The silage oils were very similar in their fatty acid fatty acid composition and compared favourably to a regular marine-based feed oil in their percentage of polyunsaturated fatty acids. However, the silage oils had a low percentage of omega-3 fatty acids and a high percentage of omega-6 fatty acids, resulting in a high omega-6:omega-3 fatty acid ratio.

A 90-day feeding trial was conducted to investigate the effects of replacing fish oil with rainbow trout (*O. mykiss*) silage oil in the diets of juvenile African catfish (*C. gariepinus*), on their growth, survival and feed conversion. The three silage oils (derived from a lactic acid x formic acid silage, lactic acid x propionic acid silage, and a commercial bacterial inoculum x molasses silage) and a marine fish oil (control) were evaluated as the feed oils in four treatment diets. The diets were fed to juvenile African catfish (1.36 ± 0.14 g) in six replicate tanks per treatment, over a period of 92 days in a temperature-controlled recirculating aquaculture system.

Overall there was no significant difference ($p>0.05$) between the four treatments on growth, survival and feed conversion ratio. The results indicate that the total dietary replacement of marine fish oil with Rainbow trout silage oil does not significantly affect the growth, survival or feed conversion of juvenile African catfish after a 92-day feeding trial, and that Rainbow trout silage oil could prove to be a viable alternative to fish oil for juvenile African catfish in the 1.36 – 53.33 g size range. This bodes well for the sustainable utilisation of Rainbow trout visceral waste as an aquafeed ingredient.

Opsomming

Vars reënboogforel (*Oncorhynchus mykiss*) viscera was gebruik om viskuilvoerolie te onttrek deur suur- en fermentasie-inkuilingsmetodes. Sowat 900L elk van gemaalde viscera is in drie lugdigte 1000 L plastiekhouersby melksuur x maursuur, melksuur x propionionsuur en 'n kommersiële kuilvoerinokulum x melasse bygevoeg. Die kuilvoerolies is geassesseer vir volume, gehalte en vetsuursamestelling met die oog daarop om as voerolie in Afrika-katvis (*Clarias gariepinus*) -diëte gebruik te word.

Na 30 dae, oor al die behandelings, was daar ‘n gemiddeld van 291L viskuilvoerolie gevorm met ‘n voedselgraadstandaard gehalte. Die kuilvoerolies was baie soortgelyk in hul vetsuur-samestelling, en is gunstig vergelyk met 'n standaard mariene olie in hul persentasie poli-onversadigde vetsure. Die kuilvoerolies het egter 'n lae persentasie omega-3-vetsure en 'n hoë persentasie omega-6 vetsure gehad, wat 'n hoë omega-6: omega-3 vetsuur verhouding tot gevolg gehad het.

'n Opvolgstudie is uitgevoer om die effekte van die vervanging van visolie met reënboogforel (*O. mykiss*) kuilvoerolie in die dieet van jong Afrika-katvis (*C. gariepinus*) te ondersoek, oor hul groei, oorlewing en voeromsetting. Die drie kuilvoerolies (afkommende van melksuur x maursuur kuilvoer, melksuur x propionionsuur kuilvoer en 'n kommersiële kuilvoerinokulum x melasse) en die mariene visolie (kontrole) is geëvalueer as die voerolies in vier behandelingsdiete. Die diëte is aan jong Afrika-katvis (1.36 ± 0.14 g) gevoer, in ses replikaat tenke per behandeling, oor 'n tydperk van 92 dae in 'n temperatuur-gekontroleerde hersirkulerende akwakultuurstelsel.

Oor die algeheel was daar geen beduidende verskil ($p>0.05$) tussen die vier behandelings op groei-, oorlewing- en voeromsettingsverhouding nie. Hierdie resultate dui daarop dat die totale dieetvervanging van mariene visolie met reënboogforel kuilvoerolie nie die groei, oorlewing of voeromsetting van jong Afrika-katvis na 'n 92-dae voedingsproef beïnvloed het nie, en dat die reënboogforel kuilvoerolie as 'n lewensvatbare alternatief vir mariene-visolie kan dien vir jong Afrika-katvis in die grootte 1.36 – 53.33 g kan dien. Dit lyk belowend vir die volhoubare benutting van reënboogforel visserale-afval as 'n akwavoer bestanddeel.

Notes

1. While this thesis is based on the dual topics of producing rainbow trout viscera silage oil and testing it as a feed oil on production parameters of juvenile African catfish, it was written as a series of separate chapters. As a result, there is some inevitable repetition of ideas, especially in the introductions to the chapters.

2. Chapter 3 should be viewed as a proof of concept report, where an industry partner, Three Streams Smokehouse, requested Stellenbosch University Aquaculture to demonstrate a workable methodology for converting tons of Rainbow trout visceral waste into viable aquafeed ingredients. The chapter is brief, sets out to demonstrate the feasibility of the method and does not make its focus the chemical composition of the fish silage.

3. The APA referencing format is followed throughout.

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To rev up one's academic engine a couple of notches, at age 50, after leaving it on idle for 27 years, is no mean feat I've discovered. It could most certainly not have been managed successfully without a great deal of support from others.

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Chapter 1: Introduction

1.1 Global aquaculture

The global aquaculture sector has grown into the single largest food production sector on earth (FAO 2018). Worldwide aquaculture is directly dependent on numerous natural resources, while simultaneously posing serious threats to the natural environment such as pollution and reducing biodiversity (Martinez-Porchas & Martinez-Cordova, 2012). It sustains the food security of millions of people (FAO, 2018), while at the same time competing for limited dietary ingredients to satisfy its demand for aquafeed (Naylor et al., 2009). The aquaculture sector employs millions of people and provides alternative livelihoods and opportunities for local economic development especially in developing nations (Edwards, Little & Demaine, 2002; Bhujel, Shrestha, Pant & Buranrom, 2008). It impacts on global health by providing a relatively affordable produce which is rich in protein and essential fatty acids required for normal development (Tacon & Metian, 2013).

The most recent report on aquaculture reveals a staggering global production of 110.2 million metric tons (MMT), of which 80 MMT was food fish (FAO, 2018). This underscores the continuing importance and the pivotal role of the international aquaculture sector in enhancing food security, providing employment and alleviating poverty (FAO, 2018). The report further asserts that aquaculture remains the fastest growing food production sector supplying about 53% of food fish for human consumption. In order to maintain this high yield an ever-increasing quantity of good quality processed aquafeed is required. This in turn demands milions of tons of fishmeal (FM; 15.8 MMT) and fish oil (FO; 0.7 MMT) annually (Auchterlonie, 2016; SEAFISH, 2018), which are the favoured sources of protein and energy, respectively. Fish oil is normally derived from oily pelagic fish species such as anchovy, blue whiting and Menhaden (Cashion, Le Manach, Zeller & Pauly, 2017; SEAFISH, 2018). FO extracted from marine pelagic fish is preferred for its high essential fatty acid (EFA) content, with a higher proportion of Omega-3 over Omega-6 PUFAs. This bias towards Omega-3 FAs is favoured to ensure that the cultured fish have access to the EFAs required for maximum growth, and so that they end up with this ratio of Omega-3 to Omega-6 FAs in their flesh, since it

has been shown to have numerous health benefits for humans (Tacon & Metian, 2013).

Furthermore, fish consumption holds other benefits for human health such as: a daily 125 g portion serving of fish provides about 40% of the daily protein requirement, which is essential for the proper development of the nervous, muscular and skeletal systems (Tilami et al., 2018). Fish also provides some of the important fats and mineral salts needed for various essential metabolic and physiological processes (Khalili Tilami & Sampels, 2018).

1.2 Aquaculture demand for fish oil

With the stagnation or decline of many marine pelagic fisheries, but the continued growth in aquaculture production (Fig. 1.1), tensions have naturally developed between the supply and demand of FO for use in aquafeed production (FAO, 2018).

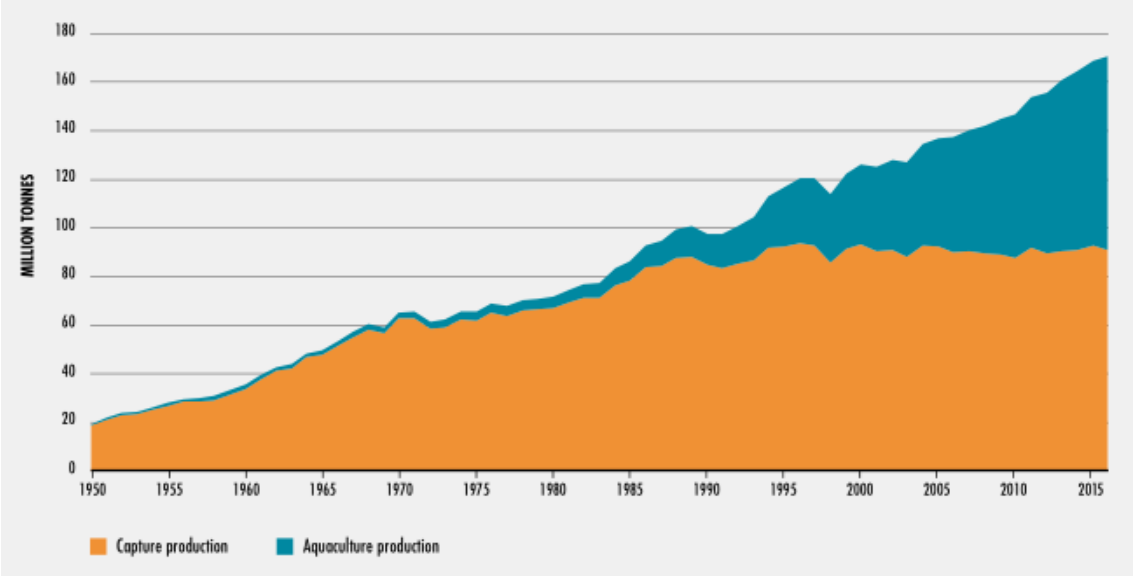


Figure 1.1 Fisheries capture production has remained the same since 2000 while aquaculture production continues to increase [source: FAO, 2018]

Furthermore, stemming from the increasing demand for the use of fish oil in various health products for human consumption, the aquafeed industry faces even further constraints on FO supply. Also, worldwide the apparent fish consumption is calculated at 20.3 kg *per capita* and increasing by 1.5% annually (FAO, 2018), which together with the annual global population increase of 1.1% (83 million; UN,

2017) puts enormous pressure on aquaculture as it continues to be a vital provider of global food security.

1.2.1 Alternatives to fish oil

In order to reduce the demand for FO, derived from pelagic marine fish species, created by a growing need for aquafeed much research is invested into finding suitable alternatives to FO that could be used as the feed oil in aquafeeds. These include marine phytoplankton, zooplankton and oily fish in the abyssal zone that are not targeted as food fish (Bell & Tocher, 2009; Pederson, Vang & Olsen, 2014), marine heterotrophic microorganisms (Chi et al., 2007; Zhu et al., 2007) and fish processing waste (Jackson and Newton, 2016).

Fish processing waste and fish by-catches represent a colossal source of raw material from which to extract FO. Currently, just taking the landed fisheries and aquaculture food fish of 80 MMT into account, this represents potentially 40MMT of fish waste for reduction to FM and FO, assuming 50% processing waste (Ferazz de Arruda, Borghesi & Oetterer, 2007; Sahu et al., 2016). The conventional method of FO extraction from fish waste is energy-expensive and requires a large capital outlay for high tech industrial equipment. The raw material is usually cooked, strained and pressed to produce an oil-rich liquid. This is heated and centrifuged to separate most of the oil from the liquid. The oil is further subjected to polishing, evaporation and centrifugation to produce various grades of soluble and insoluble oil (Arvanitoyannis & Kassaveti, 2008). Fish waste may also be hydrolysed by the addition of acids to a 1:1 minced fish in water mixture, with or without proteolytic enzymes (Yoshida, Takahashi and Terashima, 2003). This mixture is subjected to high temperature and pressure and centrifugation to produce a residue and supernatant from which the desired products are extracted by further processing.

1.2.2 Fish silage

A simpler method to extract the useful products from fish waste is by making a fish silage, which is a liquefied mixture of water, proteins, polypeptides, amino acids and lipids. Fish silage is made by mincing the available fish material, and mixing it with an acid, for an acid silage, or with a carbohydrate source and fermenting lactic acid bacteria, to produce a fermented silage (Vidotti, Viegas & Carneiro, 2003). In both methods, the acidic medium provides the low pH in which the various digestive enzymes, already present in the digestive tract and tissue cells of the fish material, break down the tissues in a process called autolytic hydrolysis (Arason, 1994; Raa, Gildberg & Olley, 1982). This reduces the fish tissues to crude fish protein hydrolysate (a mixture of water containing polypeptides and amino acids) and fish oil. The method of ensiling fish waste is especially useful in developing nations and subsistence aquaculture since it is well suited to utilising fish waste where it is only available in relatively small amounts and at irregular times, and where large-scale expensive infrastructures and big capital investments are not viable economic options (Toppe, Olsen, Peñarubia & James, 2018).

Since fish silage is similar in nutritional composition to the fish raw materials from which it was made (Vidotti et al., 2003), most researchers have used the entire by-product mixture as a component in fish diets during feeding trials (Fagbenro & Jauncey, 1995, 1998; Fagbenro, Jauncey & Haylor, 1994; Fagbenro, Jauncey & Krueger, 1997). The fish silage can either be dried and fed as is (Toppe et al., 2018) or usually co-dried with various grain meals (Fagbenro & Fasakin, 1996; Borghesi et al., 2008; Ramirez et al., 2013). The results were variable, but in the main the use of fish waste silage represents an important practise to ensure sustainable fish production, since their inclusion does not tend to promote growth but neither does it supress growth, and in some instances its inclusion reduced the cost of feed by up to 21 % (Ferraz de Arruda et al., 2007; Soltan et al., 2008). When it is mixed and co-dried with other feed meals such as soya bean meal then it readily replaces between 30 % to 50 % of fish meal in the diet without a significant effect on growth and feed utilisation (Fagbenro & Jauncey, 1995; Soltan et al., 2008; Madage et al., 2015; Soltan et al., 2017).

Very few studies have focused solely on using the FO derived from fish waste silage as a feed component in aquafeeds. In feeding trials with Mozambique tilapia (*Oreochromis mossambicus*), Goosen, de Wet, Görgens, Jacobs and de Bruyn (2014) substituted marine fish oil in the diet with silage oil, derived from rainbow trout processing waste, with no negative effect on production, an improvement in immunity and significant shortening of gastrointestinal folds. With South African abalone (*Haliotis midae*), inclusion of silage oil in the diet led to improved immunity, but negative production performance (Goosen, de Wet & Görgens, 2014), while Goosen, de Wet and Görgens (2018) reported no difference in final weight, but a significant reduction in daily weight gain (DWG) when compared to the reference diet.

The present study seeks to add to the academic conversation on this gap in the literature. As in the previous studies mentioned, it also used Rainbow trout viscera, to produce two acid silages and one fermented silage. During this process, the silage oils were extracted and used as substitutes for regular marine feed oil (the control) in three commercial aquafeed diets. The three silage oil diets were evaluated against the control diet, on the production parameters of juvenile African catfish (*Clarias gariepinus*) in a 90-day feeding trial.

The African catfish was chosen as the test species since it is popular as a food fish throughout Africa, where Nigeria has by far the largest annual global production. It is also gaining popularity as a food fish in South American, European and Asian countries (Dauda, Natrah, Karim, Kamarudin & Bichi, 2018), as is shown by the increase in its global production from 27 thousand tons in 2004 to 237 000 tons ten years later (FAO, 2010). It is also a hardy fish species which is fairly resistant to disease and can tolerate relatively poor water conditions. It can be grown equally well in earthen ponds as in a recirculating aquaculture system (RAS). It can tolerate high stocking densities, feed on many diets from raw offal to formulated complete diets and is relatively fast growing and tasty (Akinwole & Faturoti, 2007).

1.3 The scope of the research

1.3.1 The problem

The current thesis sets out to address the following related problems: In South Africa large volumes of Rainbow trout visceral waste end up in landfills and represent a wasted valuable raw material or resource; fish oil is a critical component of aquafeed and is both expensive and unsustainably derived from marine pelagic fish stocks; this raises the question if large quantities of Rainbow trout viscera could be processed by ensiling to extract fish oil for use as a feed oil in aquafeeds for juvenile African catfish?; and how the Rainbow trout viscera silage oil extracted in this way would compare to normal marine fish oil on the production parameters of juvenile African catfish?

1.3.2 The purpose of the research

The first purpose was to develop a methodology to produce fish silage from rainbow trout viscera in 1000L flobins, by employing acid- and fermented ensiling methods. This purpose flowed out of a request from the largest processor in the Rainbow trout industry, for Stellenbosch University to find a sustainable solution for dealing with the vast quantities of visceral waste that they produce annually. The second purpose was to incorporate the Rainbow trout silage oil as feed oil in juvenile African catfish diets and to conduct a feeding trial where the production performance parameters of juvenile African catfish would be evaluated on the use of silage oil versus regular marine fish oil as feed oil.

1.3.3 The rationale

Ensiling Rainbow trout waste viscera to produce silage oil represents a sustainable approach to dealing with fish waste at three levels:

Environmental – it addresses the issues of the disposal of organic waste in landfill sites that leads to degradation of organic waste which produces noxious greenhouse gaseous emissions, toxic compounds that contaminate the soil and groundwater, can lead to eutrophication in adjacent water bodies and attract disease-carrying vermin such as rats;

Socio-Economic – the by-products of ensiling rainbow trout viscera (fish silage) is a nutritious but stable mixture of water, protein, polypeptides, amino acids, minerals and lipids that can be stored for months at room temperature. It separates out naturally by density-differences into a watery fish protein component, fish protein hydrolysate (FPH) and a substantial layer of fish silage oil (SO). The FPH has been used successfully as a fish feed supplement, and as organic plant fertiliser, for which there is a growing niche market, where higher than average incomes could be earned. Such small businesses could create employment, stimulate entrepreneurship, create alternative livelihoods especially in poverty stricken areas, develop local economic activity and promote food security. The fairly low-tech approach of ensiling implies that relatively unskilled labour could be trained to develop marketable skills. The rainbow trout silage oil is a fish oil and could also be processed into various value added products such as biofuels and soaps.

Since rainbow trout silage oil is a fish oil this means that it is likely to function well as a feed oil in aquafeeds. However, since rainbow trout are freshwater fish their oil is likely to be low in Omega-3 fatty acids but (a) freshwater fish, such as African catfish, are generally known to be able to metabolically convert dietary C18 fatty acids into various longer chain C20-C22 essential fatty acids (Highly Unsaturated Fatty Acids) required for cellular metabolism, and (2) African catfish are not purchased for having flesh high in Omega-3 FAs, but rather for the other benefits derived from regular fish consumption such as high source of quality protein and all essential amino acids, other healthy fats and mineral salts.

1.4 Structure of the Thesis

The following section is a roadmap through the key issues addressed in each chapter.

Chapter 1 introduces the focus of the thesis, formulates the research questions and describes how the dissertation will be structured.

Chapter 2 is a literature review of the important background information that is required to support the arguments presented in the thesis. It starts with a brief description of the different types of FAs that are major components of fish oils and

silage oils. The important role of lipids in fish nutrition is dealt with next as well as the unsustainability of FO supply. This leads to the exploration of sources for more sustainable alternative oils, which spans microorganisms to fish waste. The reduction of fish waste to fish silage by acid and fermentation ensiling is covered in detail; followed by fish-feeding trials that tested the effects on production parameters, of the whole fish silage mixture or, less frequently, the silage oil only. Next the potential role of organic acid and probiotic residues in silage oil, on the production parameters of juvenile African catfish, is considered. Lastly, this chapter considers the qualities of the African catfish that make it a fitting aquaculture species for this trial.

Chapter 3 The chapter describes the process by which fish silage was produced using Rainbow trout viscera as the raw material, by two acid ensiling and one fermented or bacterial ensiling methods. It shows that stable fish viscera silage could be produced in 1000 L flobins using readily available appropriate technology materials and apparatus, under ambient conditions. For each of the different silage methods the quantity of silage oil and crude fish protein hydrolysate produced after 30 days, as well as the quality of the silage oil produced is reported against the standards of food grade fish oil. The success of both the acid and the fermented silage proves the reliability of the methodology used as well as the type and quantities of ingredients used.

Chapter 4 addresses whether silage oil from rainbow trout viscera, when used as feed oil, would produce statistically equivalent production performance among juvenile African catfish, as standard marine fish oil would; or if they differed, which oil would show the better performance. The chapter describes a classic 90-day feeding trial during which the three silage oils were evaluated as a FO replacement in juvenile catfish diets. The trial was conducted in an indoor temperature-controlled RAS with six replicates per treatment which were assigned in a completely randomised design. Mass and length measurements, of individual fish, were taken at about 20-day intervals, but tank averages were used as the units of measurement. The production parameters total weight gain, daily weight gain (DWG), specific growth rate (SGR), mortality, relative feed intake (RFI) and feed conversion ratio (FCR) were calculated per treatment. The means per treatment,

of final fish mass, average fish mass over time, percentage mortality, SGR and FCR were compared using a repeated measures analysis of variance (RMANOVA) model with the software package Statistica ver. 13.2 (Statsoft, Inc.); while the means per treatment of DWG were compared using a One – way ANOVA. All data sets were also subjected to Multiple Comparison Tests.

The trial was challenged by emergency repairs to the RAS resulting in intermittent poor water quality. Furthermore, antibiotic-resistant bacterial infections spread throughout the system resulting in a high mortality across all treatments. In spite of this a clear result was obtained, which showed no overall difference in juvenile catfish production parameters between the different treatments. This is a positive result for sustainable aquaculture since it shows that African catfish would grow equally well on FO derived from fragile marine pelagic fish stocks as they would on silage oil derived from rainbow trout viscera.

Chapter 5 draws together all the conclusions arrived at and evaluates the success of this thesis in adequately addressing the aims and objectives that it set out to achieve. Based on this evaluation and with a desire to address the broader issues that arise from this study, various recommendations are made about the direction in which to take this research going forward.

1.5 The specific aims and objectives of this thesis

The broad aim of this thesis is to investigate dietary ways to promote the principles of responsible aquaculture that operates within the borders of sustainability. This means to make every effort to address the three pillars of environmental sustainability, economic sustainability and social sustainability. The specific aims and objectives of this thesis and the hypotheses it sets out to test relate to the work described in Chapters 3 and 4.

In Chapter 3 the specific aim is to promote sustainable alternatives in dealing with Rainbow trout processing waste from the Three Streams trout processing facility in Franschoek. The specific objectives that were investigated and their related hypotheses are listed below:

Specific objective 1

To produce approximately 1000L of Rainbow trout viscera silage by using acid and fermented ensiling methods in an intermediate bulk container (IBC), which would remain stable for at least 30 days while exposed to ambient weather conditions. The hypotheses tested were:

- H0: All the silages will fail and decompose;
- H1: At least one of the silages will be successful and stable for 30 days

Specific objective 2

To determine the average volume of silage oil produced across the ensiling methods after 30 days. The hypotheses tested were:

- H0: No silage oil will be separated out after 30 days;
- H1: A clear volume of silage oil will be present after 30 days

Specific objective 3

To determine whether the silage oil produced, irrespective of the ensiling method, would meet the requirements for a viable food grade feed oil. The hypotheses tested were:

- H0: None of the silage oils would meet the requirements of food grade feed oils;
- H1: At least one silage oil would meet the requirements of food grade feed oil

Specific objective 4

To determine whether the silage oil produced, irrespective of ensiling method, has a fatty acid profile that is suitable for use as a feed oil for African catfish. The hypotheses tested were:

- H0: The Rainbow trout silage oils will not have a fatty acid profile suitable for use as a feed oil for African catfish;
- H1: Rainbow trout silage oil will have a fatty acid profile that makes it suitable for use as feed oil

In Chapter 4 the main aim was to determine how the three silage oils, when fed as feed oil in a commercial catfish diet, would compare with regular marine fish oil, on the production performance parameters of juvenile African catfish. The specific objectives that were investigated and their related hypotheses (based on the entire duration of the trial) are listed below:

Specific objective 1

To compare the percentage survival of catfish on the silage oil diets against those on the marine oil diet (control). The hypotheses tested were:

- H0: There will be no difference in percentage survival between treatments;
- H1: In at least one treatment the percentage survival will differ from the other treatments

Specific objective 2

To compare the growth performance parameters (final weight, DWG, SGR) of catfish on the silage oil diets against those on the marine oil diet (control). The hypotheses tested were:

- H0: There will be no difference in growth between treatments;
- H1: In at least one treatment the stated growth parameters will differ from the other treatments

Specific objective 3

To compare the feed conversion ratio (FCR) of catfish on the silage oil diets against those on the marine oil diet (control). The hypotheses tested were:

- H0: There will be no difference in the FCR between treatment diets;
- H1: In at least one treatment the FCR will differ from the other treatments

1.6 References

Akinwole, A. O., & Faturoti, E. O. (2007). Biological performance of African Catfish (Clarias gariepinus) cultured in recirculating system in Ibadan. *Aquacultural engineering*, *36*(1), 18-23.

Arason, S. (1994). Production of fish silage. In *Fisheries Processing* (pp. 244-272). Springer, Boston, MA.

Arvanitoyannis, I. S., & Kassaveti, A. (2008). Fish industry waste: treatments, environmental impacts, current and potential uses. *International Journal of Food Science & Technology*, *43*(4), 726-745.

Auchterlonie, N. (2016) Marine ingredients as a foundation for global fed aquaculture production. *Aquafeed International*, November 2016, 30-33.

Bell, M. V., & Tocher, D. R. (2009). Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. In *Lipids in aquatic ecosystems* (pp. 211-236). Springer, New York, NY.

Bhujel, R. C., Shrestha, M. K., Pant, J., & Buranrom, S. (2008). Ethnic women in aquaculture in Nepal. *Development*, *51*(2), 259-264.

Borghesi, R., Ferraz de Arruda, L., & Ôetterer, M. (2008). Fatty acid composition of acid, biological and enzymatic fish silage. *Boletim do Centro de Pesquisa de Processamento de Alimentos*, *26*(2).

Cashion, T., Le Manach, F., Zeller, D., & Pauly, D. (2017). Most fish destined for fishmeal production are food-grade fish. *Fish and Fisheries*, *18*(5), 837-844.

Chi, Z., Pyle, D., Wen, Z., Frear, C., & Chen, S. (2007). A laboratory study of producing docosahexaenoic acid from biodiesel-waste glycerol by microalgal fermentation. *Process Biochemistry*, *42*(11), 1537-1545.

Dauda, A. B., Natrah, I., Karim, M., Kamarudin, M. S., & Bichi, A. H. (2018). African Catfish Aquaculture in Malaysia and Nigeria: Status, Trends and Prospects. *Fish. Aqua. J*, *9*.

Edwards, P., Little, D. C., & Demaine, H. (2002). Issues in rural aquaculture. *Rural Aquaculture. CABI Publishing*, 323-340.

Fagbenro, O. A., & Fasakin, E. A. (1996). Citric-acid-ensiled poultry viscera as protein supplement for catfish (Clarias gariepinus). *Bioresource technology*, *58*(1), 13-16.

Fagbenro, O. A., & Jauncey, K. (1998). Physical and nutritional properties of moist fermented fish silage pellets as a protein supplement for tilapia (Oreochromis niloticus). *Animal feed science and technology*, *71*(1-2), 11-18.

Fagbenro, O., & Jauncey, K. (1995). Growth and protein utilization by juvenile catfish (Clarias gariepinus) fed dry diets containing co-dried lactic-acid-fermented fish-silage and protein feedstuffs. *Bioresource Technology*, *51*(1), 29-35.

Fagbenro, O., Jauncey, K., & Haylor, G. (1994). Nutritive value of diet containing dried lactic acid fermented fish silage and soybean meal for juvenile Oreochromis niloticus and Clarias gariepinus. *Aquatic Living Resources*, *7*(2), 79-85.

Fagbenro, O., Jauncey, K., & Krueger, R. (1997). Nutritive value of dried lactic acid fermented fish silage and soybean meal in dry diets for juvenile catfish, Clarias gariepinus (Burchell, 1822). *Journal of Applied Ichthyology*, *13*(1), 27-30.

FAO 2010-2018. Cultured Aquatic Species Information Programme. Clarias gariepinus. Cultured Aquatic Species Information Programme. Text by Pouomogne, V. In: *FAO Fisheries and Aquaculture Department* [online]. Rome. Updated 1 January 2010. [Cited 28 October 2018].

FAO. (2018). The state of world fisheries and aquaculture: meeting the sustainable development goals. Rome. Licence: CCBY-NC-SA 3.0 IGO.

Ferraz de Arruda, L., Borghesi, R., & Oetterer, M. (2007). Use of fish waste as silage: a review. *Brazilian Archives of Biology and Technology*, *50*(5), 879-886.

Goosen, N. J., de Wet, L. F., & Görgens, J. F. (2014). The effects of protein hydrolysates on the immunity and growth of the abalone Haliotis midae. *Aquaculture*, *428*, 243-248.

Goosen, N. J., De Wet, L. F., & Görgens, J. F. (2018). Effects of formic acid in abalone diets that contain ingredients derived from fish processing by-products. *Aquaculture International*, *26*(3), 857-868.

Goosen, N. J., de Wet, L. F., Görgens, J. F., Jacobs, K., & de Bruyn, A. (2014). Fish silage oil from rainbow trout processing waste as alternative to conventional fish oil in formulated diets for Mozambique tilapia Oreochromis mossambicus. *Animal Feed Science and Technology*, *188*, 74-84.

Jackson, A. & Newton R.W. (2016). Project to model the use of fisheries by-products in the production of marine ingredients, with special reference to the omega 3 fatty acids EPA and DHA. *Institute of aquaculture, University of Stirling and IFFO, the marine ingredients organisation.*

Madage, S. S. K., Medis, W. U. D., & Sultanbawa, Y. (2015). Fish silage as replacement of fishmeal in red tilapia feeds. *Journal of Applied Aquaculture*, *27*(2), 95-106.

Martinez-Porchas, M., & Martinez-Cordova, L. R. (2012). World aquaculture: environmental impacts and troubleshooting alternatives. *The Scientific World Journal*, *2012*.

Naylor, R. L., Hardy, R. W., Bureau, D. P., Chiu, A., Elliott, M., Farrell, A. P., ... & Nichols, P. D. (2009). Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences*, pnas-0905235106.

Pedersen, A. M., Vang, B., & Olsen, R. L. (2014). Oil from Calanus finmarchicus—Composition and Possible Use: A Review. *Journal of Aquatic Food Product Technology*, *23*(6), 633-646.

Raa, J., Gildberg, A., & Olley, J. N. (1982). Fish silage: a review. *Critical Reviews in Food Science & Nutrition*, *16*(4), 383-419.

Ramírez, J. C. R., Ibarra, J. I., Romero, F. A., Ulloa, P. R., Ulloa, J. A., Matsumoto, K. S., ... & Manzano, M. Á. M. (2013). Preparation of biological fish silage and its effect on the performance and meat quality characteristics of quails (Coturnix coturnix japonica). *Brazilian Archives of Biology and Technology*, *56*(6), 1002-1010.

Sahu, B. B., Barik, N. K., Paikaray, A., Agnibesh, A., Mohapatra, S., & Jayasankar, P. (2016). Fish Waste Bio-Refinery Products: Its application in Organic Farming. *International Journal of Environment, Agriculture and Biotechnology*, *1*(4).

SEAFISH. (2018). Fishmeal and fish oil facts and figures. A report compiled by Seafish with thanks to the Marine Ingredients Organisation (IFFO) for permission to use its data.

Soltan, M. A., Fouad, I. M., El-Zyat, A. M., & Zead, M. A. (2017). Possibility of Using Fermented Fish Silage as Feed Ingredient in the Diets of Nile Tilapia, Oreochromis niloticus. *Global veterinaria*, 18(1), 59-67.

Soltan, M. A., Hanafy, M. A., & Wafa, M. I. A. (2008). An evaluation of fermented silage made from fish by-products as a feed ingredient for African catfish (Clarias gariepinus). *Global veterinaria*, *2*(2), 80-86.

Tacon, A. G., & Metian, M. (2013). Fish matters: importance of aquatic foods in human nutrition and global food supply. *Reviews in Fisheries Science*, *21*(1), 22-38.

Tilami, K.S., & Sampels, S. (2018). Nutritional value of fish: lipids, proteins, vitamins, and minerals. *Reviews in Fisheries Science & Aquaculture*, *26*(2), 243-253.

Tilami, S. K., Sampels, S., Zajíc, T., Krejsa, J., Másílko, J., & Mráz, J. (2018). Nutritional value of several commercially important river fish species from the Czech Republic. *PeerJ*, *6*, e5729.

Toppe, J., Olsen, R.L., Peñarubia, O.R. & James, D.G. 2018. Production and utilization of fish silage. A manual on how to turn fish waste into profit and a valuable feed ingredient or fertilizer. Rome, FAO. 28 pp.

United Nations, Department of economic and social affairs, population division. (2017). World population prospects: the 2017 revison, key findings and advance tables. ESA/P/WP/248.

Vidotti, R. M., Viegas, E. M. M., & Carneiro, D. J. (2003). Amino acid composition of processed fish silage using different raw materials. *Animal Feed Science and Technology*, *105*(1-4), 199-204.

Yoshida, H., Takahashi, Y., & Terashima, M. (2003). A simplified reaction model for production of oil, amino acids, and organic acids from fish meat by hydrolysis under sub-critical and supercritical conditions. *Journal of chemical engineering of Japan*, *36*(4), 441-448.

Zhu, L., Zhang, X., Ji, L., Song, X., & Kuang, C. (2007). Changes of lipid content and fatty acid composition of Schizochytrium limacinum in response to different temperatures and salinities. *Process Biochemistry*, *42*(2), 210-214.

Chapter 2: Literature Review

2.1 Lipids: Basic structure and nomenclature

Lipids are a very complex group of organic compounds that are not miscible in water but are miscible in non-polar organic solvents (Petrucchi & Harwood, 1997). The familiar forms are fats, oils and waxes that are known for their roles in energy storage, insulation and water-proofing. They are a very diverse group of compounds and include other important structural and functional molecules, such as phospholipids, cholesterol, steroid hormones and eicosanoids (Bell & Koppe, 2011). There are numerous classes of lipids, but the group most relevant to an understanding of this thesis are fats, oils and fatty acids. Fats and oils are triglycerides (Fig. 2.1), consisting of glycerol and three esters of fatty acids.

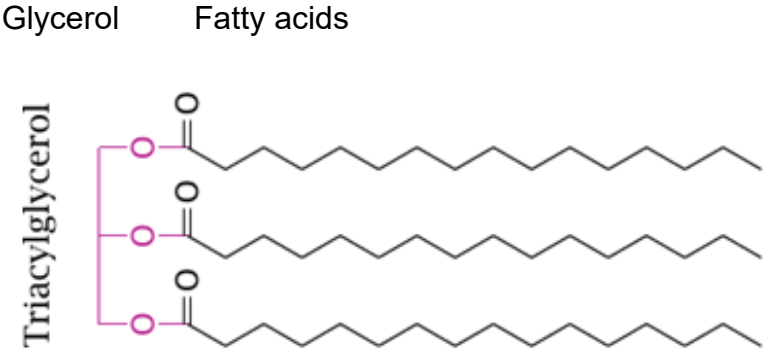


Figure 2.1 The typical structure of fats and oils

Fatty acids (FA) are the main components and basic building blocks of most of the lipid groups. They are organic acids that have a methyl group (CH_3) on one end, a hydrocarbon chain of variable length in the middle (C_xH_y), and a carboxyl (COOH) group at the other end (Bell & Koppe, 2011). With the help of Figure 2.1 and the

text that follows, the structure and names of the most common FAs referred to in this thesis are explained.

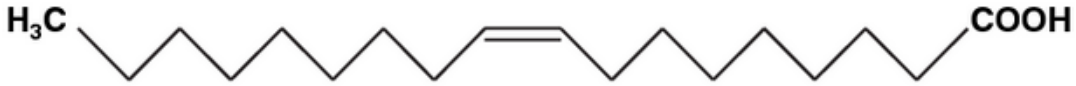
SATURATED FA (SFA)

Stearic acid (18:0)

UNSATURATED FA

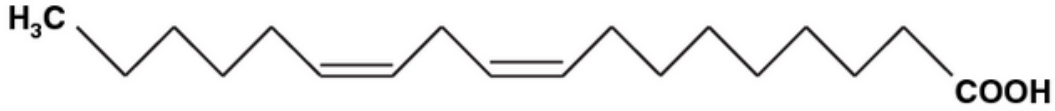
Monounsaturated FA (MUFA)

Oleic acid 18:1n-9

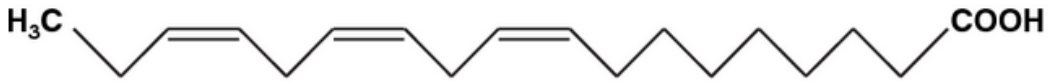


Polyunsaturated FA (PUFA)

Linoleic acid, LA, 18:2n-6



Alpha-linolenic acid, ALA, 18:3n-3



Long-chain polyunsaturated FA (LC-PUFA)

Ecosapentanoic acid, EPA, C20:5n-3;

Docosahexanoic acid, DHA, C22:6n-3

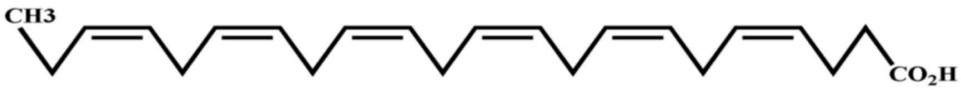


Figure 2.2 Structural representations of various FAs that maintain fish health by their involvement in vital physiological processes. (Source: Bell & Koppe, 2011)

FAs may be saturated, such as stearic acid (18:0), which means that there are only single bonds between adjacent carbon atoms (Figure 2.2). The number 18 denotes the number of carbon atoms in the FA chain, while the number 0, after the colon, denotes the number of double bonds between carbon atoms in the chain. Unsaturated FAs have at least one double bond between adjacent carbon atoms, in which case they are called monounsaturated FAs, such as Oleic acid (18:1n-9). However, unsaturated FAs may also have two double bonds, such as Linoleic acid (LA; 18:2n-6), three double bonds such as Alpha-linolenic acid (ALA; 18:3n-3), five double bonds such as Ecosapentanoic acid (EPA; C20:5n-3) and six double bonds such as Docosahexanoic acid (DHA; C22:6n-3).

All the unsaturated FAs with two or more double bonds are polyunsaturated FAs (PUFAs), which include LA, ALA, EPA and DHA from the list above; while long chain PUFAs (LC-PUFAs) generally contain 20 or more C-atoms and three or more double bonds, represented by EPA and DHA above. The naming of the Omega-3 (ALA 18:3n-3, EPA C20:5n-3, DHA C22:6n-3) and Omega-6 (LA 18:2n-6) fatty acids is based on the relative position of the first carbon to carbon (C=C) double bond. When counting from the methyl (CH_3) end of the FA, Omega-3 FAs have their first double bond starting at the 3rd carbon atom, while Omega-6 FAs have their first double bond starting at the 6th carbon atom (Bell & Koppe, 2011).

2.2 Role of lipids in fish nutrition

Lipids constitute an indispensable ingredient in fish diets. The bulk of dietary lipid is in the form of fatty acids, while others that are not fatty acids are no less important, for example cholesterol and other sterols (Tocher & Glencross, 2015). They provide energy for growth and other vital metabolic activities, are a source of essential fatty acids (EFA) that are necessary for normal growth and development and assist in the absorption of the fat-soluble vitamins A, D, E and K (NRC, 2011). They form a fundamental part of cell membranes (phospholipid bilayer) that are a major structural component of all living organisms, are involved in cell-signalling and direct the physiology and development of organisms in the form of steroid hormones (Sargent, Tocher & Bell, 2002). Lipids provide fuel for respiration, structural support at cellular level, and key molecules involved in the control and regulation of various metabolic activities as diverse as stress responses,

vasoconstriction and dilation, sexual development, oestrus cycle and nerve conduction (De Silva, David & Tacon, 2011).

Essential fatty acids (EFAs) are those omega-3 and omega-6 PUFAs that are required for normal development and good health but cannot be synthesised from simpler molecules and must therefore be obtained in the diet (Bell & Koppe, 2011). According to Sargent et al. (2002), the EFA in the omega-3 series is generally represented by ALA (18:3n-3) while the EFA in the omega-6 series is typically represented by LA (18:2n-6). Marine fish species usually require LA, ALA and the LC-PUFAs EPA and DHA, since they cannot convert the C-18 PUFAs into LC-PUFAs, while freshwater fish usually require only one or more of the C-18 PUFAs, which they use to biosynthesise the LC-PUFAs (Sargent et al., 2002).

FO derived from pelagic marine sources provide the full spectrum of EFAs required by pivotal life stages in the AC industry, such as brood stock, fry and early juveniles (Glencross & Turchini, 2011). EFAs are essential components of phospholipids in all biological membranes and some, such as Arachidonic acid (ARA; 20:4n-6) and EPA, serve as precursors for the production of eicosanoids that have numerous important metabolic functions. Eicosanoids are signalling-molecules embedded in the cell membranes where they control inflammation and other immune responses, regulate the growth of cells, regulate acclimation to ambient temperature changes, control blood pressure and the differential blood flow to body tissues (Monroig, Tocher and Castro, 2018). Signs of EFA deficiency include various skin disorders such as lesions and fin rot, shock syndrome, myocarditis, reduced growth rate, reduced feed efficiency and increased mortality (De Silva et al., 2011). A balanced diet must, therefore, provide sufficient lipids and the necessary variety to perform all the crucial metabolic functions and maintain good health (Tocher & Glencross, 2015).

2.3 Sources of fish oil for aquaculture: Issues of sustainability

Aquaculture is greatly dependent on marine pelagic fisheries for the fish meal and fish oil that provide key dietary nutrients for aquafeed production. In 2006 it utilised 3.7 MMT (68.2%) and 88.5% of the total global production of fish meal and fish oil, respectively, which was derived from 16.6 million metric tons (MMT) of pelagic fish

stocks (Tacon & Metian, 2008). According to Jackson and Newton (2016) currently about 20MMT of fish raw material is reduced to FM and FO, 14 MMT from whole pelagic fish, 3.7MMT from capture fisheries by-product and 1.9Mt from aquaculture by-product. The FAO (2018) puts the total mass of fish raw material reduced for this purpose at 15 MMT. The FO produced amounts to approximately 1 MMT (5% by mass) of this raw material, which according to a World Bank report (World Bank, 2013) is expected to remain unchanged up to 2030. However, Jackson and Newton (2016) assert that globally a further 12 MMT of fisheries and aquaculture by-product could be collected if every fishing region makes an extra effort; this would add another 1.5 MMT of FO to the global supply.

But, the availability of dietary lipids from global capture fisheries, the main source of dietary lipids and FAs, continues to face an uncertain future. Several related factors, such as: a decline in global reduction fisheries, climate change, unpredictable El Nino events, human population growth, increased per capita fish consumption, increased direct human consumption of FO health products and the continued growth of aquaculture combine to put pressure on the finite fish oil resources (Naylor et al., 2009; IFFO, 2016; FAO, 2018). Professor Krishan Rana, of Sterling University, delivering a keynote address at the World Aquaculture Society conference in Cape Town, South Africa, in July 2017 warned that the aquaculture industry may be heading into a FO trap, where the demand for FO would outstrip supply, especially in the aquaculture of carnivorous species, thus leading to the collapse of these aquaculture sectors. The ever-increasing demand for FM and FO by world aquaculture against the finite supply by capture fisheries is clearly not sustainable and demands alternative sources (Huntington & Hasan, 2009). Jackson (2010) argues that, while the volumes of pelagic forage fish extracted for use in aquafeed may appear alarming, these pelagic fisheries are suited to such high-level annual exploitation because of the high turnover and short life cycle of its species. However, this is dependent on these fisheries being managed in a sustainable manner; but recent statistics reveal that about 33% of capture fisheries are being managed unsustainably (FAO, 2018).

Addressing the social sustainability and ethical aspects of this debate, Naylor et al. (2009) contend that even when these capture fisheries are managed

sustainably, the proportion used for aquafeeds is too high when considering the nutrient needs of the poor. They point out that the bulk of this capture goes to aquafeeds for high value carnivorous species, such as salmon and trout, and ironically, these species are less nutrient rich than the pelagic fish from which they derive their FM and FO. Furthermore, since they are so expensive, they are out of reach of the majority of poor, from who's very coastal waters the pelagic fish are extracted that enable their production.

On the basis of increasing costs, Tacon and Metian (2008) predicted a decrease in the inclusion levels of FO in compound aquafeeds. They stated that the limited supply of FO would be extended by using it more sparingly in costly speciality aquafeeds for critical life stages during production, such as broodstock, starter feeds and finishes. They also suggested that in order to sustain the growth rate of aquaculture, the production and supply of FM and FO would have to increase at similar rates to meet the demand from aquaculture. However, while aquaculture has continued to grow (IFFO, 2017; FAO, 2018), the quantity of FM and FO it has used remained relatively stable over the past 20 years (Jackson 2010; FAO 2018). This situation has been achieved by an increased use of alternate feed oils and a greater utilisation of fisheries and aquaculture by-products for fish oil production (Turchini, Ng, & Tocher, 2011).

There is a clear declining trend in the annual production of FO as more food fish is used for direct human consumption and less for non-food products (SEAFISH, 2018). Currently aquaculture still uses the bulk (75%) of the global FO produced, with 18% used in human health products (SEAFISH, 2018). More than half (60%) of the FO used by global aquaculture is used to raise Salmonids (IFFO, 2017). However, there is also a declining inclusion of FO in feeds for Salmonids, with vegetable oil making up 66% and FO 33% of the feed oil content in 2013 (SEAFISH, 2018). Furthermore, there has been significant reductions in the fish in: fish out (FIFO) ratios, standing at 0.22 for aquaculture as a whole in 2015, and the forage fish dependency ratio (FFDR fish oil) among major aquafeed suppliers, at 0.93 for BioMar, 1.7 for Skretting and 1.97 for EWOS (SEAFISH, 2018).

2.4 Alternatives to fish oil for aquafeeds

Finding alternative feed oils may reduce both the pressure on pelagic marine fisheries and the price of aquafeeds. A variety of sources that include algae, plants, terrestrial and aquatic animals and their by-products, have been utilised as alternatives to FO in providing feed oil for fish diets. However, terrestrial animal lipids are high in saturated FAs and very low in EFAs (n-3 and n-6), while seed oils only contain a high percentage of n-6 unsaturated FAs; the best, cost effective source of long chain highly unsaturated FAs remain fish oils (Tocher, 2015).

To satisfy their EFA requirements all fish species need some C18 PUFAs in their diet while others also need additional LC-PUFAs. Both of these types of EFAs are abundantly present in fish oil of marine origin and its inclusion in formulated fish diets is sufficient to supply the EFA needs of any cultured species (Tocher, 2015). The globally expanding middle class is demanding fish meat that contains high concentrations of n-3 PUFAs, especially EPA and DHA (Tocher, 2015) that have been shown to promote cardiovascular and neural health and reduce cancers and inflammatory diseases (Gil & Gil, 2015). Simopoulos (2011) contends that the typical modern diet is already greatly imbalanced towards a very high intake of n-6 PUFAs and agrees that there is a need for an increased intake of n-3 LC-PUFAs, which is abundant in oily fish.

Unless the alternative oils are able to supply sufficient omega-3 PUFAs to maintain optimum fish health and growth, as well as provide sufficient omega-3 PUFAs in the fish flesh for optimum consumer health, aquaculture products will face increasing scrutiny. So, while we may be able to recover large quantities of fish oil by processing fish by-product waste, from both fisheries and aquaculture, and by utilising more of the by-catch, the omega-3 content may not be sufficient to enhance the fish meat to the levels desired for human health. Fish oils recovered in this way, especially from freshwater species may have to be blended with oils rich in n-3 PUFAs in order to raise its n-3 PUFA levels. Various vegetable oils containing high concentrations of n-3 PUFAs have been successfully included in aquafeeds to raise the levels of n-3 in fish flesh. Terrestrial oilseed plants *Camelina sativa* (Camelina), were genetically modified to produce oil that was rich in the LC-PUFAs EPA and DHA, which was evaluated in a feeding trial on Atlantic salmon

(*Salmo salar*); the transgenic Camelina oil performed no differently to FO on growth and health parameters, but raised the n-3 PUFAs in the fish flesh by almost double the amount of the FO (Betancor et al., 2017).

In order to operate sustainably aquafeed companies have to seek both alternative ingredients as well as a thorough understanding of the specific FA requirements and the biochemical pathways by which the major cultured species metabolise LC-PUFAs (Turchini et al., 2011). Following a reduction in the use of FO in aquafeeds, research continues to seek ways to enhance omega-3 LC-PUFA concentrations in the flesh of farmed fish. These include the use of precisely formulated diets (Randall et al., 2013), genetic modification (Kabeya et al., 2014) and artificial selection for heritable traits that increase the concentration of LC-PUFAs in the flesh of farmed fish (Gjedrem, 2000). However, the gain in flesh LC-PUFAs with each of these methods has been so slight that Tocher and Glencross (2015) concludes that the only effective answer is probably to be found in enhancing the dietary concentration of LC-PUFAs of farmed fish.

The reason that marine seafood is the richest source of n-3 LC-PUFAs is because of the bioaccumulation of these FAs in the tissues all along the marine food chain (Bell & Tocher, 2009). As a result of marine phytoplankton and zooplankton storing lipids in the form of LC-PUFAs, such as EPA and DHA, pelagic fish that feed on them store fish oils that are the best source of LC-PUFAs for aquafeeds. But, since these fish yields are highly variable (Naylor et al., 2009) and in many cases unsustainably fished (FAO, 2018), it makes sense to investigate opportunities further down the marine food chain. Marine zooplankton, notably krill and copepods, that store oils with a high LC-PUFA content (Olsen, Andersen, Gismervik & Vadstein, 2011) and have a short life-cycle and rapid turnover, could provide a regular and abundant harvest. However, these zooplankton form the base of a complex food web that supports pelagic fisheries, as well as numerous apex predators, such as whales and sharks, and great care must be taken to harvest them sustainably (Branch and Branch, 1982). Since the phytoplankton and zooplankton from the photic zone sink down into the abyssal regions to form the base of the food chains there, the largely untapped biomass of fish in the aphotic

bathypelagic zone, that store large quantities of lipids rich in n-3 LC-PUFA, (Olsen, Waagbø, Melle, Ringø & Lall, 2011) could also be targeted.

Because photosynthetic marine microalgae require only sunlight energy and inorganic nutrients, to produce oils rich in EPA and DHA, they represent one of the better potential sources of marine oil. However, the photobioreactor technology to produce these microalgae cheaply in large quantities still needs to be developed (Perez-Garcia, Escalante, de-Bashan & Bashan, 2011). But, a select group of heterotrophic microalgae and related microorganisms hold great promise for the future sustainable supply of oils rich in omega-3 FAs to the aquaculture industry (Chi et al., 2007). They are cultured aerobically, in the dark in large vats, where they are supplied with carbon-rich nutrients such as glucose, glycerol or acetate (Zhu et al., 2007). One example, *Schizochytrium sp*., is relatively easily cultured and produces oil that is rich in DHA and is set to be genetically modified to produce EPA as well (Sprague, Betancor & Tocher, 2017). Already oil, rich in Omega-3 PUFAs, produced in this way is included in select diets by various top aquafeed companies and it has been shown to maintain growth, while increasing flesh n-3 LC-PUFA concentrations (Miller, Nichols & Carter, 2008; Glencross and Rutherford, 2011). However, even though this technology is in use by various companies, it is still an expensive process and the oils supply niche markets that are prepared to pay high prices for nutraceuticals and feeds for critical life stages of high value fish species.

Recent research has focused on terrestrial oilseed crop plants that have been trans-genetically modified by insertion of algal genes coding for n-3 LC PUFAs (Ruiz-Lopez, Haslam, Napier & Sayanova, 2014). One example is *C. sativa* that was genetically modified to synthesize oils rich in EPA and DHA. This is perhaps the most viable alternative technology since the inputs are minimal, and the existing infrastructure for extracting, processing and packaging seed oils are well established and widespread (Betancor et al., 2017).

While all of the alternatives to FO explored above require effort in directions that point away from fisheries and aquaculture, one of the most easily accessible alternatives is probably to utilise fish by-products more effectively. In a recent publication, Jackson and Newton (2016) propose a model that predicts the

potential for increasing the volume of fisheries and aquaculture by-product, for reduction to fish oil rich in EPA and DHA. At that time, they predicted that an annual 20 MMT of raw material (14 MMT from whole fish and 6 MMT from by-products) was reduced to FM and FO. They further predicted that in ten years' time, the volume of by-product for reduction to FM and FO would exceed the volume of whole pelagic fish; with half of the by-product being from aquaculture and the other half from wild caught fish. Therefore, they predicted that by 2026 the production of FO would increase by 5-10%, and the production of EPA and DHA remain unchanged or decrease slightly as the result of there being fewer oily marine pelagic fish in the raw material, and because of the lower omega-3 FO content, especially of the freshwater aquaculture species. Lastly, they concluded that there was about 12 MMT of fish by-products, available but not used for reduction into fish oil, and that this situation was likely to remain over the next 10 years. The reasons behind this include the cost of collection from remote processing plants, the small and irregular volumes supplied, the capital investment needed to set up a reduction plant and the cost involved in treating the effluents and emissions produced (Jackson & Newton, 2016). These negatives make a strong positive case for the reduction of such by-product waste by ensiling; a technology which is much less costly to set up, is suited to handling smaller volumes, has minimum emissions and the effluents, namely the fish protein hydrolysate and fish oil, are its commercial products (Ferraz de Arruda, Borghesi & Oeterrer, 2007).

2.5 Fish silage

Ensiling presents an opportunity to utilise fish waste and convert it into a well preserved, stable product that has a composition and nutritional profile similar to the raw materials from which it was made (Ferraz de Arruda et al., 2007). This product is fish silage (FS), which refers to a liquid mixture of whole fish or fish processing by-products that have been preserved by the addition of acid or by anaerobic bacterial fermentation. The general procedure for producing fish silage is to homogenise the fish waste by mincing or chopping. This serves to increase the surface area of the fish tissue in contact with the chemical or biological reagents, which accelerate the ensiling process. Once all the reagents have been added to the fish, everything must be thoroughly mixed together to prevent the

development of any pockets of spoilage microorganisms. To prevent the oil in the silage from becoming rancid, and reduce its nutritional value as a feed, an antioxidant is added (Toppe, Olsen, Peñarubia & James, 2018).

The two most common fish silages are acid silage and fermented silage.

2.5.1 Acid silage

An acid silage is made by adding either inorganic acid or organic acids to macerated whole fish or any part of the fish. Inorganic acids used are sulphuric acid, hydrochloric acid or phosphoric acid, while the organic acids formic acid or propionic acid is commonly used. The acid lowers the pH of the mixture to the point where spoilage by micro-biota is prevented. Proteolytic digestive enzymes that occur naturally in the fish tissue and viscera are activated by the low pH and break down the fish tissues into a liquid mass. This 'self-digestion' process is referred to as autolytic hydrolysis or autolysis (Raa & Gildberg, 1982). The rate of autolytic decomposition of the tissues is largely dependent on the pH and temperature of the silage, with the optimum pH and temperature ranges being between pH 2 to 4 and 20 °C to 40 °C, respectively (Hertrampf & Piedad-Pascual, 2000; Toppe et al., 2018).

Organic acid silages are preferred over inorganic acid silages for several reasons: Organic acids prevent microbial spoilage at a higher pH (4 to 4.5) than inorganic acids (pH 2), which means it can be presented as feed without the need to add a base. With inorganic acid silages 20-50 kg of calcium carbonate may have to be added per ton of silage to raise the pH before it can be served as feed; but this produces large quantities of salt that renders the silage less palatable as a feed (Arason, 1994). Furthermore, the weak organic acids propionic acid and formic acid have antimicrobial effects at pH 4.5 and pH 3.5, respectively, with propionic acid being very effective at preventing the growth of fungi in fish silage; this includes the highly toxic aflatoxin-producing fungus, *Aspergillus flavus*. The organic acids are soluble in lipids and may produce silage oils that are more acidic than when inorganic acids are used. However, when these organic acids are included in aquafeeds they could potentially promote growth and boost immunity (De Wet, 2005; Goosen, Görgens, De Wet & Chenia, 2011).

2.5.2 Fermented silage

Fermented fish silage is made when digestible sugars and fermenting microorganisms such as *Lactobacillus spp.* are added, under anaerobic conditions, to the minced fish (Fagbenro, 1994). The lactobacilli covert the sugars into lactic acid, which preserves the fish and creates a favourable pH (~4.5; Hertrampf et al., 2000) for silage formation by autolysis of the organic molecules in the silage. Some lactobacilli produce antibiotics, which further enhances their preservation effect (Schroder, Clausen, Sandberg & Raa, 1980; Van Wyk & Heydenrych, 1985), also helping to prevent oxidation of fats (Raa and Gildberg, 1982).

Furthermore, antibiotics administered therapeutically in the diet are also known for their effects as growth promoters in fish (Sanchez-Martinez, Pérez-Castañeda, Rábago-Castro, Aguirre-Guzmán & Vázquez-Sauceda, 2008). Fermented silage can be less expensive than organic acid silage, especially if locally available agricultural waste such as molasses and whey are used in its production. Various researchers have also used plain yoghurt as the source of *Lactobacillus sp.* (Soltan, Hanafy & Wafa, 2008) and the cost could be further reduced if a lactobacillus source culture is kept going in the form of natural yoghurt, from which other cultures could be started.

2.5.3 Silage quality

It is commonly reported that the nutritional value of silage diminishes over time in storage as a result of the amino acid tryptophan breaking down in the acid pH; but, except for tryptophan, the other amino acids are stable in acid silages (Ferraz de Arruda et al., 2007; Raa & Gildberg, 1982; Toppe et al., 2018). The lipid component of the silage is also prone to becoming rancid resulting mainly from the oxidation of polyunsaturated FAs, which leads to the formation of fatty acid peroxides. However, numerous studies have shown that fish silage can remain stable over many months provided that certain conditions are met. It is important to exclude air, since aerobic conditions promotes oxidation of lipids as well as the growth of pathogens and food-spoiling microbes such as *Clostridium* species and yeasts. The temperature should not exceed 40 ºC for extended periods; and the pH should remain below 4.5 to prevent putrefaction and the formation of biogenic amines

(Espe & Lied, 1999; Fagbenro, 1994). The high levels of unsaturated FAs readily react with oxygen to form hydroperoxides. The addition of an antioxidant will reduce the rate of these oxidation reactions and prevent the oil from turning rancid. Free fatty acids (FFAs) lower than or equal to 3 % in oil signifies good quality oil suitable for refining for various edible purposes (Tatterson, 1982). Windsor and Barlow (1981) state that to protect the oil quality, it is best to separate it from the silage as soon as possible after its production. Lastly, the quality of the silage also depends on the freshness and quality of the raw material and toxins already present are unlikely to be destroyed during the ensiling process (Raa & Gildberg, 1982).

2.6 Feeding trials: Silage on fish growth

Numerous feeding trials have been executed (Table 2.1-Table 2.3) in which various silage-based diets were evaluated against the growth and feed conversion of farmed fish species. In these feeding trials the most common acid fish silage was made by adding 85 % concentrated formic acid at 2.5 – 4 % (w/w) inclusion to the macerated fish tissue (Goosen et al., 2014; Goosen, de Wet & Gorgens, 2016; Haider et al., 2016; Madage et al, 2015), while the most common fermented fish silage was made by adding sugar molasses at 5 – 15% (w/w) and a *Lactobacillus plantarum* inoculum at 2 % (w/w) inclusion to the ground fish tissue (Fagbenro et al., 1994; Fagbenro & Jauncey, 1995; Fagbenro, Jauncey & Kreuger, 1997). Sometimes the Lactobacillus species are provided at 5 % (w/w) in the form of yoghurt (Soltan et al 2008; Soltan, Fouad, El-Zyat & Zead, 2017).

Since fish silage is a runny mixture and the cost of drying it for inclusion in aquafeeds is very high (Ferraz de Arruda et al., 2007) it is usually mixed with other feed ingredients, such as soya bean meal, to produce a drier, more manageable paste (Fagbenro, Jauncey & Haylor, 1994; Fagbenro & Jauncey, 1995). In feeding trials with fish silage this mixture is mainly co-dried and mixed with other conventional ingredients to replace various percentages of fishmeal. In many of the feeding trials (Fagbenro et al., 1997; Madage et al., 2015; Soltan et al., 2017) fish silage included in this way has been shown to supplement up to 50 % of the dietary protein (Table 2.2) in aquafeeds without a significant effect on growth, feed conversion, meat quality or health of the cultured species.

Table 2.1 Silage feeding trials with freshwater fish, mainly Tilapia species. Whole silage included with dietary meals commonly found in commercial fish diets

Species fed	Waste & Silage	Inclusion levels & Results	Reference
Mirror carp *Cyprinus carpio carpio*	Minced whiting; 3 % formic acid (FA)	Fish silage (FS) lower specific growth rate (SGR)* & higher feed conversion ratio (FCR) than cooked fish	Wood et al., 1985
Nile tilapia *Oreochromis niloticus*	Whole minced tilapia; 2 % *Lactobacillus spp.* (LB) & 5% Molasses	FS:soyabean meal (SBM), 75% inclusion; No difference on FCR, protein efficiency ratio (PER), SGR	Fagbenro et al., 1994
Nile tilapia *O. niloticus*	Whole minced tilapia; 5 % LB & 15 % Molasses	Suitable as on-farm feed (no feeding trial)	Fagbenro & Jauncey, 1988
Nile tilapia *O. niloticus*	Whole & waste tilapia; 3% 1:1 FA:propionic acid 1.4 % LB & 18 % Molasses Exogenous enzymes	FS:SBM up to 30 % in acid and fermented silage	Borghesi et al., 2008
Red tilapia *O. aureas x O. mossambicus*	Tilapia by-products; 4 % formic acid	Up to 50 % inclusion No difference in weight gain, SGR, FCR, PER	Madage et al., 2015
Rohu *Labeo rohita*	Various fish minced; 3 % Formic acid	75 % FS inclusion better than PBM on SGR, FCR, WG*	Haider et al., 2016
Nile tilapia *O. niloticus*	Various fish by-products; 5 % Yoghurt & 5 % Molasses	Up to 50 % inclusion; no difference in growth & feed utilisation; Feeding cost reduced by 16 %	Soltan et al., 2017
Mozambique tilapia *Oreochromis mossambicus*	Farmed rainbow trout viscera; 2.5 % Formic acid	Low silage (16 %) improved cellular non-specific immunity; high silage (29 %) decrease in growth*; Formic acid had no effect on growth	Goosen, de Wet & Gorgens, 2016

Table 2.2 Silage feeding trials with African catfish. Whole fish silage included with various meals commonly used in commercial fish diets

Species fed	Waste & Silage	Inclusion levels & Results	Reference
African catfish fry *C. gariepinus*	Minced herring 3% Formic acid	41 % fish silage (FS) best feed conversion ratio (FCR)	Ayinla & Akande, 1988
Nile tilapia; African catfish	Minced whole tilapia 2 % Lactobacillus spp. (LB) & 5 % Molasses	FS:SBM up to 75 % replace protein; No difference in FCR, PER, ADG, SGR	Fagbenro et al., 1994
African catfish	Minced whole tilapia 2 % LB & 5 % Molasses	FS:SBM & FS:PBM replaced up to 50 % dietary protein No effect on FE, growth or health	Fagbenro & Jauncey, 1995
African catfish	Defatted poultry viscera 5 % Citric acid & propionic acid (PA)	85:15 poultry silage:SBM; Up to 30 % replacement of poultry blood meal; No effect on growth, feed utilisation or flesh quality	Fagbenro & Fasakin, 1996
African catfish juveniles	Whole minced tilapia 5 % LB & 15 % Molasses	FS stored 15 days best growth response mean weight gain (MWG), ADG, SGR; Compared to minced fish (control) & silage stored 30 days	Fagbenro & Jauncey, 1994
African catfish	Minced stunted tilapia 2 % LB & 5 % Molasses	1: 1, FS:SBM (No fishmeal); Reduced growth rate, FCR, PER, lower flesh protein, lower haematocrit, but health not compromised	Fagbenro et al., 1997
African catfish	Fish by-products 5 % Yoghurt (LB) & 5 % Molasses & 30% orange-peel filler	Up to 50 % replace FM; No loss in growth performance (weight gain (WG), SGR) and feed utilisation (FCR, PER); Reduced feed costs /kg by 20.8 % and cost/kg WG by 25 %	Soltan et al., 2008

SBM – soyabean meal; PER – protein efficiency ratio; ADG – average daily growth; SGR – specific growth rate; PBM – poultry blood meal

Table 2.3 Silage feeding trials in which only the silage oil was used in fish diets

Species fed	Waste & Silage	Inclusion levels & Results	Reference
Mozambique tilapia *Oreochromis mossambicus*	Farmed rainbow trout viscera; 2.5 % Formic acid	Silage oil (SO) substituted fish oil with no negative effect on production; Improved cellular non-specific immunity; Shortening* of intestinal folds	Goosen, et al., 2014
SA abalone *Haliotis midae*	Farmed rainbow trout viscera; 2.5 % Formic acid	SO 25 g/kg inclusion in formulated diet; Negative production performance on specific growth rate, weight gain & feed conversion ratio; improved cellular immune function	Goosen, de Wet and Gorgens, 2014
	Farmed rainbow trout viscera; 2.5 % Formic acid	No difference on final weight with inclusion of SO or SO+Formic Acid; but DWG* differs from reference diet; Formic Acid inclusion with raw viscera mitigates production performance	Goosen, de Wet and Gorgens, 2018

*The asterisk denotes significant differences among means; DWG – daily weight gain

There are only a few feeding trials where fish silage oil only, as opposed to the whole fish silage, was evaluated on the growth and feed performance of aquaculture species (Table 2.3). It was shown by Goosen et al. (2014) that the inclusion of rainbow trout viscera silage oil, in formulated diets, improved the non-specific cellular immunity in Mozambique tilapia, as well as in South African abalone (Goosen, de Wet & Gorgens, 2014). Furthermore, the inclusion of silage oil produced no negative effect on production parameters in Mozambique tilapia, but for South African abalone it performed negatively in SGR, WG and FCR (Goosen et al., 2014) as well as significantly lower in DWG when compared to a reference diet (Goosen, de Wet & Gorgens, 2018).

2.7 Role of probiotic *Lactobacilli spp.* and organic acid residues on fish growth & health

Since the organic acids used to prepare the acid silages are soluble in lipids the resultant silage oils should contain residues of these organic acids. Similarly, the silage oil from a fermented silage is likely to contain residues of Lactobacilli and other species present in the inoculum.

2.7.1 Probiotic *Lactobacilli spp.*

In the wake of the ban on the use of antibiotic growth promoters by the EU in 2006, there was renewed interest in the use of alternative growth promoting factors such as probiotics and organic acids in aquaculture (Hoseinifar, Sun & Caipang, 2017). Probiotics are microorganisms, such as *Lactobacilli sp.* and yeasts, which generally have a beneficial impact on cultured species when included in diets or in the water. In the gut they establish fast growing populations that lower the number of pathogenic bacteria by competitive exclusion (Zorriehzahra et al., 2016). Some species produce nutrients and enzymes that aid digestion (Balcazar et al., 2006) thus enhancing growth. Certain *Lactobacilli sp*. strains are known for enhancing the non-specific immune response of the host (Mohapatra et al., 2013) while also waging chemical warfare by producing substances such as antibiotics, hydrogen peroxide and bacteriocins that inactivate pathogenic bacteria, viruses and fungi. The addition of gram-positive bacteria to the system water has also shown improvements in water quality (Qi et al., 2009). Al-Dohail et al. (2009) showed that by including *Lactobacillus acidophilus* at $3x10^7$ cfu/g diet in a regular catfish diet, it led to significantly better growth performance (SGR), feed utilisation (FCR, PER) and survival. Furthermore, numerous haematological parameters were also significantly better. In *Labeo rohita* (Rohu) dietary inclusion of the bacterium, *Sendomonas aerugimosa* (10^7 – 10^9 cfu/g) led to significantly higher survival rates after being challenged with an *Aeromonas hydrophila* infection, indicating its role in improving disease resistance and immunity (Giri, Sukumaran & Oviya, 2013).

2.7.2 Organic acids

The dietary inclusion of organic acids such as lactic acid, formic acid, butyric acid and propionic acid, are known for their positive influence on fish health and

performance (Luckstadt, 2008). The pure organic acids are corrosive and more expensive and may be substituted by their salts, such as potassium diformate, which are not always equally effective (De Wet, 2005; Luckstadt, 2008). Vasquez, González and Murado (2005) showed through a series of *in vitro* trials that the presence of lactic acid bacteria cultures inhibited the growth of pathogenic bacteria from fish intestines mainly by their secretions of organic acids. Organic acids counter soya-induced hindgut enteritis by promoting cell generation in the intestine mucosa and villi growth (De Wet, 2005). They have bacteriocidic properties since, being non-polar acids, they are able to cross the polar cell membranes of pathogens where they disassociate at the appropriate pKa, leading to an accumulation of H+ ions and the eventual death of the pathogen by metabolic energy depletion (De Silva et al., 2011). They lower the pH in the intestinal tract thus promoting proteolytic enzyme activity and increasing digestion and absorption of nutrients (Luckstadt, 2008).

In a feeding trial with rainbow trout fingerlings comparing the effect of dietary organic acids against antibiotic growth promoters, De Wet (2005) found that with a 1,5 % blend of sorbic acid and formic acid growth performance increased with the increasing dietary inclusion levels (0.5-1.5 %). At 1.0 and 1.5 % inclusion the SGR was significantly better than the control (no antibiotics or organic acids) and the FCR lower than the AB treatment group. Similarly, work on Atlantic salmon (*Salmo salar*) demonstrated that a dietary inclusion of 1.4% potassium diformate, improved growth, feed efficiency and uniformity in size (Christiansen and Luckstadt, 2008). Omosowone, Dada & Adeparusi (2015) evaluated various dietary levels of fumaric acid in African catfish, finding that 1 $g.kg^{-1}$ diet produced better growth and feed utilisation than other inclusion levels. On the other hand, Goosen, de Wet and Gorgens (2016) found that inclusion of dietary formic acid had no effect on the growth of Mozambique tilapia, while Goosen, de Wet and Gorgens (2018) found that formic acid included with silage oil in South African abalone commercial diet had no effect on final weight, but mitigated production performance when included with a raw visceral meal.

2.8 The importance of African catfish

2.8.1 African catfish as an aquaculture species

Clarias gariepinus originated in North Africa (Pillay & Kutty, 2005) and has been widely relocated for aquaculture (FAO, 2010) in Africa (23 countries), Europe (4 countries), Asia (10 countries) and South America (1 country). Here it is raised in virtually all formats of production systems, on small and large scales, in monoculture or polyculture, extensively or intensively in earthen ponds, raceways, cages and recirculating aquaculture systems. In 2007, Catfish production was 47 428 tons in 2009 with 79 % of that share being in Nigeria and 9 % in the Netherlands, while in 2015 Nigerian production had increased to about 246 000 tons (Dauda, Natrah, Karim , Kamarudin & Bichi, 2018).

The African catfish is a good choice for aquaculture in areas where access to sufficient and nutritious food is limited. Its flesh provides sufficient protein and lipids to satisfy the dietary requirements for essential amino acids and energy (Osibona, Kusemiju & Akande, 2009) and would therefore prevent deficiency diseases such as kwashiorkor and marasmus. It also contains the EFAs EPA and DHA (Chauke et al., 2008; Osibona et al., 2009) that have been linked to a reduction in heart disease, diabetes, cancer and total mortality (Watters, Edmonds, Rosner, Sloss & Leung, 2012). A further benefit is that the ratio of n-6:n-3 FAs for both wild (1:0.9 & 1:3) and cultured (1:1) catfish fall within the ratio that is considered healthy for the human diet (Russo, 2009). Chauke et al. (2008), therefore suggest that food-insecure communities that have access to African catfish as a dietary supplement should be encouraged to catch it from local rivers and dams or culture it in ponds, so that it could become a regular item in the diet.

The African catfish is suitable for smallholder fish farmers, who constitute the greatest number of fish farmers in developing regions (Musa, Aura, Ngugi & Kundu, 2012), because it can be cultured at high stocking densities (Nyina-Wamwiza, Wathelet & Kestemont, 2007) and has a high growth rate (Van Weerd, 1995), which together lead to a good yield. Catfish are omnivores, naturally feeding on a wide range of plant and animal food items (Van Weerd, 1995). Spawning in captivity is relatively easily achieved, which ensures a perennial supply of seed (Okechi, 2004; Musa et al., 2012). African catfish are successfully used in polyculture with tilapia to control the number of tilapia fingerlings, which ensures a harvest of both larger tilapias and catfish (De Graaf & Janssen, 1996). Catfish

sustain large natural populations across diverse habitats and climate zones, from temperate to tropical regions (Nyina-Wamwiza et al., 2007). They are resilient against adverse water quality, tolerating low oxygen, high ammonia and nitrite levels (De Graaf & Jansen, 1996) and disease (Nyina-Wamwiza et al., 2007). Furthermore, a profitability assessment by Okechi (2004) concluded that the culture of African catfish is financially viable even on a relatively small scale.

2.8.2 Catfish water quality requirements

Table 2.4 Recommended water quality for culture of African catfish

Parameter	Requirement
Temperature (°C)	26 - 33
pH	6.5 - 8
DO (mg/L)	>3.0
NH_3 (mg/L)	<2.5 (pH=7)
CO_2 (mg/L)	<15
NH_4^+ (ppm)	<8.8 (pH=7)
NO_3^- (ppm)	<250
NO_2^- (ppm)	<0.25
salinity (ppt)	0 - 2.5

Source: Fagbenro, 1996

2.8.3 Catfish nutrition requirements

African catfish (*C. gariepinus*) are naturally euryphagous, feeding on a wide variety of plants and animals (Musa et al., 2012) present in the lotic and lentic ecosystems that they inhabit. Subsequently, numerous ingredients of plant and animal origin could be used in feeds for this species. Numerous potential foodstuffs that have been tested as feed ingredients include algae, plants, plant meals and plant by-products, animals, animal meals and animal by-products and chemically processed products such as fish silage (Fagbreno, Adeparusi & Fapohunda, 2003). Furthermore, African catfish accept aqua feeds in a variety of forms, which include processed extruded feeds, as well as on-farm mixes of dry and moist, floating or sinking feed. Its propensity for such a diverse selection of foodstuffs

makes *C. gariepinus* a suitable candidate species for trials on alternative dietary lipid sources.

However, determining the nutrient requirements of the catfish is a complex matter because many different variables need to be considered. These include, among others, the origin or source of the ingredients and the way they were processed, for example if heat was applied or chemicals used to extract nutrients; the presence of anti-nutritional factors, how the ingredients were stored and for how long. Further criteria, and possibly more important, are a knowledge of the concentration of nutrients in the ingredients, their digestibility by the fish and the biological availability of the nutrients once digested or even after being absorbed into the body tissues. Professor Dominique Bureau (NRC, 2011) makes the point that crude protein or crude lipid values do not hold much meaning if the quantities and availability of the essential AAs and EFAs are not known as well. He also cautions that the nutrient requirements stated are often mere “guestimates” because they are taken from a specific life stage, sex and weight class of a fish, under a very specific set of experimental conditions. While in the biological organism the nutrient requirements will differ across all of these variables, which only represent a subset of all possible sources of variance (NRC, 2011). He further argues that in an effort to increase our accuracy it is important that feeding experiments are standardised and contain minimum requirements for experimental design, procedures and data collection that will ensure the validity and reliability of the trial, allowing it to add to the international body of knowledge. In addition to all the constraints already mentioned, it is also paramount that aquafeed ingredients are sourced and used cost-effectively, with minimum damage to the environment and with maximum social gain.

Since we used a commercial feed pellet as our basal diet, that was formulated to meet the basic nutritional requirements of juvenile catfish in this feeding trial, I will briefly tabulate (Table 2.5) the dietary recommendations for *C. gariepinus* based on the work of numerous researchers and summarised in Hecht (2013).

Table 2.5 Nutrient requirements and dietary recommendations of the African catfish

Dietary component	Recommended quantity
Crude protein (% min)	40 - 43
Least cost / appetite feeding protein (%)	35 - 38
Crude lipid (% min)	10 - 12
Carbohydrate (%)	15-35
Digestible energy (min, kJ/g)	14 - 16
Metabolizable energy (min, kJ/g)	13
Gross energy (min, kJ/g)	22 - 24
Protein to energy ratio (mg/kJ)	22 - 30
Lipid to carbohydrate ratio (g/g)	2.47

Furthermore, for optimal growth, Uys (1988) recommended that 10 % of the total dietary lipid should be fish oil which is generally accepted for *C. gariepinus* together with a ratio of n3:n6 FA of 1:1. While Ng, Wang, Kstchimenin and Yuen (2003 & 2004) found that African catfish needed some plant-based lipids in their diet, since a purely fish oil-based diet had a negative effect on growth.

2.8.4 Catfish lipid requirements and the ability to convert certain fatty acids to others

Warm water fish such as the African catfish need lipids for energy provision but are also able to utilise a greater quantity of carbohydrate to supply its energy needs than cold water fish (NRC, 2011). The percentage of dietary lipid also depends on the protein and energy content of the feed, as too much lipid may lead to an imbalance in the digestible energy to crude protein (DE:CP) ratio. This could result

in the excessive deposition of fat in the body, which would have a negative impact on the yield, quality and shelf life of the flesh (Ali & Jauncey, 2005).

As in other vertebrates, catfish cannot synthesis the omega-6 fatty acid linoleic acid, (LA, C18:2n-6), or the omega-3 fatty acid alpha-linolenic acid (ALA, C18:3n-3) from scratch, meaning that one or both of these must be supplied in the diet (Monroig et al., 2018). In common with other freshwater fish they need either LA and / or ALA and other essential fatty acids, from which they are able to synthesise the additional fatty acids that they require. For example, channel catfish require 1 to 2 % LA plus 0.5 to 0.75% EPA and / or DHA; common carp require 1 % LA plus 1 % ALA; Nile tilapia require only 0.5 % LA and African catfish require both LA and ALA (Sargent et al., 2002).

It is clear from the list above that certain freshwater fish species only require the C18 FAs LA and / or ALA in the diet, even though all fish need LC-PUFAs to maintain normal metabolic activities and good health (Bell and Koppe, 2010). These fish must therefore possess the genes and resultant enzymes to metabolically convert omega-3 and-6 C18 FAs into longer chain EFAs (Oboh, Betancor, Tocher & Monroig, 2016). Researchers (Agabah et al., 2005; Oboh et al., 2016) confirmed that the African catfish contained the genes that would allow it to convert dietary ALA and LA into various LC-PUFAs endogenously. The elongase enzyme, Elovl5, increases the length of the C-chain in molecules at various places in this metabolic pathway, while the desaturation enzyme, Fads 2 increases the number of double bonds along this metabolic pathway; so that for example, ALA (C18:3n-3) could be converted either into EPA (C20:5n-3) and / or DHA (C22:6n-3).

The main advantage to aquaculture of farming fish species such as the African catfish that have the capacity to endogenously convert C18 precursors into LC-PUFAs, is a decrease in the dependence on including dietary FO in aquafeeds. FO derived from unsustainable marine pelagic sources could be substituted by oils rich in LA and ALA from more sustainable, even plant based, sources. The importance of this for the culture of *C. gariepinus* is that it could effectively utilise VO diets that are rich in C18 PUFAs to satisfy its physiological need for LC-PUFAs, without the addition of the latter, such as EPA and DHA, to its diet (Tocher, 2015).

However, the crucial demand from discerning consumers is not only that the catfish satisfy its own physiological needs required for good health and growth but also that an excess of the LC-PUFAs , known to have beneficial effects on human health, are deposited in the flesh of the catfish, in sufficient quantities and in the desired ratios of n-6:n-3 that infer health benefits to the consumers.

2.9 Conclusion

Marine fish oil derived from pelagic fisheries is still the most readily available and cost effective source of feed oil for aquafeeds that provides the full spectrum of essential fatty acids for optimum health, growth and acceptable omega-3:omega-6 flesh levels. However, pelagic fisheries are under pressure with 33% being fished unsustainably, and aquaculture currently uses 75 % of global fish oil, another unsustainable practice given the increasing demand for fish oil from other sectors such as the human health products industry.

Sustainable alternatives are being sought with the most viable alternatives to FO including oils derived from transgenetically modified oilseed crops, heterotrophic microorganisms, marine zooplankton and fisheries and aquaculture by-products. It is estimated that by 2026 the volume of by-products for reduction to FM and FO will exceed the volume of whole pelagic fish, with half of this derived from aquaculture. This will increase the production of FO by 5-10 % but the volume of DHA and EPA will not increase because of the predominance of freshwater species. Twelve million metric tonnes of fish by-products are available but not reduced to FO, mainly in economically less developed countries. This presents a substantial opportunity for small scale businesses to extract the FO by either acid or fermented ensiling.

The most common acid fish silage is made using formic acid and fermented silage, using *Lactobacillus sp*. and sugar molasses, and it is a cost-effective way of preserving fish by-product waste. The silage remains stable for months and contains all the nutritional benefits of the fish waste it was derived from. Furthermore, the presence of *Lactobacilli spp.* and organic acids in aquafeeds have been shown to promote the health and growth of aquaculture species, and it

is possible that these benefits may extend to species fed on diets containing fermented or organic acid silage components.

Numerous feeding trials have been performed using fish silage as a dietary ingredient, usually mixed or co-dried with various plant or animal based meals, supplementing up to 50 % of dietary protein without a significant effect on growth, feed conversion, meat quality or growth of the cultured species. However, there are very few feeding trials where silage oil is the dietary ingredient under investigation, and more work is required to determine conclusively whether silage oil could partially or fully replace marine FO as feed oil in the diets of the main aquaculture species.

This investigation therefore enters into that gap in the literature where silage oil as feed oil has only been trialled on South African abalone and Nile tilapia. It aims to make a contribution on the effect of replacing 100 % marine FO with silage oil in the diets of juvenile African catfish, which has been selected for its importance in food security throughout Africa and for its hardiness. Furthermore, African catfish are genetically predisposed to convert dietary C18 PUFAs (ALA & LA) to satisfy their LC-PUFA requirements. Therefore, VO or silage oil that meets the C18 needs would be adequate as the only dietary lipids.

2.10 References

Agaba, M. K., Tocher, D. R., Zheng, X., Dickson, C. A., Dick, J. R., & Teale, A. J. (2005). Cloning and functional characterisation of polyunsaturated fatty acid elongases of marine and freshwater teleost fish. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *142*(3), 342-352.

Ali, M. Z., & Jauncey, K. (2005). Approaches to optimizing dietary protein to energy ratio for African catfish Clarias gariepinus (Burchell, 1822). *Aquaculture Nutrition*, *11*(2), 95-101.

Al-Dohail, M. A., Hashim, R., & Aliyu-Paiko, M. (2009). Effects of the probiotic, Lactobacillus acidophilus, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (Clarias gariepinus, Burchell 1822) fingerling. *Aquaculture Research*, *40*(14), 1642-1652.

Arason, S. (1994). Production of fish silage. In *Fisheries Processing* (pp. 244-272). Springer, Boston, MA.

Ayinla, O. A., & Akande, G. R. (1988). Growth response of Clarias gariepinus (Burchell 1822) on silage-based diets. NIOMR Technical Paper No. 37. *Nigerian Institute for Oceanography and Marine Research, Lagos. 19pp*.

Ayoola, S. O., Ajani, E. K., & Fashae, O. F. (2013). Effect of probiotics (Lactobacillus and Bifidobacterium) on growth performance and hematological profile of Clarias gariepinus juveniles. *World journal of fish and Marine Sciences*, *5*(1), 1-8.

Balcázar, J. L., De Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D., & Muzquiz, J. L. (2006). The role of probiotics in aquaculture. *Veterinary microbiology*, *114*(3-4), 173-186.

Bell, J.G. & Koppe, W. (2011) Lipids in aquafeeds. In G. M. Turchini, , W. K. Ng, & D. R. Tocher (Eds.), *Fish oil replacement and alternative lipid sources in aquaculture feeds*. CRC Press.

Bell, M.V., and Tocher, D.R. (2009). Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. In M. Arts, M. Brett, & M. Kainz (Eds.), Lipids in aquatic ecosystems. Springer Dordrecht, Heidelberg, London, New York.

Betancor, M. B., Li, K., Sprague, M., Bardal, T., Sayanova, O., Usher, S., ... & Tocher, D. R. (2017). An oil containing EPA and DHA from transgenic Camelina sativa to replace marine fish oil in feeds for Atlantic salmon (Salmo salar L.): Effects on intestinal transcriptome, histology, tissue fatty acid profiles and plasma biochemistry. *PloS one*, *12*(4), e0175415.

Borghesi, R., Ferraz de Arruda, L., & Ôetterer, M. (2008). Fatty acid composition of acid, biological and enzymatic fish silage. *Boletim do Centro de Pesquisa de Processamento de Alimentos*, *26*(2).

Branch, G. & Branch, M. (1982). *The living shores of southern Africa*. Struik publishers.

Chauke, E., Cukrowska, E., Thaela-Chimuka, M. J., Chimuka, L., Nsengimana, H., & Tutu, H. (2008). Fatty acids composition in South African freshwater fish as indicators of food quality. *Water SA*, *34*(1), 119-126.

Chi, Z., Pyle, D., Wen, Z., Frear, C., & Chen, S. (2007). A laboratory study of producing docosahexaenoic acid from biodiesel-waste glycerol by microalgal fermentation. *Process Biochemistry*, *42*(11), 1537-1545.

Christiansen, R., & Lückstädt, C. (2008). Effects of different dosages of potassium diformate in fishmeal on the performance of Atlantic salmon Salmo salar. Abstract CD-Rom. *World Aquaculture Society* WAS Conference, Busan, Korea, May 2008. Accessed online: http://www.wfish.de/fulltext/WAS08_Potassium-diformate-in-salmon-feed_Lueckstaedt.pdf.

Dauda, A. B., Natrah, I., Karim, M., Kamarudin, M. S., & Bichi, A. H. (2018). African Catfish Aquaculture in Malaysia and Nigeria: Status, Trends and Prospects. *Fish. Aqua. J*, *9*.

De Graaf, J. G., & JANSSEN, H. C. (1996). Artificial reproduction and pond reaning of the African catfish, Clarias gariepinus in sub-Saharan Africa A Handbook (No. Mi 362). FAO.

De Silva, S.S., David, S.F. & Tacon G.J. (2011) Fish oils in aquaculture. In G. M. Turchini, W. K. Ng, & D. R. Tocher (Eds.), *Fish oil replacement and alternative lipid sources in aquaculture feeds*. CRC Press.

De Wet, L.F. (2005). Organic acids as performance enhancers. *Aqua Feeds: Formulation Beyond 2*: 2-14.

Espe, M., & Lied, E. (1999). Fish silage prepared from different cooked and uncooked raw materials: chemical changes during storage at different temperatures. *Journal of the Science of Food and Agriculture*, *79*(2), 327-332.

Fagbenro, O. A. (1994). Studies on the use of fermented fish silage in diets for juvenile tilapia (Oreochromis niloticus) and catfish (Clarias gariepinus). Thesis submitted for the degree of Doctor of Philosophy, Institute of aquaculture, university of Stirling, Stirling, Scotland.

Fagbenro, O. A., Adeparusi, E., & Fapohunda, O. (2003). Feedstuff and dietary substitution for farmed fish in Nigeria. *In Proceedings of the National Workshop on Fish Feed Development and Feeding Practices in Aquaculture*, (NWFFDFPA'03), *National Fresh Water Fisheries Research Institute* (pp. 60-65).

Fagbenro, O., & Jauncey, K. (1994). Growth and protein utilization by juvenile catfish (Clarias gariepinus) fed moist diets containing autolysed protein from stored lactic-acid-fermented fish-silage. *Bioresource Technology*, *48*(1), 43-48.

Fagbenro, O., & Jauncey, K. (1995). Growth and protein utilization by juvenile catfish (Clarias gariepinus) fed dry diets containing co-dried lactic-acid-fermented fish-silage and protein feedstuffs. *Bioresource Technology*, *51*(1), 29-35.

Fagbenro, O. A., & Jauncey, K. (1998). Physical and nutritional properties of moist fermented fish silage pellets as a protein supplement for tilapia (Oreochromis niloticus). Animal feed science and technology, 71(1-2), 11-18.

Fagbenro, O., Jauncey, K., & Haylor, G. (1994). Nutritive value of diet containing dried lactic acid fermented fish silage and soybean meal for juvenile Oreochromis niloticus and Clarias gariepinus. *Aquatic Living Resources*, *7*(2), 79-85.

Fagbenro, O., Jauncey, K., & Krueger, R. (1997). Nutritive value of dried lactic acid fermented fish silage and soybean meal in dry diets for juvenile catfish, Clarias gariepinus (Burchell, 1822). *Journal of Applied Ichthyology*, *13*(1), 27-30.

Fagbenro, O. A., & Fasakin, E. A. (1996). Citric-acid-ensiled poultry viscera as protein supplement for catfish (Clarias gariepinus). *Bioresource technology*, *58*(1), 13-16.

FAO. (2010). Cultured Aquatic Species Information Programme. Clarias gariepinus. Cultured Aquatic Species Information Programme. Text by Pouomogne, V. In: *FAO Fisheries and Aquaculture Department* [online]. Rome. [Cited 31 October 2018].

FAO. (2018). The state of world fisheries and aquaculture: meeting the sustainable development goals. Rome. Licence: CCBY-NC-SA 3.0 IGO.

Ferraz de Arruda, L. F. D., Borghesi, R., & Oetterer, M. (2007). Use of fish waste as silage: a review. *Brazilian Archives of Biology and Technology*, *50*(5), 879-886.

Giri, S. S., Sukumaran, V., & Oviya, M. (2013). Potential probiotic Lactobacillus plantarum VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, Labeo rohita. *Fish & shellfish immunology*, *34*(2), 660-666.

Gjedrem, T. (2000). Genetic improvement of cold-water fish species. *Aquaculture research*, *31*(1), 25-33.

Glencross, B., & Rutherford, N. (2011). A determination of the quantitative requirements for docosahexaenoic acid for juvenile barramundi (Lates calcarifer). *Aquaculture Nutrition*, *17*(2), e536-e548.

Glencross, B.D. & Turchini, G.M. (2011) Fish oil replacement in starter, grow-out and finishing feeds for farmed aquatic animals. In G. M. Turchini, , W. K. Ng, & D. R. Tocher (Eds.), *Fish oil replacement and alternative lipid sources in aquaculture feeds*. CRC Press.

Goosen, N. J., de Wet, L. F., & Görgens, J. F. (2014). Rainbow trout silage oil as immunity enhancing feed ingredient in formulated diets for South African abalone Haliotis midae. *Aquaculture*, *430*, 28-33.

Goosen, N. J., de Wet, L. F., & Görgens, J. F. (2014). The effects of protein hydrolysates on the immunity and growth of the abalone Haliotis midae. *Aquaculture*, *428*, 243-248.

Goosen, N. J., de Wet, L. F., & Görgens, J. F. (2016). Rainbow trout silage as immune stimulant and feed ingredient in diets for M ozambique tilapia (O reochromis mossambicus). *Aquaculture research*, *47*(1), 329-340.

Goosen, N. J., De Wet, L. F., & Görgens, J. F. (2018). Effects of formic acid in abalone diets that contain ingredients derived from fish processing by-products. *Aquaculture International*, *26*(3), 857-868.

Goosen, N. J., de Wet, L. F., Görgens, J. F., Jacobs, K., & de Bruyn, A. (2014). Fish silage oil from rainbow trout processing waste as alternative to conventional fish oil in formulated diets for Mozambique tilapia Oreochromis mossambicus. *Animal Feed Science and Technology*, *188*, 74-84.

Goosen, N. J., Görgens, J. F., De Wet, L. F., & Chenia, H. (2011). Organic acids as potential growth promoters in the South African abalone Haliotis midae. *Aquaculture*, *321*(3-4), 245-251.

Haider, M. S., Ashraf, M., Azmat, H., Khalique, A., Javid, A., Atique, U., ... & Akram, S. (2016). Nutritive evaluation of fish acid silage in Labeo rohita fingerlings feed. *Journal of applied animal research*, *44*(1), 158-164.

Hecht, T. (2013). A review of on-farm feed management practices for North African catfish (Clarias gariepinus) in sub-Saharan Africa. *On-farm feeding and feed management in aquaculture*, 463-479.

Hertrampf, J. W., & Piedad-Pascual, F. (2000). *Handbook on ingredients for aquaculture feeds*. Springer Science & Business Media.

Hoseinifar, S. H., Sun, Y. Z., & Caipang, C. M. (2017). Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view. *Aquaculture Research*, *48*(4), 1380-1391.

Huntington, T.C. & Hasan, M.R. (2009). Fish as feed inputs for aquaculture–practices, sustainability and implications: a global synthesis. In M.R. Hasan and implications. FAO Fisheries and Aquaculture Technical Paper. No. 518. Rome, FAO.

Jackson, A. & Newton R.W. (2016). Project to model the use of fisheries by-products in the production of marine ingredients, with special reference to the omega 3 fatty acids EPA and DHA. Institute of aquaculture, University of Stirling and IFFO, the marine ingredients organisation.

Luckstadt, C. (2008). The use of acidifiers in fish nutrition. *Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 3(044),* 1-8.

Madage, S. S. K., Medis, W. U. D., & Sultanbawa, Y. (2015). Fish silage as replacement of fishmeal in red tilapia feeds. *Journal of Applied Aquaculture*, *27*(2), 95-106.

Miller, M. R., Nichols, P. D., & Carter, C. G. (2008). n-3 Oil sources for use in aquaculture–alternatives to the unsustainable harvest of wild fish. *Nutrition Research Reviews*, *21*(2), 85-96.

Mohapatra, S., Chakraborty, T., Kumar, V., DeBoeck, G., & Mohanta, K. N. (2013). Aquaculture and stress management: a review of probiotic intervention. *Journal of animal physiology and animal nutrition*, *97*(3), 405-430.

Monroig, O., Tocher, D.R. & Castro L.F.C. (2018). Polyunsaturated Fatty Acid Biosynthesis and Metabolism in Fish. In Burdge, G.C. (Ed.), Polyunsaturated fatty acid metabolism (pp. 31-60). London, U.K., AOCS press.

Musa, S. M., Aura, C. M., Ngugi, C. C., & Kundu, R. (2012). The effect of three different feed types on growth performance and survival of African catfish fry (Clarias gariepinus) reared in a hatchery. *ISRN Zoology*, *2012*.

National Research Council. (2011). *Nutrient requirements of fish and shrimp*. National academies press.

Naylor, R. L., Hardy, R. W., Bureau, D. P., Chiu, A., Elliott, M., Farrell, A. P., ... & Nichols, P. D. (2009). Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences*, pnas-0905235106.

Ng, W. K., Lim, P. K., & Boey, P. L. (2003). Dietary lipid and palm oil source affects growth, fatty acid composition and muscle α-tocopherol concentration of African catfish, Clarias gariepinus. *Aquaculture*, *215*(1-4), 229-243.

Ng, W. K., Wang, Y., Ketchimenin, P., & Yuen, K. H. (2004). Replacement of dietary fish oil with palm fatty acid distillate elevates tocopherol and tocotrienol

concentrations and increases oxidative stability in the muscle of African catfish, Clarias gariepinus. *Aquaculture*, *233*(1-4), 423-437.

Nyina-wamwiza, L., Wathelet, B., & Kestemont, P. (2007). Potential of local agricultural by-products for the rearing of African catfish Clarias gariepinus in Rwanda: effects on growth, feed utilization and body composition. *Aquaculture Research*, *38*(2), 206-214.

Oboh, A., Betancor, M. B., Tocher, D. R., & Monroig, O. (2016). Biosynthesis of long-chain polyunsaturated fatty acids in the African catfish Clarias gariepinus: Molecular cloning and functional characterisation of fatty acyl desaturase (fads2) and elongase (elovl2) cDNAs7. *Aquaculture*, *462*, 70-79.

Okechi, J. K. (2004). Profitability assessment: A case study of African catfish (Clarias gariepinus) farming in the Lake Victoria basin, Kenya (Master's thesis, The United Nations Univeristy).

Olsen, R.E., Waagbø, R., Melle, W., Ringø, E., Lall, S.P. (2011). Alternative marine resources, in: Turchini, G.M., Ng, W.-K., Tocher, D.R. (Eds.), Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. Taylor & Francis, CRC Press, Boca Raton, pp. 267-324.

Olsen, Y., Andersen, T., Gismervik, I., & Vadstein, O. (2011). Marine heterotrophic bacteria, protozoan and metazoan zooplankton may experience protein N or mineral P limitation in coastal waters. *Marine Ecology Progress Series*, *436*, 81-100.

Omosowone, O., Dada, A., & Adeparusi, E. (2015). EFFECTS OF DIETARY SUPPLEMENTATION OF FUMARIC ACID ON GROWTH PERFORMANCE OF AFRICAN CATFISH Clarias gariepinus AND Aeromonas sobria CHALLENGE. *Croatian Journal of Fisheries*, *73*(1), 13-19.

Osibona, A. O., Kusemiju, K., & Akande, G. R. (2009). Fatty acid composition and amino acid profile of two freshwater species, African catfish (Clarias gariepinus) and tilapia (Tilapia zillii). *African Journal of Food, Agriculture, Nutrition and Development*, *9*(1), 608-621.

Perez-Garcia, O., Escalante, F. M., de-Bashan, L. E., & Bashan, Y. (2011). Heterotrophic cultures of microalgae: metabolism and potential products. *Water research*, *45*(1), 11-36.

Petrucci, R.H. & Harwood, W.S. (1997). *General chemistry principles and modern applications* (7th ed. / Ralph H. Petrucci, William S. Harwood). Upper Saddle River, N.J Prentice Hall.

Pillay, T. V. R., & Kutty, M. N. (2005). *Aquaculture: principles and practices* (No. Ed. 2). Blackwell publishing, pp. 1–61.

Qi, Z., Zhang, X. H., Boon, N., & Bossier, P. (2009). Probiotics in aquaculture of China—current state, problems and prospect. *Aquaculture*, *290*(1-2), 15-21.

Raa, J., Gildberg, A., & Olley, J. N. (1982). Fish silage: a review. *Critical Reviews in Food Science & Nutrition*, *16*(4), 383-419.

Randall, K. M., Drew, M. D., Øverland, M., Østbye, T. K., Bjerke, M., Vogt, G., & Ruyter, B. (2013). Effects of dietary supplementation of coriander oil, in canola oil diets, on the metabolism of [1-^{14}C] 18: 3n-3 and [1-^{14}C] 18: 2n-6 in rainbow trout hepatocytes. *Comparative biochemistry and physiology*.

Ruiz-Lopez, N., Haslam, R. P., Napier, J. A., & Sayanova, O. (2014). Successful high-level accumulation of fish oil omega-3 long-chain polyunsaturated fatty acids in a transgenic oilseed crop. *The Plant Journal*, *77*(2), 198-208.

Russo, G. L. (2009). Dietary n− 6 and n− 3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. *Biochemical pharmacology*, *77*(6), 937-946.

Sanchez-Martínez, J. G., Pérez-Castañeda, R., Rábago-Castro, J. L., Aguirre-Guzmán, G., & Vázquez-Sauceda, M. L. (2008). A preliminary study on the effects on growth, condition, and feeding indexes in channel catfish, Ictalurus punctatus, after the prophylactic use of potassium permanganate and oxytetracycline. *Journal of the World Aquaculture Society*, *39*(5), 664-670.

Sargent, J.R., Tocher, D.R. & Bell, J.G. (2002). The lipids. In J.E. Halver & R.W. Hardy (Eds.), Fish nutrition (pp. 182-246). London, U.K.: Academic press.

Schroder, K., Clausen, E., Sandberg, A. M., & Raa, J. (1980). Psychrotrophic Lactobacillus plantarum from fish and its ability to produce antibiotic substances. In *Advances in fish science and technology: papers presented at the Jubilee conference of the Torry Research Station, Aberdeen, Scotland, 23-27 July 1979, edited by JJ Connell and staff of Torry Research Station*. Farnham, Surrey, England, Fishing News Books, 1980.

SEAFISH. (2018). Fishmeal and fish oil facts and figures. A report compiled by Seafish with thanks to the Marine Ingredients Organisation (IFFO) for permission to use its data.

Simopoulos, A. P. (2011). Importance of the omega-6/omega-3 balance in health and disease: evolutionary aspects of diet. In *Healthy agriculture, healthy nutrition, healthy people* (Vol. 102, pp. 10-21). Karger Publishers.

Soltan, M. A., Fouad, I. M., El-Zyat, A. M., & Zead, M. A. (2017). Possibility of Using Fermented Fish Silage as Feed Ingredient in the Diets of Nile Tilapia, Oreochromis niloticus. *Global veterinaria*, 18(1), 59-67.

Soltan, M. A., Hanafy, M. A., & Wafa, M. I. A. (2008). An evaluation of fermented silage made from fish by-products as a feed ingredient for African catfish (Clarias gariepinus). *Global veterinaria*, *2*(2), 80-86.

Sprague, M., Betancor, M. B., & Tocher, D. R. (2017). Microbial and genetically engineered oils as replacements for fish oil in aquaculture feeds. *Biotechnology letters*, *39*(11), 1599-1609.

Tacon, A. G., & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*, *285*(1-4), 146-158.

Tacon, A.G.J. (1992). Nutritional fish pathology. Morphological signs of nutrient deficiency and toxicity in farmed fish. FAO Fish Technical Paper. No. 330. Rome, FAO. 75 pp.

Tatterson, I. N. (1982). Fish silage—preparation, properties and uses. *Animal Feed Science and Technology*, *7*(2), 153-159.

Tocher, D. R. (2015). Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. *Aquaculture*, *449*, 94-107.

Tocher, D. R., & Glencross, B. D. (2015). Lipids and fatty acids. *Dietary nutrients, additives, and fish health*, 47-94.

Toppe, J., Olsen, R.L., Peñarubia, O.R. & James, D.G. (2018). Production and utilization of fish silage: A manual on how to turn fish waste into profit and a valuable feed ingredient or fertilizer. Rome, FAO. 28pp.

Turchini, G. M., Ng, W. K., & Tocher, D. R. (2011). Fish oil replacement and alternative lipid sources in aquaculture feeds. CRC Press.

United Nations Millennium Development Goals. (2015). Retrieved from http://www.un.org/millenniumgoals/.

Uys, W. (1988). Nutrition. In T. Hecht, W. Uys and P. J. Britz, (Eds.), The c*ulture of sharptooth catfish, Clarias gariepinus in southern Africa*. South African national scientific programmes, report No. 153, Foundation for Research Development: CSIR.

Van Weerd, J. H. (1995). Nutrition and growth in Clarias species-a review. *Aquatic Living Resources*, *8*(4), 395-401.

Van Wyk, H. J., & Heydenrych, C. M. (1985). The production of naturally fermented fish silage using various lactobacilli and different carbohydrate sources. *Journal of the Science of Food and Agriculture*, *36*(11), 1093-1103.

Vázquez, J. A., González, M., & Murado, M. A. (2005). Effects of lactic acid bacteria cultures on pathogenic microbiota from fish. *Aquaculture*, *245*(1-4), 149-161.

Watters, C. A., Edmonds, C. M., Rosner, L. S., Sloss, K. P., & Leung, P. (2012). A cost analysis of EPA and DHA in fish, supplements and foods. *J Nutr Food Sci*, *2*(8), 1-5.

Windsor, M., & Barlow, S. (1981). *Introduction to fishery by-products*. Fishing News Books Ltd.

Wood, J. F., Capper, B. S., & Nicolaides, L. (1985). Preparation and evaluation of diets containing fish silage, cooked fish preserved with formic acid and low-temperature-dried fish meal as protein sources for mirror carp (Cyprinus carpio). *Aquaculture*, *44*(1), 27-40.

World Bank. (2013). Fish to 2030: Prospects for Fisheries and Aquaculture. Washington, DC: World Bank.

Zhu, L., Zhang, X., Ji, L., Song, X., & Kuang, C. (2007). Changes of lipid content and fatty acid composition of Schizochytrium limacinum in response to different temperatures and salinities. *Process Biochemistry*, *42*(2), 210-214.

Zorriehzahra, M. J., Delshad, S. T., Adel, M., Tiwari, R., Karthik, K., Dhama, K., & Lazado, C. C. (2016). Probiotics as beneficial microbes in aquaculture: an update on their multiple modes of action: a review. *Veterinary Quarterly*, *36*(4), 228-241.

Chapter 3

Assessment of a feasible methodology for the large-scale production of silage oil from rainbow trout (*Oncorhynchus mykiss*) viscera using acid and fermentation ensiling methods

3.1 Abstract

Fresh rainbow trout (*O. mykiss*) viscera was used to extract fish silage oil by acid and fermentation ensiling methods. Lactic acid x formic acid, lactic acid x propionic acid, and a commercial bacterial inoculum x molasses was added to about 900 L each of minced viscera, in three airtight 1000 L containers, respectively. The silage oils produced were assessed for their volume, quality and fatty acid composition with the view to being used as feed oil in African catfish (*Clarias gariepinus*) diets. After 30 days an average volume of 291 L of silage oil was produced across the three treatments, with a quality deemed favourable by feed grade standards. The fatty acid composition of the silage oils compared favourably to a regular marine-based feed oil in their percentage of polyunsaturated fatty acids. However, the silage oils had a low percentage (1.7 %) of omega-3 fatty acids and a high percentage (31.3 %) of omega-6 fatty acids, resulting in a high (9.0) omega-6:omega-3 fatty acid ratio. These results indicate that rainbow trout viscera ensiled by either organic acid or fermentation methods would produce fish oil that could potentially be used in the production of fish feed and other commercially viable products. The trial represented a proof of concept for ensiling large quantities of fish visceral waste under low-tech ambient conditions. The success of the method, using readily available apparatus, demonstrates its feasibility and potential for producing viable fish feed ingredients from local fish waste.

3.2 Introduction

As the biggest food production sector, producing some 80 million metric tons (MMT) of food fish (FAO, 2018), the aquaculture sector has an enormous demand for fish oil (FO), which is used as feed oil in diets to provide the energy and necessary fatty acids (FAs) required for fish growth (Bell & Koppe, 2011).

Marine FO remains the preferred oil in marine fish diets because it supplies essential fatty acids, required for the necessary physiological functions of these fish. It also supplies sufficient omega-3 FAs to give the fish flesh the favourable Omega-6:Omega-3 FA profile considered to be of optimal benefit for human health (Russo, 2009). But much of the marine FO is obtained from unsustainably fished marine pelagic fish populations that have remained static or have slowly declined since the year 2000 (Tacon & Metian, 2008; FAO, 2018). This situation is further exacerbated by the global aquaculture sector's continuous annual growth of 4.6 % (FAO, 2018), demanding an increasing quantity of FO, while the yield from the natural supply sources are dwindling. Furthermore, since a minimum daily intake of omega-3 oil containing EPA and DHA has been found to have numerous health benefits to humans there is a growing demand for marine FO, for the production of human health products; thus, placing a greater pressure on the already limited resource (Tacon & Metian, 2015).

Consequently, various oils are continually evaluated for inclusion in aquafeeds in an attempt to find a more sustainable and cheaper alternative to fish oil. This would reduce both the negative environmental impact and economic demand that the aquaculture sector is responsible for. Some potential alternatives being investigated include vegetable oil rich in n-3 PUFAs, heterotrophic microorganism oil, unfished marine stocks in the ocean's abyssal zone, transgenic oilseed plants and a greater effort to recover more fish by-product waste (Glencross & Turchini, 2011).

Fish processing waste which includes skin, head, gills, viscera and frames constitutes, on average, about 60 % of the fish biomass (Villamil, Váquiro & Solanilla, 2017). An approximate calculation based on the most recent statistics (FAO, 2018) will reveal that fish waste represents a potentially gargantuan quantity of raw material from which FO could be extracted; 80 MMT food fish x 60 % equals

48 MMT. However, this calculation assumes that all food fish produced by aquaculture is processed and that the waste is accessible for collection and further reduction into FO. The reality is more complex, for example, the bulk of aquaculture production is from rural China, where fish waste processing facilities are few and there is a prevailing culture among consumers of trading in whole fresh live fish (Jackson & Newton, 2016). Furthermore, the bulk of aquaculture production is freshwater fish, which has a relatively low FO content with low EPA and DHA fatty acids (De Silva et al., 2011). In Europe where there are many fish processing plants, a high percentage of the fish waste is reduced to FO. Here, a large quantity of the cultured fish belongs to the *Salmonidae* that also have a good omega-3 profile with EPA:DHA of approximately 2:1 and therefore produces a good quality oil (Jackson & Newton, 2016). In the developing nations where there may be a lack of infrastructure to transport and process fish waste, and where quantities of by-product may be available in small quantities in remote areas, the fish waste is usually discarded since it is highly perishable on account of its high protein content and abundant hydrolytic enzymes in the viscera, skin and flesh (Toppe et al., 2018).

Where fish waste is utilised, it is usually for relatively low-value products such as animal feed, while substantial amounts of high-quality compounds could be extracted from the waste (Villamil et al., 2017). These include up to 90 % proteins, polypeptides and amino acids, various bioactive compounds, PUFAs, endogenous enzymes, collagen, gelatine, and oils rich in omega-3 and omega-6 FAs. During the hydrolysis process the functional properties of the proteins in fish protein hydrolysates (FPH) are enhanced, making them suitable to be used in pharmaceuticals, nutraceuticals, cosmetic and food products. Functional properties refer to characteristics such as their water- and oil-holding capacity, solubility, foaming and emulsifying capacities that would allow them to be applied in food products and cosmetics (Taheri, Anvar, Ahari & Fogliamo, 2013). Fish viscera are a rich source of proteolytic enzymes that have high levels of activity and present a biotechnological alternative to conventional enzymes used in industry. Fish viscera protein hydrolysates (FVPH) shows numerous potential health benefits even though these remain to be tested in large scale human clinical trials. The potential health benefits include their use as antioxidants in foods,

hypotensive effects, antimicrobial and anti-inflammatory functions, calcium-binding ability, anticoagulant and antitumour activity (Benjakul, Yarnpakdee, Senpahn, Halldorsdottir & Kristinsson, 2014).

This high-end processing and use of fish waste are very costly and its specialised products supply large, ever-growing niche markets located in a high-income sector of society, where the quality and functionality of the products and not their price determine sales. Processing the same fish waste into fish silage requires much less costly inputs in the form of cheaper chemical resources, simpler infrastructure and less-skilled labour, which makes it more affordable and suited to waste processing in developing countries (Soltan & Tharwat, 2006). The enzymatic hydrolysis of fish viscera during silage production takes place at lower temperatures and pressures than conventional chemical and mechanical processes. This ensures that the end-products suffer minimum damage and maintain their nutritional value. In fact, fish viscera protein hydrolysate contains the full spectrum of essential and non-essential amino acids. For this reason, ensiling fish by-product waste by acid or fermentation means have proven to be a useful way to prevent spoilage and yet preserve sufficient nutrients for it to be used as a component in fish feed. The most common acid silage would appear to be formic acid silage, which is made using 85% strength formic acid at a 2.5 % to 3 % inclusion level (Borghesi et al., 2008; Goosen et al., 2014; Wood et al., 1985), while the most common fermented silage uses inclusion levels of 2 % *Lactobacillus plantarum* and 5 % sugar molasses (Fagbenro et al., 1994; Fagbenro et al., 1997; Fagbenro & Jauncey, 1995).

Fish processing waste streams, across the world, are being managed more sustainably than before but there is still much room for improvement (Jackson & Newton, 2016). This is also true for South Africa where a lot of fish waste is used directly as low-grade animal feed, with much of it still ending up in the oceans and landfill sites. Here it decomposes easily releasing green house gas emissions that enhance global warming and leachates that poison groundwater. This nutrient-rich waste also attracts vermin such as rats and cockroaches that can spread disease in poor communities that often live proximal to landfill sites.

The fish production sector, and especially the rainbow trout production sector in South Africa, on whose processing waste this thesis is focused, is relatively small (Britz, Hara, Weyl, Tapela & Rouhani, 2015), yielding a total of 2254 tons per annum (DAFF, 2018). Therefore, it lends itself more to ensiling the processing waste rather than investing in large high-tech waste processing plants. By ensiling the fish waste, it is preserved and does not decompose. Over time it breaks down into good quality silage oil and crude fish protein hydrolysate, rich in polypeptides and amino acids. Both of these components are useful raw materials for further processing into value-added products such as biofuel, biofertilisers, biofeed, health supplements, soap products and animal feed oil (Bastidas-Oyanedel et al., 2016). Some of these products can be directed back into the aquaculture industry and possibly lead to cost savings on formulated feed ingredients. The range of useful products could also be used as the basis for economic development and to address unemployment and food insecurity (Toppe et al., 2018).

This research task developed from the request of an industry partner of the university, Three Streams Smokehouse, to develop a methodology to manage their waste streams by ensiling Rainbow trout visceral waste to produce useful products. It documents the preliminary research to develop a proof of concept to test the feasibility that large volumes (1000 L) of fish silage could be produced using readily available reagents and equipment under ambient conditions. This chapter documents the process by which approximately 3000 L of Rainbow trout viscera was processed to produce three distinct viscera silages, using two organic acid silages (lactic acid x formic acid, and lactic acid x propionic acid) and one fermented (commercial bacterial inoculant mixture; Bactosile® x molasses) silage. The ensiling methods were evaluated for their viability, the average volume of silage oil produced, the quality of the silage oil and the FA profile of the oil. *In lieu* of testing each silage oil as feed oil in African catfish diets, the silage oils were compared to a standard marine FO in regard to their FA profiles.

3.3 Materials and methods of silage preparation

3.3.1 Site and raw materials

The study was conducted mainly on the premises of the Three Streams Smokehouse, which is situated in Franschoek, Western Cape, South Africa; after 30 days the silage containers were transported to Stellenbosch University where the silage oils were decanted. Three Streams is the largest Rainbow trout processor in South Africa and produces large volumes of processing waste, which includes heads, frames, skin and viscera. The raw materials used to produce the fish silage in this study, were about three tons of viscera, from freshly gutted rainbow trout. The complete viscera were used, which contained the liver, pancreas, stomach, intestine, gonads and some visceral fat. The chilled viscera were used directly from a 1000 L container that was kept in the shade (Figure 3.1) and used completely by the end of a day. If viscera from the previous day's evisceration was used, it was kept refrigerated overnight and processed the following day while still cool. These precautions were essential since fish viscera are rich in nutrients and live bacteria, and therefore highly susceptible to putrefaction.

Figure 3.1 Chilled viscera in a shaded area ready for ensiling. Three intermediate bulk containers (IBC) in the background with equal volumes of minced viscera

3.3.2 Procedures

Three separate silages were prepared from minced Rainbow trout viscera. The silages included two organic acid silages, namely lactic acid x formic acid (LF), lactic acid x propionic acid (LP), and a fermented silage made with a commercial bacterial inoculant, Bactosile (BAC).

3.3.2.1 Equipment

Each silage was prepared in an airtight plastic 1000 L intermediate bulk container (IBC). An airlock, consisting of a flexible 10mm hose submerged in a 500 ml bottle of water, allowed gases to escape while maintaining the anaerobic conditions. The volume of silage prepared was at least 40x the usual 25 L volume produced during similar fish feeding trials. The three IBCs were placed outside in a shaded spot underneath a patio.

3.3.2.2 Preparing the raw material

The fresh Rainbow trout viscera was minced in a small industrial mincer (Bizerba, 50 W, model 220218) through a 4.5 mm die. The mincing mixed the various visceral components into a viscous liquid (Figure 3.2) that was homogeneous throughout. Mincing also greatly increased the surface area of the viscera that was in contact with the ensiling reagents, which increased the efficiency of the ensiling process and reduced the chance of putrefaction (Toppe et al., 2018). To ensure the homogeneity of the raw materials in each of the treatment IBCs, the minced viscera from each day's batch was divided equally between the three IBCs. This was done specifically because the viscera came from the Rainbow trout grown on different trout production farms in the Western Cape.

As a result of the relatively small size and power rating of the mincer, the almost 3000 L of viscera was minced over a period of 10 days. The different reagents were therefore also added periodically in the correct proportions to the volume of minced viscera. After each addition of viscera and reagents the mixtures in each IBC was mechanically stirred by a paint mixing paddle which was driven by an electric hand drill. The reach of the paint mixing paddle was extended to 1.2 m by having an extra piece of metal rod welded onto it. This allowed every corner of the silage to be stirred thoroughly from the 150mm diameter opening in the top of the

IBC. Additionally, a forklift was used intermittently to mix the contents of each IBC thoroughly. During such mixing periods the relative position, left, right or centre, of the three IBCs were changed so that each IBC could experience the same exposure to the ambient weather conditions.

Figure 3.2 Mincing Rainbow trout viscera to homogenise and increase surface area of viscera

3.3.2.3 Safety measures for reagents

Formic acid and propionic acid are highly corrosive and a health hazard if inhaled or makes contact with the eyes or skin (EFSA 2014; EFSA 2015). Due precautions in the form of personal protective equipment consisting of a chemical cartridge respirator (with organic vapour cartridges), a Perspex full face piece helmet, and long acid resistant gloves were worn when handling these acids. A spray bottle containing a weak solution of bicarbonate of soda was kept at hand in the event of possible eye exposure.

3.3.2.4 Silage formulas

The organic acid mixtures (Bitek industries, South Africa), which were mixtures of the respective acids (52 %; v/v) with lactic acid (48 %; v/v), were added in small amounts in proportion to the volume of viscera (Table 3.1). They were decanted and added to the minced rainbow trout viscera outside, in the open air (100 % ventilation), until they represented 25 L (2.8 %) of the silage. Oxiban L (BITEK industries, South Africa), an antioxidant, was added at 1 L per 900 L viscera. The bacterial inoculant solution, Bactosile®, was supplied by Nutritionhub (South

Africa) and the animal feed grade sugar cane molasses (Voermol molasses syrup, Voermol S.A.) obtained at a local farmer supply store, AgriMark in Franschoek. The lactic acid, formic acid and propionic acid were supplied as 80 %, 85 % and 99 % concentrations, respectively.

Table 3.1 Proportions of reagents used in the acid and fermented silages

Ingredients	**Treatments**					
	LF		**LP**		**BAC**	
	Litres	**%**	Litres	**%**	Litres	**%**
Trout viscera	874	**97.1**	874	**97.1**	838	**93.1**
Lactic x Formic acid	25	**2.8**				
Lactic x Propionic acid			25	**2.8**		
Bactosile®					25	**2.8**
Feed molasses					36	**4.0**
Oxiban*	1	**0.1**	1	**0.1**	1	**0.1**
Total	900	**100**	900	**100**	900	**100**

LF – lactic acid x formic acid silage; LP – lactic acid x propionic acid; BAC – commercial bacterial innoculum Bactosile®

3.3.3 Sampling procedures, measurements and calculations

The temperature and pH of the developing silages were taken over a period of a month, using a portable pH meter (HI 8424, HANNA instruments). Three silage samples were drawn randomly, at varying depths, from each IBC and diluted 5:1 (v/v) with distilled water, before pH readings were taken. The pH and temperature probes were also placed directly into the silage in the IBCs in order to ensure that the sample pH and temperature accurately reflected that of the bulk silage.

The volume of silage oil, formed in each silage, was taken as the oil layer that had formed above the fish protein hydrolysate layer after 30 days. This volume was measured indirectly by measuring the average depth of the oil and multiplying this by the width and breadth of the oil layer in each container. The volume of the fish

protein hydrolysate was determined by subtracting the volume of oil from the total volume of 900 L in each IBC. Some of the silage oil was siphoned off into 25 L containers and stored in a cool room until it was used as feed oil in catfish diets. After remaining outside for a further seven months the three 1000 L silages were moved back to Welgevallen Experimental Farm where the excess oil was decanted and the FPH disposed of. There was no evidence of putrefaction since the silages still had their original sweet-sour odours.

3.4 Results

3.4.1 Silage pH and temperature

The pH of the acid silages was maintained below pH 4.5 while the fermented silage pH increased close to pH 6 by the end of the 30-day period. The mean pH readings were pH 3.19 for LF, pH 3.94 for LP and pH 4.94 for BAC (Figure 3.3 A). The temperatures of all three silages were similar, at about 25˚C on average. They ranged between 20 ˚C to 30 ˚C and increased steadily over the 30-day period (Figure 3.3 B). Since a methodology that tested the viability for producing silage at ambient conditions was being evaluated, the temperatures were not modified as long as they remained within the acceptable temperature range for fish silage below 40 ˚C (Dapkevicius, 2002).

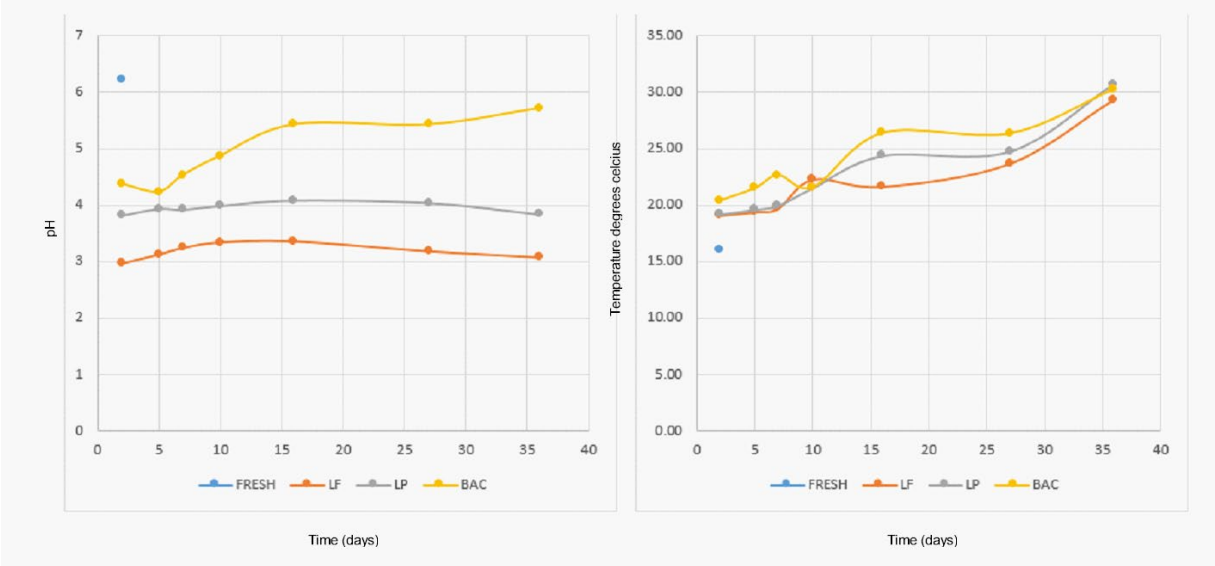


Figure 3.3 A & B. pH (A) and temperature (B) measurements of the acid and fermented silages over the first month

3.4.2 Silage oil and crude fish protein hydrolysate volumes

The relative proportions of silage oil and fish protein hydrolysate varied between silage treatments. The formic acid silage (LF) yielded the least silage oil (LFSO; 21 %), while the propionic acid silage (LP) yielded an intermediate volume of silage oil (LPSO; 36 %) and the fermented silage (BAC) yielded the most silage oil (BACSO; 40 %), (Figure 3.4). The resultant fish protein hydrolysate to silage oil ratios for LF, LP and BAC silages, were 79:21, 64:56 and 60:40, respectively. The average ratio of fish protein hydrolysate to silage oil was 68:32.

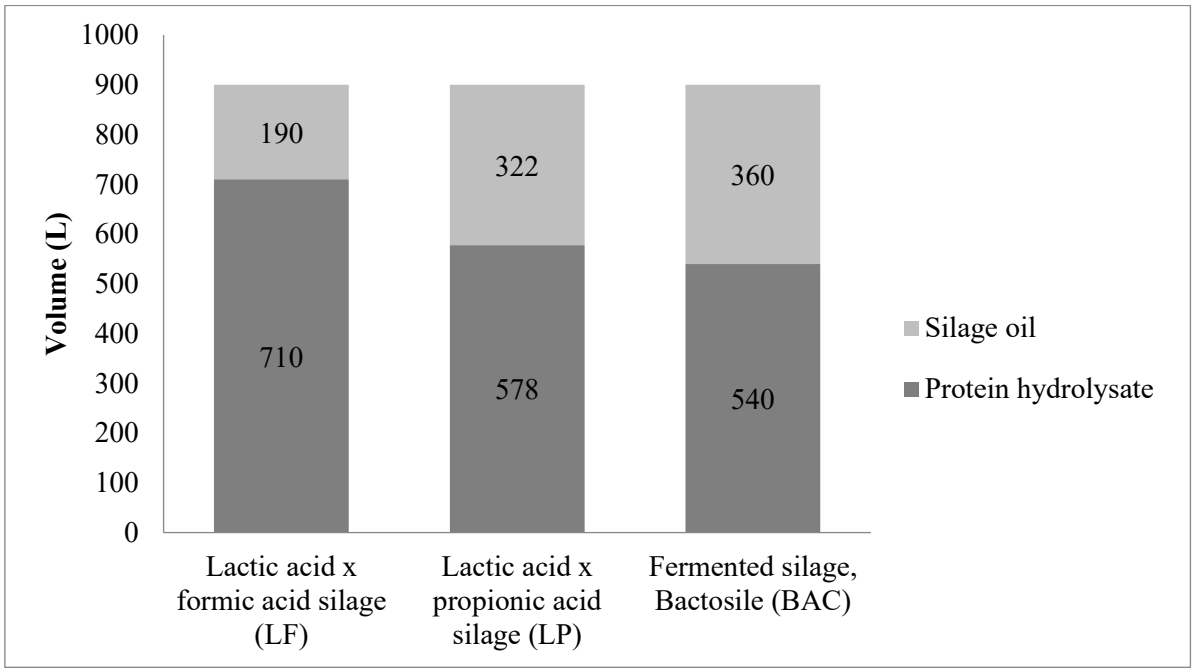


Figure 3.4 Relative volumes of silage oil and crude fish protein hydrolysate obtained in each of the acid fish silages and fermented fish silage

3.4.3 Quality criteria of silage oils

The quality criteria for the silage oils, against food quality criteria, were determined by an independent laboratory (SGS Agricultural Laboratory, Cape Town, Somerset West, South Africa). The quality of the three silage oils was reasonably good (Table 3.2) with levels of moisture (<1 %), insoluble impurities (<0.01 %) and peroxide values well below 5 milliequivalents per kg. Furthermore, the acid silages had acceptable micro-organism loads, while the fermented silage exceeded the acceptable number of colony forming units per g (according to the minimum value of <100 cfu/g set by the processing laboratory) for total plate count and yeasts, respectively. However, since the microorganism were not identified taxonomically

they could very well have been the same probiotic bacteria and yeast cells present in the bacterial inoculant used in the preparation of the fermented silage.

3.4.4 Fatty acids in silage oils

All three silage oils have equally low quantities of Omega-3 FAs (3-4%), with EPA being undetected. The percentage of PUFA is high (34-36 %), represented by high quantities (31-32 %) of omega-6 FAs; this coupled with the low quantities of omega-3 result in relatively high n-6 / n-3 ratios. The combined LA and ALA represent 32 to 33 % of each silage oil, which are the C18 FAs needed by *Clarias sp* to manufacture essential long chain PUFAs. The saturated FAs (23-25 %) and MUFAs (41-42 %) are also well represented among all the silage oils (Table 3.3).

Table 3.2 Silage oils compared to food oil quality criteria

		Silage oils		
Quality criteria	Min. values	LFSO	LPSO	BACSO
Free fatty acids	< 3 %	4.33	7.66	13.8
Moisture	< 1 %	0.11	0.19	0.18
Insoluble impurities	< 0.05 %	< 0.01	< 0.01	< 0.01
Peroxide value	< 5 meq/kg	3.3	1.9	< 1
Total plate count	< 100 cfu/g	ND	ND	6800
Yeasts	< 1000 cfu/g	ND	ND	3680
Moulds	< 1000 cfu/g	40	ND	ND

LFSO – lactic acid x formic acid silage oil; LPSO – lactic acid x propionic acid silage oil; BACSO – fermented silage oil

Table 3.3 Fatty acid compositions of the three silage oils compared to standard marine fish oil

Fatty acid		Oils (g/100g)			
		FO	LFSO	LPSO	BACSO
C14:0		4.4	1.0	1.0	1.1
C16:0		5.9	14.9	14.9	15.3
C18:0		1.3	5.2	5.3	5.1
C20:0		ND	1.1	1.1	1.1
C22:0		ND	1.2	1.2	ND
C16:1		ND	3.0	3.0	3.1
C18:1n9c		3.0	38.1	38.5	38.9
C18:2n6c	(LA)	0.1	30.8	31.3	31.9
C18:3n3	(ALA)	0.4	1.4	1.4	1.5
C20:5n3	(EPA)	28.1	ND	ND	ND
C22:6n3	(DHA)	ND	1.9	2.1	2.1
SFA		13.1	24.8	23.6	22.6
MUFA		26.1	41.1	41.6	41.9
PUFA		60.7	34.1	34.8	35.5
PUFA:SFA		4.6	1.4	1.5	1.6
(n-6)/(n-3)		1.1	9.3	8.9	8.9

FO – standard marine fish oil derived from pelagic fish such as South African pilchard (Supplier unknown, Wegevallen Experimental Farm Aquaculture); BacSO – silage oil derived from fermented silage; LFSO – silage oil derived from lactic x formic acid silage; LPSO – silage oil derived from lactic x propionic acid silage

3.5 Discussion

In summary, the primary aim of this silage investigation was to develop a feasible methodology to convert thousands of tons of rainbow trout visceral waste into fish silage, from which to extract silage oil with the potential to be used as an aquafeed ingredient. This had to be achieved with organic acid silages and fermented silage using readily available equipment under ambient conditions. The secondary aim was to determine preliminary data on the volumes of silage oil produced by each method; the quality of each silage oil and their respective FA profiles. And to compare the FA profiles of the silage oils to a standard marine fish oil.

For the silage oils produced by organic acid (LFSO & LPSO) and fermentation (BACSO) methods, the following hypotheses were tested: hypothesis 1 – that there would be no difference in the volume of oil produced; hypothesis 2 – there is no difference in respect of oil quality and FA composition; hypothesis 3 – there is no difference between the silage oils and pelagic marine fish oil in respect of the total percentage of PUFAs, EPA + DHA, and the Omega-6/Omega-3 ratios. However, as a result of the lack of replication of the three silages, the comparisons remain unreliable. They act merely as baseline data and as signposts for future research.

The lack of replications in this research stemmed from (1) the primary aim being to develop a feasible methodology and (2) limited research funding and logistical constraints in scaling up the total silage volume to 3000 L.

3.5.1 Fish are what they eat

An important consideration for research in this field is that since fish by-product waste may show great variability, it is very important to state the source of the waste as well as the composition of the fish diet as far as is possible. When the silage oil produced from formic acid in this trial (LFSO) is compared to silage oil which was also derived from formic acid ensiling and from rainbow trout viscera sourced at the same processing facility (Goosen, et al., 2014; Goosen, de Wet and Gorgens, 2014) some important differences in FA composition are detected (Table 3.4). The SFA, MUFA and PUFA content is very similar, but the omega-6/omega-3 level is vastly different, which is mainly due to the much higher omega-3 FA and much lower omega-6 FA content of the silage oils produced by these researchers.

This results primarily from the contributions from DPA (~11% vs 0; either undetected in LFSO or not tested for), DHA (~11 % vs 2 %) and LA (~12 % vs 31 %).

Table 3.4 Comparison of fatty acids between silage oils derived from viscera from the same source, using similar ensiling methods

	Silage oils (g/100g)		
Fatty acid	LFSO*	SO (a)**	SO (b)***
C18:2n6c (LA)	30.8	11.7	12.2
C20:5n3 (EPA)	ND	0.2	0.1
C22:5n3 (DPA)	ND	11.5	11.4
C22:6n3 (DHA)	1.9	11.4	11.0
SFA	24.8	25.4	27.0
MUFA	41.1	37.6	37.0
PUFA	34.1	36.9	36.1
PUFA/SFA	1.4	1.5	1.4
(n-6/n-3)	9.3	0.5	0.6

*LFSO – silage oil from formic acid ensiling of rainbow trout viscera in this study; SO (a)** - silage oil from formic acid ensiling of rainbow trout viscera by Goosen, et al., 2014; SO (b)*** - silage oil from formic acid ensiling of rainbow trout viscera by Goosen, de Wet and Gorgens, 2014

These apparently anomalous results raise an important consideration for researchers in this field, which is that every trial must be evaluated on its own merit and that comparisons between what appears to be very similar treatments and raw material sources must be made with caution. The feed quality and feeding regime used at the site from which the fish waste is sourced, must be taken into account, since the fatty acid profile of the feed will invariably be reflected in the fatty acid profile of the tissues of the cultured species (Barrado et al., 2003). When the raw materials are sourced from different sites and mixed in unknown proportions it becomes difficult to set standards for comparative studies (Holliger et al., 2016).

On the other hand, one of the objectives here was to produce silage oils by two different organic acid silages and one fermented silage method, of sufficient quantity and quality, in order for them to be used in a feeding trial on juvenile African catfish; and although we will be comparing the performance of the one silage oil treatment against the other on the catfish production parameters, simply having an alternative feed oil (any of the three treatments) produced from fish waste would already constitute a considerable gain for sustainable aquaculture.

3.5.2 The importance of using fresh viscera

Fish viscera have a very high proportion of moisture, are rich in nutrients and may even have resident pathogenic and putrefaction microorganisms in the intestines, which makes it very susceptible to decay (Dapkevicius, 2002; Olsen & Toppe, 2017). It is therefore essential that the viscera is used fresh or kept chilled in a refrigerator until it is ensiled. In the first attempt at a fermented silage during this trial about 300 L of Rainbow trout viscera that had stood out overnight, without refrigeration, was minced and added to another 200 L of fresh viscera. The entire batch decomposed, producing extremely foul-smelling gases and did not recover despite the addition of sufficient bacterial inoculant, molasses and an antioxidant. The experiment had to be aborted and wasted a lot of resources in the process. Toppe et al., (2018) therefore emphasise that unless the viscera are used within a very short time after evisceration one risks losing it to decomposition and it is unlikely to be fit for use as animal feed. In the subsequent large volumes of silages prepared in this trial (900 L x three) the viscera were used freshly eviscerated or refrigerated overnight (one night only), added to the IBCs ,100-150 L per day, along with the chemical reagents, and mixed through thoroughly (Ferraz de Arruda et al., 2007) on the same day. These precautions prevented any further putrefaction and resulted in the successful production of three stable treatment silages.

3.5.3 Management of silage parameters

The pH and temperature of silages are meant to remain below 4.5 and 35 ˚C, respectively to prevent the growth of pathogenic and fouling microorganisms (Hertrampf & Piedad-Pascual, 2000). The pH of the fermented silage (BAC) was

above this (Figure 3.3), most of the time with a mean of 4.94, ranging from 4.07 to 5.73. This may have allowed some fouling microorganisms to develop since both the IBC and the container that stored the oil were usually more swollen than the acid silage treatments, even though there was no smell of decomposition. Furthermore, the number of colony forming units present in this oil greatly exceeded the number deemed safe compared to food grade oil (SGS Agriculture Laboratory) ; in fact, the fermented silage had 68 times and 37 times the acceptable number of cfu/g of total plate count and yeasts, respectively (FSAI, 2016). However, the absence of a foul smell could indicate that these colonies were simply formed by the microorganisms already present in the active bacterial inoculum used to make the fermented silage and that the gas that caused the container to swell was CO_2. This is especially likely since Bactosile® contains Brewer's yeast (*Saccharomyces cerevisiae*), which would ferment the sugars to form carbon dioxide. Fermented fish silages are commonplace and invariably report successful trials when the pH is maintained at or below 4.5 even over long periods of time. However, while the percentage addition of inoculant (2.8 %; v/v) in this trial exceeded that used in most trials (2 %), the percentage molasses added (4 %; v/v) was unintentionally lower than what was normally added (5 %) in similar trials (Fagbenro, Jauncey & Haylor, 1994; Fagbenro et al, 1994). Some researchers increased the bacterial inoculant to 5 % and the fermentable carbohydrates from 10 % to 18 % (Fagbenro & Jauncey, 1994, 1998; Hertrampf & Piedad-Pascual, 2000; Ramirez-Ramirez et al., 2013). Van Wyk and Heydenrych (1985), who also made two semi-commercial 1-ton batches of minced hake silage, used 5 % bacterial inoculum with 10 % carbohydrate. Since the metabolic conversion of sugars in the molasses, by microorganisms in the inoculum, is what produces the lactic acid that determines the pH, a lower quantity of molasses may have led to the higher pH values. According to Van Wyk & Heydenrych (1985) when yeasts are present in fish silage under anaerobic conditions, they cause a reduction in carbohydrates by fermenting the carbohydrates into ethanol and CO_2. This would lead to less lactic acid being produced per mole of carbohydrate and possibly to the observed rise in pH. In order to prevent the growth of fungi various researchers sprayed a 1 % potassium sorbate solution onto the walls of the silage container and onto the surface of the silage (Fagbenro & Jauncey, 1988;

Fagbenro, Jauncey & Haylor, 1994; Van Wyk & Heydenrych, 1985). In spite of a number of potential shortcomings in the fermented silage mentioned above, the fact that it did not putrify suggests that the microorganism assemblage in Bactosile® was effective in controlling potentially putrefying microorganisms in this silage. However, since the silage was not tested for microbial strains present, it cannot be stated with any certainty that it did not contain pathogenic strains.

From another perspective, all trials in the literature, bar one (Van Wyk and Heydenrych, 1985) that produced silages for use in fish feeding trials have been conducted on a much smaller scale, usually 5 L to 25 L (e.g. Fagbenro et al., 1994; Fagbenro & Jauncey, 1995; Fagbenro, Jauncey & Kreuger, 1997; Soltan et al 2008; Soltan, Fouad, El-Zyat & Zead, 2017). The current trial prepared about 900 L of viscera silage which added to the difficulty of ensuring a homogeneous mixture of visceral substrate with the associated reagents. With its much larger volume, a lower percentage addition of molasses, and the successful formation of the silage, this trial has set an important baseline, for larger volumes of fermented fish viscera silage, on which further investigations could build.

For the acid silages the pH remained well below 4.5, with the mean pH for LF (3.19) and LP (3.94), thus ensuring a stable silage.

The mean temperatures of the different silages, BAC (25.33 °C), LF (23.33 °C) and LP (24.32 °C) were well within the 30 - 40 °C range, with an optimum of 25 – 30 °C, required for a stable silage (Dapkevicius, 2002). Even though the IBCs containing the silages stood out of the direct sunlight the silage temperatures approximated the mean ambient air temperature (23.0 °C ± 3.0) recorded over that period. This means that large volumes of fermented and acid silages will be stable even if kept outside, provided that the mean ambient temperature does not exceed 40 °C during the ensiling period. If that is likely to be the case, then contingency plans such as draping the IBCs in wet cloth to promote evaporative cooling should be made.

Despite the failure of the pilot fermented rainbow trout viscera silage, likely the result of using day-old viscera, all three subsequent 900 L silages were successful after 30 days.

3.5.4 Differences between silage oils in respect of oil quality and fatty acid composition

The three silage oils, LFSO, LPSO and BACSO are very similar in their fatty acid compositions. This is evident from Table 3.5 where the average percentage composition per fatty acid was calculated across the three silage treatments (Ave SO) as well as the standard deviation of the means (SD). The standard deviation gives one an idea of the distribution of data around the mean, and since they appear to be quite small it shows that there was not much difference between the percentage of each fatty acid in the different silage oils, whether they were prepared by organic acid ensiling or by fermented ensiling. However, since the silages have no replication the result represents only a snapshot, which has no statistical reliability and further trials with replicates are needed to verify or nullify this assertion.

3.5.5 Fatty acid profiles of rainbow trout viscera silage oil

The silage oils produced from Rainbow trout viscera in this trial have relatively larger percentage compositions (Table 3.3) of PUFAs than SFAs; 1.5 times on average in the silage oils. These lower quantities of SFAs in the human diet have generally been considered to be the healthier option for preventing excessive weight gain and cardiovascular disease (Siri-Tarino et al., 2010). In the silage oils, on average, the PUFA fraction is comprised mainly (95 %) of Linoleic acid, an n-6 PUFA, and only small quantities (5 %) of omega-3 PUFAs. This makes the average n6:n3 ratio (Table3.5) of the silage oils (9.0) quite large and the silage oil may require an addition of oil rich in Omega 3 FAs to improve this ratio.

Table 3.5 Comparison of fatty acid compositions between the average rainbow trout viscera silage oils (independent of treatment) and marine fish oil

Fatty acid	FO	Ave SO*	± SD	SO-FO**
C14:0	4.41	1.0	0.1	-3.4
C16:0	5.9	15.0	0.2	9.1
C18:0	1.3	5.2	0.1	3.9
C20:0	0.0	1.1	0.0	1.1
C22:0	0.0	0.8	0.7	0.8
C16:1	0.0	3.0	0.1	3.0
C18:1n9c	3.0	38.5	0.4	35.5
C18:2n6c (LA)	0.1	31.3	0.6	31.2
C18:3n3 (ALA)	0.4	1.4	0.1	1.0
C20:5n3 (EPA)	28.1	0.0	0.0	-28.1
C22:6n3 (DHA)	0.0	2.0	0.1	2.0
SFA	13.1	23.7	1.1	10.6
MUFA	26.1	41.5	0.4	15.4
PUFA	60.7	34.8	0.7	-25.9
PUFA:SFA	4.6	1.5	0.1	-3.1
(n-6)/(n-3)	1.1	9.0	0.2	7.9

*average SO values represented by averages of LFSO, LPSO & BACSO values; **negative numbers indicate a greater concentration of the specific FA in FO, while positive numbers indicate a greater concentration in the SO

The fish oils of marine origin contain among the highest levels of n-3 PUFAs and when they are included in fish diets, these FAs are usually assimilated by the fish and end up in its tissues (Ng, Lim & Boey, 2003). The low levels of n-3 PUFAs in the three silage oils therefore indicate that the rainbow trout viscera used for silage oil production probably did not contain high levels of n-3 PUFAs to start with. It has been known for a long time that freshwater fish fed on a diet of plant oils have reduced levels of LC-PUFAs in their tissues. For example, Toyomizu et al (1963) showed that rainbow trout whose diet contained soybean oil instead of FO had undetectable levels of EPA and DHA, in their body tissues. Hixson, Parrish & Anderson (2014) report a significant reduction in EPA and DHA in the flesh of rainbow trout as they replaced FO in the diet with 50 % and 100 % Camelina seed oil. Bell, Henderson, Tocher, & Sargent (2004), working on Atlantic salmon (*Salmo*

salar), indicate that fish fed vegetable oil had approximately 65 % lower omega-3 LC-PUFA tissue levels when compared to fish fed FO.

The trend is, clearly, that when vegetable oils replace FOs in fish diets, there is a significant reduction in omega-3 PUFAs, noticeably EPA and DHA, in the tissues. It is therefore likely, especially in view of the rising cost of FO, that the rainbow trout in this study were fed on diets low in FO and high in vegetable oils such as sunflower oil (De Wet, personal communication, 2017) resulting in low EPA and DHA in its viscera. The FA profile of the raw rainbow trout viscera was not done as part of this investigation, but could be done in a follow up study to test this hypothesis.

3.6 Conclusion

Since the acid and fermented silages did not spoil during the period of the trial and continued to be stable for seven months afterwards it validates the reagents, the formulation and the process employed in this trial. However, the high concentration of yeasts, the raised pH and swollen storage container of the fermented silage point to the likelihood of alcoholic fermentation. In future trials with fermented fish silages a wider range of the carbohydrate molasses concentration should be evaluated, for example 5 – 15 % (w/w) in order to determine the optimum sugar volume required to produce sufficient lactic acid to lower the pH of the silage; an antimycotic agent, such as potassium sorbate, could also be tested against unwanted yeast growth.

3.7 References

Arruda, L. F. D., Borghesi, R., & Oetterer, M. (2007). Use of fish waste as silage: a review. *Brazilian archives of Biology and Technology*, *50*(5), 879-886.

Barrado, E., Jimenez, F., Prieto, F., & Nuevo, C. (2003). The use of fatty-acid profiles of the lipids of the rainbow trout (Oncorhynchus mykiss) to differentiate tissue and dietary feed. *Food chemistry, 81(1)*, 13-20.

Bastidas-Oyanedel, J. R., Fang, C., Almardeai, S., Javid, U., Yousuf, A., & Schmidt, J. E. (2016). Waste biorefinery in arid/semi-arid regions. *Bioresource technology, 215*, 21-28.

Bell, J. G., Henderson, R. J., Tocher, D. R., & Sargent, J. R. (2004). Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (Salmo salar) using a fish oil finishing diet. *Lipids, 39*(3), 223-232.

Bell, J.G. & Koppe, W. (2011) Lipids in aquafeeds. In G. M. Turchini, , W. K. Ng, & D. R. Tocher (Eds.), Fish oil replacement and alternative lipid sources in aquaculture feeds. CRC Press.

Benjakul, S., Yarnpakdee, S., Senphan, T., Halldorsdottir, S. M., & Kristinsson, H. G. (2014). Fish protein hydrolysates: production, bioactivities and applications. *Antioxidants and functional components in aquatic foods, 1st ed*. Reykjavik, Iceland: Matil Ltd, 237Á83.

Borghesi, R., Portz, L., Oetterer, M., & Cyrino, J. E. P. (2008). Apparent digestibility coefficient of protein and amino acids of acid, biological and enzymatic silage for Nile tilapia (Oreochromis niloticus). *Aquaculture Nutrition, 14(3),* 242-248.

Britz, P. J., Hara, M. M., Weyl, O. L. F., Tapela, B. N., & Rouhani, Q. A. (2015). Scoping study on the development and sustainable utilisation of inland fisheries in South Africa. *Report to the Water Research Commission. Volume 1.* Research Report.

Dapkevicius, M. E. (2002). Biological ensilage of fish-optimization of stability, safety and functionality. [sn]. PhD Thesis Wageningen University, Wageningen, The Netherlands.

De Silva, S.S., David, S.F. & Tacon G.J. (2011) Fish oils in aquaculture. In G. M. Turchini, , W. K. Ng, & D. R. Tocher (Eds.), Fish oil replacement and alternative lipid sources in aquaculture feeds. CRC Press.

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food). (2014). Scientific Opinion on the re-evaluation of propionic acid (E 280), sodium propionate (E 281), calcium propionate (E 282) and potassium propionate (E 283) as food additives. *EFSA Journal;12(7):*3779, 45 pp.

EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed). (2015). Scientific Opinion on the safety and efficacy of formic acid, ammonium formate and sodium formate as feed hygiene agents for all animal species. *EFSA Journal; 13(5)* :4113, 24pp.

Fagbenro, O., & Jauncey, K. (1995). Growth and protein utilization by juvenile catfish (Clarias gariepinus) fed dry diets containing co-dried lactic-acid-fermented fish-silage and protein feedstuffs. *Bioresource Technology, 51(1)*, 29-35.

Fagbenro, O., Jauncey, K., & Haylor, G. (1994). Nutritive value of diet containing dried lactic acid fermented fish silage and soybean meal for juvenile Oreochromis niloticus and Clarias gariepinus. *Aquatic Living Resources, 7(2),* 79-85.

Fagbenro, O., Jauncey, K., & Krueger, R. (1997). Nutritive value of dried lactic acid fermented fish silage and soybean meal in dry diets for juvenile catfish, Clarias gariepinus (Burchell, 1822). *Journal of Applied Ichthyology, 13(1),* 27-30.

FAO. (2018). The state of world fisheries and aquaculture: meeting the sustainable development goals. Rome. Licence: CCBY-NC-SA 3.0 IGO.

FSAI (2016). Guidance Note No. 3: Guidelines for the Interpretation of Results of Microbiological Testing of Ready-to-Eat Foods Placed on the Market (Revision 2). Food Safety Authority of Ireland, Dublin.

Glencross, B.D. & Turchini, G.M. (2011) Fish oil replacement in starter, grow-out and finishing feeds for farmed aquatic animals. In G. M. Turchini, , W. K. Ng, & D. R. Tocher (Eds.), Fish oil replacement and alternative lipid sources in aquaculture feeds. CRC Press.

Goosen, N. J., de Wet, L. F., & Görgens, J. F. (2014). Rainbow trout silage oil as immunity enhancing feed ingredient in formulated diets for South African abalone Haliotis midae. *Aquaculture, 430*, 28-33.

Goosen, N. J., de Wet, L. F., Görgens, J. F., Jacobs, K., & de Bruyn, A. (2014). Fish silage oil from rainbow trout processing waste as alternative to conventional fish oil in formulated diets for Mozambique tilapia Oreochromis mossambicus. *Animal Feed Science and Technology, 188*, 74-84.

Hertrampf, J. W., & Piedad-Pascual, F. (2000). Handbook on ingredients for aquaculture feeds. Springer Science & Business Media.

Hixson, S. M., Parrish, C. C., & Anderson, D. M. (2014). Full substitution of fish oil with camelina (Camelina sativa) oil, with partial substitution of fish meal with camelina meal, in diets for farmed Atlantic salmon (Salmo salar) and its effect on tissue lipids and sensory quality. *Food chemistry, 157*, 51-61.

Holliger, C., Alves, M., Andrade, D., Angelidaki, I., Astals, S., Baier, U., ... & Ebertseder, F. (2016). Towards a standardization of biomethane potential tests. *Water Science and Technology, 74(11),* 2515-2522.

Jackson, A. & Newton R.W. (2016). Project to model the use of fisheries by-products in the production of marine ingredients, with special reference to the omega 3 fatty acids EPA and DHA. Institute of aquaculture, University of Stirling and IFFO, the marine ingredients organisation.

Ng, W. K., Lim, P. K., & Boey, P. L. (2003). Dietary lipid and palm oil source affects growth, fatty acid composition and muscle α-tocopherol concentration of African catfish, Clarias gariepinus. *Aquaculture, 215(1-4*), 229-243.

Olsen, R. L., & Toppe, J. (2017). Fish silage hydrolysates: Not only a feed nutrient, but also a useful feed additive. *Trends in food science & technology*, *66*, 93-97.

Ramírez, J. C. R., Ibarra, J. I., Romero, F. A., Ulloa, P. R., Ulloa, J. A., Matsumoto, K. S., ... & Manzano, M. Á. M. (2013). Preparation of biological fish silage and its effect on the performance and meat quality characteristics of quails (Coturnix coturnix japonica). *Brazilian Archives of Biology and Technology*, *56*(6), 1002-1010.

Russo, G. L. (2009). Dietary n− 6 and n− 3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. *Biochemical pharmacology, 77(6*), 937-946.

Siri-Tarino, P. W., Sun, Q., Hu, F. B., & Krauss, R. M. (2010). Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *The American journal of clinical nutrition, 91(3),* 535-546.

Soltan, M. A., & Tharwat, A. A. (2006). Use of fish silage for partial or complete replacement of fish meal in diets of Nile tilapia (Oreochromis niloticus) and African catfish (Clarias gariepinus). *Egyptian J. Nutrition and Feeds, 9(2),* 299-314.

Soltan, M. A., Fouad, I. M., El-Zyat, A. M., & Zead, M. A. (2017). Possibility of Using Fermented Fish Silage as Feed Ingredient in the Diets of Nile Tilapia, Oreochromis niloticus. *Global veterinaria*, 18(1), 59-67.

Soltan, M. A., Hanafy, M. A., & Wafa, M. I. A. (2008). An evaluation of fermented silage made from fish by-products as a feed ingredient for African catfish (Clarias gariepinus). *Global veterinaria*, *2*(2), 80-86.

Tacon, A. G., & Metian, M. (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture, 285(1-4),* 146-158.

Tacon, A. G., & Metian, M. (2015). Feed matters: satisfying the feed demand of aquaculture. Reviews in *Fisheries Science & Aquaculture, 23(1),* 1-10.

Taheri, A., Anvar, S. A. A., Ahari, H., & Fogliano, V. (2013). Comparison the functional properties of protein hydrolysates from poultry by-products and rainbow trout (Onchorhynchus mykiss) viscera. *Iranian Journal of Fisheries Sciences, 12(1),* 154-169.

Toppe, J., Olsen, R.L., Peñarubia, O.R. & James, D.G. (2018). Production and utilization of fish silage: A manual on how to turn fish waste into profit and a valuable feed ingredient or fertilizer. Rome, FAO. 28pp.

Toyomizu, Masamichi & Kawasaki, Kenji & Tomiyasu, Yukio. (1963). Effects of dietary oil on fatty acids composition of rainbow trout oil. NIPPON SUISAN GAKKAISHI. 29. 957-961.

Van Wyk, H. J., & Heydenrych, C. M. (1985). The production of naturally fermented fish silage using various lactobacilli and different carbohydrate sources. *Journal of the Science of Food and Agriculture*, *36*(11), 1093-1103.

Villamil, O., Váquiro, H., & Solanilla, J. F. (2017). Fish viscera protein hydrolysates: Production, potential applications and functional and bioactive properties. *Food Chemistry, 224*, 160-171.

Wood, J. F., Capper, B. S., & Nicolaides, L. (1985). Preparation and evaluation of diets containing fish silage, cooked fish preserved with formic acid and low-temperature-dried fish meal as protein sources for mirror carp (Cyprinus carpio). *Aquaculture, 44(1),* 27-40.

Chapter 4

Evaluating the effect on the production parameters of juvenile African catfish of replacing fish oil with rainbow trout silage oil in their diet.

4.1 Abstract

To investigate the effects of replacing fish oil with rainbow trout (*Oncorhynchus mykiss*) silage oil in the diets of juvenile African catfish (*Clarias gariepinus*), on their production performance parameters, three silage oils were obtained by ensiling fresh rainbow trout viscera. The silage oils (LFSO, LPSO and BACSO) were obtained from a lactic acid x formic acid silage, lactic acid x propionic acid silage, and a bacterial inoculum x sugar cane molasses fermented silage, respectively. The three silage oils and a standard marine dietary fish oil (control; FO) were evaluated as the feed oils in four treatment diets (LFSO-D, LPSO-D, BACSO-D and the control FO-D), which were made by the post-extrusion addition of the silage oils and marine fish oil to regular commercial catfish feed. The treatment diets were fed to juvenile African catfish (1.36 ± 0.14 g) in six replicate tanks per treatment, over a period of 92 days in a temperature-controlled recirculating aquaculture system. Over the entire duration of the trial there was no significant difference ($p>0.05$) between the treatment means in growth (4.1 ± 3.6 to 4.6 ± 3.7 %BM/d; $p=0.8$), survival (21.5 ± 2.6 to 28.0 ± 3.1 %; $p=0.34$) and feed conversion ratio (1.5 ± 1.5 to 2.1 ± 1.8; $p=0.45$). The silage oils were found to be a rich source of polyunsaturated fatty acids (34.8 ± 0.6g/100g) and matched marine fish oil as a viable feed oil for meeting the dietary and physiological needs of juvenile African catfish. Rainbow trout viscera silage oil could prove to be a viable alternative to fish oil in aquafeeds for juvenile African catfish, which bodes well for the sustainable utilisation of rainbow trout visceral waste as an aquafeed ingredient.

4.2 Introduction

Lipids are an indispensable ingredient in all formulated fish diets. They provide essential fatty acids and energy for growth and general metabolism, facilitate the absorption of nutrients, are the major component of all biological membranes, are required to build specialised molecules that regulate the immune response, thermoregulation and sexual development, to name only a few (NRC, 2011).

Marine fish oil of pelagic origin is the gold standard among feed oils for various aquaculture species. The reason for this is two-fold; on the one hand, marine fish oil contains sufficient essential fatty acids (EFAs), which ensures the good health, maximum growth and excellent feed conversion of the cultured species, while on the other hand it supplies sufficient omega-3 FAs that ensure that the flesh has adequate quantities of this type of FA to which numerous health benefits for humans have been ascribed (Pike, 1990). A regular intake of omega-3 FAs have been shown, in observational studies in humans, to decrease blood pressure, lower blood triglycerides, reduce inflammatory and autoimmune disease, reduce depression and improve cardiovascular health (Simopoulos, 2002).

The high demand for marine fish oil for use in human omega-3 health supplements coupled with the high demand from aquafeed producers that fuel the global aquaculture industry put enormous and in many cases unsustainable pressure on global pelagic fisheries (Jenkins et al., 2009); with 33% of pelagic fisheries currently being unsustainably fished (FAO, 2018). The human demand for fish oil supplements keeps growing, along with an increasing demand for food fish, which is presently 20.3kg per capita globally, with a 1.5% increase p.a. (FAO, 2018). When this is added to population growth, which is 1.1% or 83 million people p.a. and an increasing number of higher income middle-class world population (UN, 2017), there is an exponentially increasing demand on fish oil (FO), but a pelagic marine fish resource that has been stagnant since 2000 (FAO, 2018) or more seriously possibly declining since the 1990s (Pauly and Zeller, 2016).

This apparent mismatch between a supply source that has plateaued and a demand that appears to grow exponentially, has been referred to as the ‘FO Trap’ that is seen as being grossly unsustainable, and could therefore only lead to the collapse of the pelagic fisheries and certain sectors of aquaculture. That this

debate still continues about 18 years after pelagic fisheries have plateaued obviously means that some solutions have been found which are preventing the collapse of pelagic fisheries and aquaculture. Various alternatives to fish oil are being used, such as rapeseed oil, and alternative strategies such as feeding mainly VO and using FO sparingly, only in broodstock, early developmental stages and finishing diets (Woitel, Trushenski, Schwarz & Jahncke, 2014). Furthermore, much more fish waste and by-catch is being utilised for fish oil production than before (Jackson & Newton, 2016) and research into alternatives to fish oil is on-going, including areas such as marine photosynthetic and heterotrophic microorganisms, oily fish in the ocean depths and transgenic terrestrial oilseed crops (Miller, Nichols & Carter, 2011; Olsen et al., 2011). However, the fact that approximately 33 % of marine pelagic oily fish stocks are unsustainably fished (FAO, 2018), points out that the apparent 'balancing act' between supply and demand of fish oil, that keeps both the omega-3 health supplement industry and the aquaculture industry supplied, is probably a very fragile one that could 'tip off its tightrope' at any moment, with catastrophic effects for global food security and health.

Numerous studies have trialled fish waste silage in an attempt to find alternative protein and lipid sources, so as to reduce the quantities of FM and FO that are added to aquafeeds (Fagbenro & Jauncey, 1994, 1995, 1998; Fagbenro, Jauncey & Haylor, 1994; Fagbenro, Jauncey & Krueger, 1997; Fagbenro & Fasakin, 1996; Borghesi et al., 2008; Ramirez et al., 2013; Soltan et al., 2008; Madage et al., 2015; Soltan et al., 2017). The fish silage has the same nutritional composition of the fish waste that was used to produce it so that the silage could naturally vary widely between studies (Vidotti, Viegas & Carneiro, 2003). Also, marine fish waste would be preferred to freshwater fish waste because of the much higher concentrations of omega-3 LC-PUFAs, especially EPA and DHA, in marine fish (Henning & Hoffman, 2017).

Some of these feeding trials used fermented silage with *Lactobacilli spp.* and molasses to provide the milieu for preservation and autolysis (Fagbenro et al., 1994; Fagbenro & Jauncey, 1995; Soltan et al, 2017), while others produced an acid silage using primarily formic acid (Ayinla & Akande, 1988; Goosen, De Wet & Gorgens, 2018; Madage et al., 2015). Organic acid silage oils usually contain

residues of the organic acid ingredients used, while in fermented silages the oil may contain some of the beneficial microorganisms used in the inoculant. Goosen, et al. (2014), using silage oil derived from formic acid ensiling as a feed oil, demonstrated an effect on boosting immunity and gastrointestinal repair in Mozambique tilapia, while de Wet (2005) demonstrated the importance of *Lactobacilli spp.* for their role as probiotics in fish nutrition.

There are only a few studies (e.g. Goosen, de Wet and Görgens, (2014); Goosen, et al., (2014); Goosen, de Wet and Görgens, (2018)) where the silage oils were deliberately separated from the rest of the silage mixture to be used as feed oils in feeding trials. In these studies, the silage oil replaced marine FO in food fish diets and was evaluated on production parameters of growth, feed utilisation and immunity. It was the aim of this study to evaluate silage oils, derived from rainbow trout viscera by acid and fermentation ensiling, as alternatives to marine FO in the diets of juvenile African catfish. The evaluation would be based on the outcomes of standard production performance parameters and feed utilisation.

4.3 Materials and methods

4.3.1 Fish and experimental facility

The African catfish (*C. gariepinus*) fingerlings used in the feeding trial were obtained from a commercial hatchery (Rivendell, Grahamstown) in the Eastern Cape.

The feeding trial was conducted at Welgevallen Experimental Farm of the University of Stellenbosch, South Africa, where a temperature-controlled recirculating aquaculture system (RAS) was used.

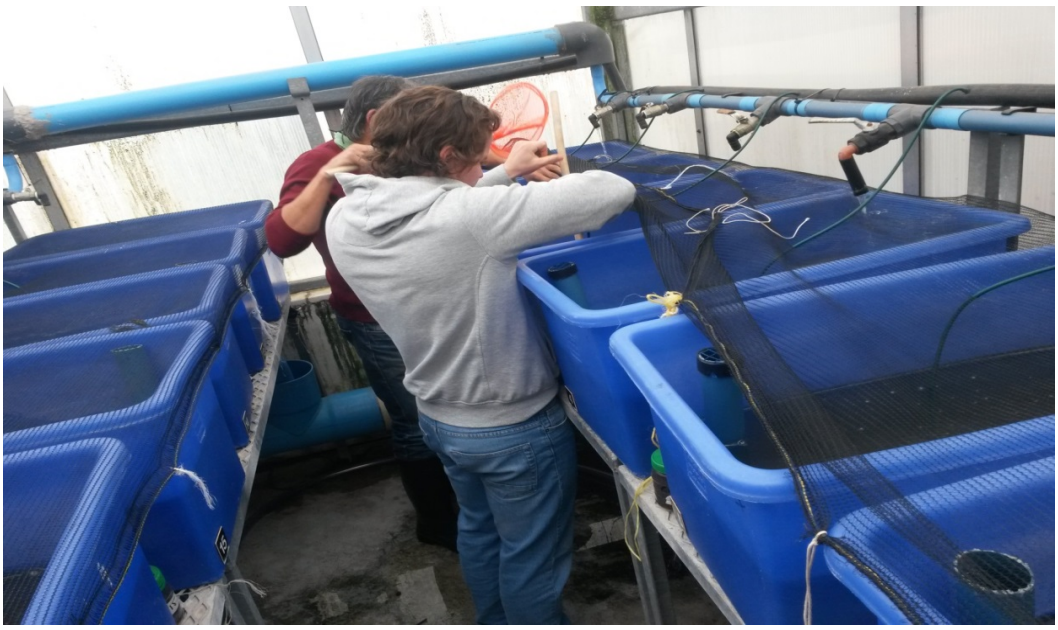


Figure 4.1 Experimental units in part of the RAS at Welgevallen Experimental Farm

The RAS has 88 x 100 L tanks (Figure 4.1) that served as experimental units, of which 26 were used in this trial.

The water source (Table 4.1) was from the local Eerste River.

Table 4.1 Physico-chemical parameters of water source (Eersteriver), and normal range of water quality for *C. gariepinus.*

Parameter	Summer	Winter	Catfish requirement (*)
pH	6.57	7.47	6.5 – 8.0
Ammonium (ppm)	0.08	0.11	<8.8 (pH 7)
Ammonia (mg/L)	-	-	<2.5 (pH 7)
Nitrite (ppm)	0.005	0.004	<0.25
Nitrate (ppm)	0.11	0.04	<250
Salinity (ppt)	0.1	0.1	0 – 2.5
Temperature ºC			26 - 33
DO (mg/L)			>3

*Source: Fagbenro, O. A. (1994). Studies on the use of fermented fish silage in the diets of juvenile tilapia (Oreochromis niloticus) and catfish (Clarias gariepinus) (Doctoral thesis, University of Sterling, Sterling, Scotland). Retrieved from http://hdl.handle.net/1893/1924

4.3.2 Experimental design

In view of the potentially different ambient conditions, specifically temperature and light intensity that a tank might experience depending on its location in the RAS, a completely randomised design was selected for allocating the four test diets (FO-D, LFSO-D, LPSO-D and BACSO-D) to their replicate tanks in the RAS (Figure 4.2). The control diet (fish oil; FO-D) was replicated eight times, while the other silage oil diets (LFSO-D, LPSO-D & BACSO-D) were allocated six replicates each, for a total of 26 experimental units. The fingerlings (1.36 ± 0.14 g) were assigned randomly, 31 fish per tank.

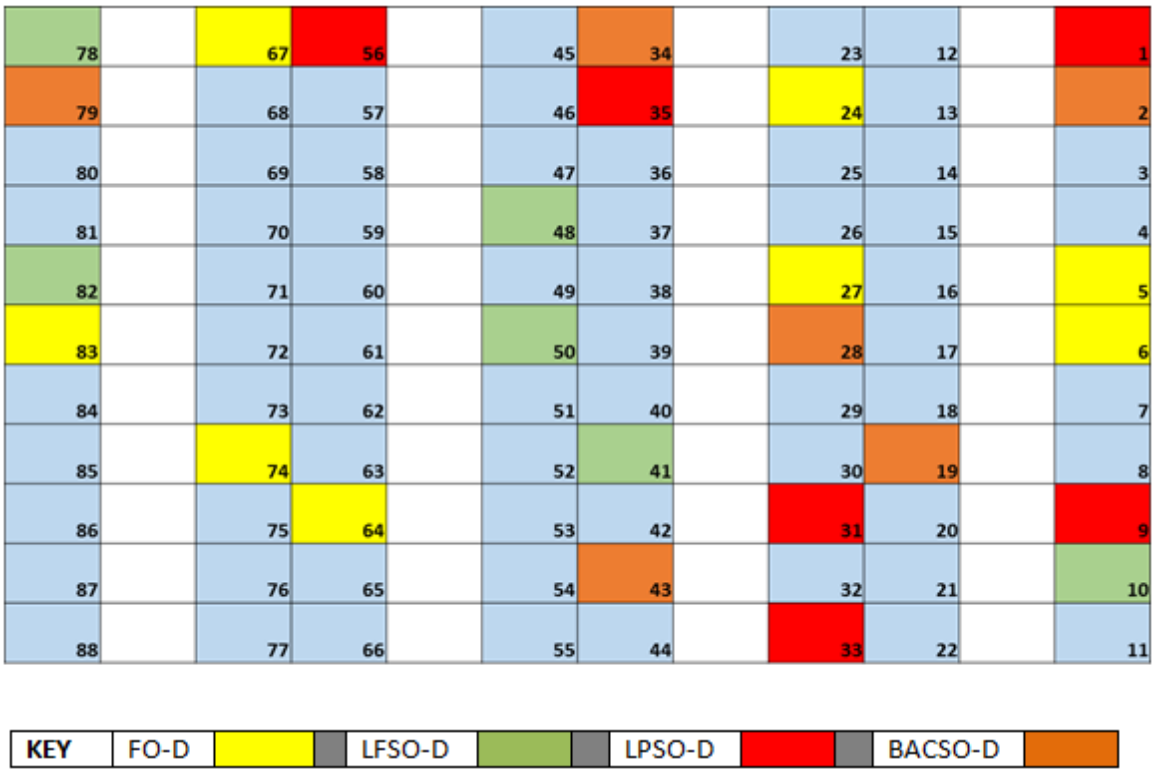


Figure 4.2 Completely randomised design in which treatments and fish were randomly assigned to replicate experimental units (tanks)

4.3.3 Fatty acid profiles of treatment oils and feeds

All the silage oils are very similar in their FA profiles, which differ quite markedly from the control oil in many aspects. The control oil has a much lower saturated fatty acid (SFA) content (13 g/100g) than the silage oils (23-25 g/100g), and similarly for the monounsaturated fatty acid (MUFA) content with the control at 26.1 g/100g and the silage oils between 41-42 g/100g (Table 4.2). This pattern is

reversed for the polyunsaturated fatty acids (PUFAs) where the control has nearly twice as much (60.7 g/100g) as the silage oils (34-36 g/100g). The PUFA:SFA ratio of the control (4.6) is about 2.5 times larger than that of the silage oils (1.4-1.6). In the control oil the quantity of Omega-6 and Omega-3 FAs is nearly equal (n6:n3 ratio of 1.1) while the silage oils have about nine times more Omega-6 than Omega-3 (n6:n3 ratio of ~9). Further noteworthy differences among individual FAs include the relatively large amount of EPA (C20:5n3) in the control (28.1 g/100g) and undetectable amounts in the silage oils, while DHA (C22:6n3) is undetectable in the control and present in small quantities in the silage oils (~2.0 g/100g). Quantities of C18:1n9, LA and ALA are all much higher in the silage oils (~39, ~32 and ~1.5 g/100g) than in the control oil (3.0, 0.1 and 0.4 g/100g), respectively.

The FA composition of the control diet (FO-D, commercial catfish feed coated in marine fish oil) and the silage oil coated diets (LFSO-D, commercial catfish feed coated in lactic x formic acid silage oil; LPSO-D, commercial catfish feed coated in lactic x propionic acid silage oil and BACSO-D, commercial catfish feed coated in bacterial innoculant x molasses silage oil) was also determined. The FA analysis of the feeds is similar with regard to SFA, MUFA and PUFA. The main difference is found in the higher Omega-3 value (16.4 g/100g) in the control feed and its associated lowest n6:n3 ratio (Table 4.3). The LPSO-D feed is uncharacteristic of the other silage oil based feeds, having a high Omega-3 content (14 g/100g) and a relatively low n6:n3 ratio (2.9).

Table 4.2 Fatty acid composition of the experimental oils

Fatty acid		Oils (g/100g)			
		FO *	**LFSO**	**LPSO**	**BACSO**
C14:0		4.4	1.0	1.0	1.1
C16:0		5.9	14.9	14.9	15.3
C18:0		1.32	5.2	5.3	5.1
C20:0		ND	1.1	1.1	1.1
C22:0		ND	1.2	1.2	ND
C16:1		ND	3	3	3.1
C18:1n9c		3.0	38.1	38.5	38.9
C18:2n6c	(LA)	0.11	30.8	31.3	31.9
C18:3n3	(ALA)	0.4	1.4	1.4	1.5
C20:5n3	EPA	28.1	ND	ND	ND
C22:6n3	DHA	ND	1.9	2.1	2.1
SFA		13.1	24.8	23.6	22.6
MUFA		26.1	41.1	41.6	41.9
PUFA		60.7	34.1	34.8	35.5
PUFA:SFA		4.6	1.4	1.5	1.6
(n-6)/(n-3)		1.1	9.3	8.9	8.9

*The fatty acid (FA) analysis of the control fish oil was performed at a different laboratory which yielded additional information, which accounts for the apparent discrepancies when tallying the types of FAs in the control column. FO – marine fish oil; LFSO – silage oil derived from lactic x formic acid silage; LPSO – silage oil derived from lactic x propionic acid silage; BACSO – silage oil derived from fermented silage using Bactosile®

Table 4.3 Fatty acid composition of experimental diets

Fatty acids	Catfish diets			
	FO-D	**LFSO-D**	**LPSO-D**	**BACSO-D**
C12:0	0.0	0.3	0.2	0.2
C14:0	2.7	1.5	1.6	1.5
C15:0	0.0	0.7	0.7	0.5
C16:0	6.8	17.0	16.5	15.3
C18:0	3.6	5.4	5.6	5.0
C21:0	4.7	2.2	1.7	2.2
C18:1n9c	6.1	12.2	12.8	14.2
C20:1	0.0	0.8	0.5	0.4
C24:1	17.6	7.9	5.9	6.4
C18:2n6c	1.5	0.0	0.2	29.4
C18:2n6t	3.4	26.1	24.7	2.4
C18:3n6	0.0	0.8	1.2	1.4
C18:3n3	1.5	2.5	2.3	2.4
C20:3n6	5.2	0.7	0.5	0.5
C20:3n3	0.0	1.1	7.4	1.1
C20:4n6	6.7	7.2	6.2	5.2
C20:5n3	14.9	5.3	4.3	3.8
C22:2n6	23.8	8.3	7.7	8.2
SFA	19.4	27.0	26.2	24.7
MUFA	23.7	20.8	19.2	20.9
PUFA	56.9	52.1	54.6	54.4
PUFA:SFA	2.9	1.9	2.1	2.2
n-6	40.6	43.2	40.5	47.1
n-3	16.4	8.9	14.1	7.3
(n-6)/(n-3)	2.5	4.8	2.9	6.5

FO-D – commercial catfish feed coated in marine fish oil; LFSO-D – catfish feed coated in silage oil derived from lactic x formic acid silage; LPSO-D – catfish feed coated in silage oil derived from lactic x propionic acid silage; BACSO-D – catfish feed coated in silage oil derived from fermented silage using Bactosile®

4.3.4 Feed composition and feeding

The basal diet was a standard catfish starter diet (HiSoy Catfish Grower, Montego, Hermanus, South Africa) provided without post extrusion oil-addition to allow for experimental oil addition. The experimental control diet (FO-D) consisted of the

basal catfish starter diet to which six percent (w/w) marine fish oil was added post extrusion. In a similar way the silage oil treated diets (LFSO-D, LPSO-D and BACSO-D) were basal diets coated with the silage oils (LFSO, LPSO and BACSO), respectively; these were produced by ensiling rainbow trout viscera with a Lactic acid x Formic acid mixture, a Lactic acid x Propionic acid mixture and a bacterial inoculant (Bactosile, Nutritionhub) x sugar cane molasses mixture. The dietary crude lipid (10-12 g/100g) recommended for juvenile catfish (Hecht, 2013) was achieved by adding 6 g/100g experimental oil to the basal diet that already contained 7 g/100g crude lipid. Oil addition was done post-extrusion by means of a non-vacuum mixer. A mechanical bread dough mixer was used to mix the oil with the dry pellets until they were visibly, thoroughly coated. Each batch of feed was prepared in the same way with the mixer blade carefully wiped clean of oil between consecutive batches of feed. Small batches of feed were mixed to last approximately one month and they were stored under cool conditions in a feed store in airtight plastic bags inside black plastic storage containers. The feed treatment for each tank was dispensed into a 250 ml screw-top jar and placed next to the relevant tank. Each feed jar was clearly labelled with the tank number and treatment name.

Proximate analyses

Proximate analyses, in duplicate, of the feed samples (Table 4.4) were performed at the laboratory of the department of Animal Sciences, Stellenbosch University, according to the methodology prescribed in the AOAC official method (AOAC International, 2002) to determine moisture, ash, crude fat and crude fibre. Crude protein was determined by an independent laboratory (Bemlab, Strand, South Africa) and the gross energy and carbohydrates (NFE) were determined by calculation.

Sample preparation

Approximately 100 g feed from each treatment was randomly extracted from storage containers and ground in a hammer mill with a 1.5 mm sieve.

Moisture (AOAC official method 934.01)

Dry a clean porcelain crucible for 2 h at 100 °C, then place in desiccator and allow to cool for 30 min. Weigh the empty crucible (A). Weigh 2 g of feed sample (B). Place crucible with sample in oven at 100-105 °C for 24 h, allow to cool in desiccator and weigh crucible with moisture free sample (C). Calculate:

$$\text{Percentage moisture} = \frac{(A+B) - C \times 100}{B}$$

Ash (AOAC official method 942.05)

The moist free sample known mass (B) after percentage moisture determination and crucible of known mass (A) were placed into a furnace at 500 °C for 6 h. Allow to cool for at least 2 hours overnight, then place crucible in desiccator to cool for 30 min and determine mass (D). Calculate:

$$\text{Percentage ash} = \frac{D - A \times 100}{B}$$

Crude fat (AOAC official method 954.02)

Crude fat was determined by acid hydrolysis with HCl. Small glass beakers were dried, desiccated and weighed. About 2 g of sample was placed in a test tube to which 2 ml of ethanol and 10 ml of HCl were added. This was boiled for 30 min in a water bath, cooled to room temperature for 30 min. The boiled sample was emptied into a separating funnel, to which: 25 ml of diethyl ether was added and shook for 1 min, then 25 ml petroleum ether added and shaken for 1 min (repeat twice adding 15 ml of the ethers). Pour the upper portion from the separating funnel into a glass beaker (beaker with fat) and place onto a sand bath for 2 h for the ether to evaporate. Cool the beakers in a desiccator for 30 min and weigh. Calculate:

$$\text{Percentage crude fat} = \frac{(\text{mass of beaker with fat}) - (\text{mass of beaker})}{\text{Mass of sample}} \times 100$$

Crude fibre (AOAC official method 962.09)

Crude fibre was determined by using an ANKOM fibre analyser (ANKOM 220, USA). Ether, acid and alkalis are added to dissolve all organic moecules except for fibre. A 1 g mass of sample was sealed in an ANKOM filter bag and soaked in petroleum ether for fat extraction. It was then air dried at room temperature and placed into a fibre analyser vessel, to which 0.25 molar sulphuric acid was added for 40 min, after which they were rinsed twice in hot water. A solution of sodium hydroxide (0.3 molar) was added for another 40 min, after which the samples were again washed in hot water and soaked in acetone for 5 min. The samples were oven dried (105 °C) and ashed in a furnace at 500 °C for 2 h. Calculate (corrected for mass of filter bag):

$$\text{Percentage crude fibre} = \frac{\text{Residue after drying g} - \text{Residue after ashing g}}{\text{Sample mass g}}$$

Crude protein was determined by an independent laboratory (Bemlab, Strand, Western Cape, South Africa) as percentage nitrogen, which was converted to crude protein by multiplying by 6.25 (FAO, 2003). For example, the control (FO-D) was reported to have a percentage nitrogen of 6.15, which translated into a crude protein content of (6.15 x 6.25) 38.4 g/100 g.

Gross energy

Gross energy was calculated based on the known energy value per unit of fat (37 MJ/kg), protein (17 MJ/kg) and carbohydrates (17 MJ/kg), respectively.

Calculated:

Gross energy = (37 MJ/kg x crude fat) + (17 MJ/kg x crude protein) + (17 MJ/kg x NFE)

Carbohydrates (NFE) were calculated (FAO, 2003). Nitrogen free extract, representing soluble carbohydrates as follows:

NFE = 100 – (moisture + ash + crude fat + crude protein + crude fibre) g/100 g

For example, for the control feed (FO-D):

NFE = 100 – (6.8 + 9.0 + 10.5 + 38.4 + 6.3) = 29.0 g/100 g

Table 4.4 Composition of the experimental diets

Feed ingredient (g/100g)	Diet (g/100g)			
	FO-D	**LFSO-D**	**LPSO-D**	**BACSO-D**
Basal diet	94	94	94	94
Fish oil (FO; Control)	6	0	0	0
Silage oil (BACSO)	0	0	0	6
Silage oil (LFSO)	0	6	0	0
Silage oil (LPSO)	0	0	6	0
Feed composition (g/100g)				
Moisture	6.8	8.0	8.2	8.3
Ash	9.0	8.8	8.8	9.0
Crude fat	10.5	11.1	11.0	10.9
Crude protein	38.4	37.1	37.1	37.1
Crude fibre	6.3	6.1	6.0	6.0
NFE (Carbohydrates, calculated)	29.0	28.9	28.9	28.7
Gross Energy (MJ/kg)*	15.3	15.3	15.3	15.2

*Calculated: Gross energy = (37kJ/g x crude fat) + (17kJ/g x crude protein) + (17kJ/g x NFE)

Daily feed allowance was calculated based on the total mass of fish per treatment multiplied by the percentage body weight (%BW) feeding rate. The fish were fed, by hand, a percentage of their body mass daily according to the aquafeed management software Feedflow® (www.feedflow.co.za, NutritionHub, 2015), over a 92-day period. The recommended feed allocations were used as a guide, adjusted according to the feeding response of the fish to allow for apparent *ad libitum* feed intake. Initially over the first two weeks the feed was divided out over four feedings per day, which was reduced to two feedings daily, at about 9h00 and 17h00, respectively.

4.3.5 Sample collection and analytical procedures

4.3.5.1 Water quality parameters

The water quality physico-chemical parameters that were collected daily were temperature and dissolved oxygen (DO). These readings were collected directly

from the sump by using a portable YSI ProODO Optical Dissolved Oxygen instrument and occasionally from individual tanks to ensure that they matched the sump readings. Just before each sampling of the fish, approximately every three weeks, pH and ammonia readings were taken. The pH was measured using a bench-top pH meter (Hannah, pH 211 microprocessor) and ammonia and nitrite levels were measured using a portable benchtop calorimeter (Hach, DR/850).

4.3.5.2 Fish mass and length

Mass and length measurements were taken of the catfish fingerlings, on six occasions, approximately two weeks apart (Day 0, 14, 35, 55, 71 and 92) over a period of 92 days. On Day 0 the batch mass (total simultaneous mass of all fish in a tank) per tank was taken and the average mass per fish obtained by dividing the batch mass by the number of fish in each tank. This was done to reduce mortalities caused by handling stress because the fish were still very small (average mass 1.36 ± 0.14 g). On Day 14 mortalities were caused by the use of clove oil as an anaesthetic in the initial tanks and a decision was taken to measure only individual fish mass and not length as well. The un-anaesthetised fish were placed on a drying towel and thereafter weighed individually. On subsequent days (Day 35, 55, 71 and 92) the fish were anaesthetised using clove oil and individual mass and length measurements were taken. Fish mass was measured in grams to two decimal places using an electronic scale (KERN, PLE-N), while fish length was measured as total length to the nearest millimetre on a fixed aluminium ruler. The number of mortalities per tank and the body mass of each dead fish was recorded daily throughout the duration of the trial.

4.3.5.3 Feed intake

The feed fed during the trial was taken as the feed intake of the fish since it was not possible to measure the mass of uneaten feed remaining in the tanks. The mass of feed that was fed during a period e.g. day 0-14 was measured by subtracting the mass of the feed jar at the end from its mass at the start.

4.3.5.4 Haematocrit

On day 92 blood was removed from the caudal vein of one fish per tank to obtain haematocrit values. Since the size variation among fish in a tank was quite wide in

certain tanks and haematocrit values are correlated with fish size (Ojogbo, 2016) the fish were selected, as far as was possible, to be similar in size. Blood was placed in a heparinised capillary tube and spun for five minutes in a microhaematocrit centrifuge at 14000 x g; and the haematocrit or packed cell volume was measured as a percentage of total blood volume in the capillary tube aided by the scaled divisions on the haematocrit reader (Nwani et al., 2014).

4.3.5.5 Fatty acid analyses of feed oils and treatment diets

Fatty acid analyses of the feed oils as well as the four treatments diets were performed by SGS Agriculture Laboratory, Somerset West, and Stellenbosch University Central Analytical Facility, respectively. Both laboratories used gas chromatography (GC) with SGS Agri Food Laboratory based on the method: WI-AN-011 based on AOCS Ce 1b-89. Stellenbosch University Central Analytical Facility describe their method as follows: “Column: B 7HG-G027-11, ZB-Wax (30 m, 0.25 mm ID, 0.25 µm film thickness); Approximately 100mg of the sample was weighed and 1000 µl of hexane was added. Heptadecanoic (C17) was added as internal standard (100ul of 100ppm). 1ml of 20% (v/v) sulphuric acid in methanol was added into the tube. The mixture was incubated in the oven maintained at a temperature of 80 °C for 1 hour. After incubation, the mixture was allowed to cool to room temperature. 2 ml of 20 % (w/v) NaCl was added to extract the fatty acids methyl esters (FAMES). The samples were shaken vigorously followed by centrifuging to facilitate phase separation. The upper hexane phase (hexane containing FAMES) was transferred to a GC vial. Chromatographic separation: Separation was performed on a gas chromatograph (6890N, Agilent technologies network) coupled to an Agilent technologies inert XL EI/CI Mass Selective Detector (MSD) (5975B, Agilent technologies Inc., Palo Alto, CA). The GC-MS system was coupled to a CTC Analytics PAL autosampler. Separation of fatty acids was performed on a polar ZB-Wax (30 m, 0.25 mm ID, 0.25 µm film thickness) capillary column. Helium was used as the carrier gas at a flow rate of 1 ml/min. The injector temperature was maintained at 250°C. 1µl of the sample was injected in a split ratio was set at 5:1 split ratio. The oven temperature was programmed as follows: 50 °C for 2 min; and then ramped up to 180 °C at a rate of 25 °C/min and held for 5 min and finally ramped up to 250 °C at a rate of 3 °C/min and held for 2 min. The

MSD was operated in a full scan mode and the source and quad temperatures were maintained at 230 °C and 150 °C, respectively. The transfer line temperature was maintained at 250 °C. The mass spectrometer was operated under electron impact mode at ionization energy of 70 eV, scanning from 35 to 500 m/z."

4.3.6 Calculations and statistical methods

The growth performance parameters that were calculated were initial fish mass (Wi; g), final fish mass (Wf; g), daily weight gain (DWG; g/day) and specific growth rate (SGR; %BM/day), while feed utilisation parameters calculated were relative feed intake (RFI; %BM/day) and feed conversion ratio (FCR; dry feed intake/wet mass gained). Initial body mass and final body mass per treatment were calculated using the tank means on Day 0 and Day 92, respectively.

The following formulae (Kirimi, Musalia, Magana & Munguti, 2016; Lugert, Thaller, Tetens, Schulz & Krieter, 2016) were used to calculate the production performance parameters, where Wi is the initial fish mass and Wf is the final fish mass after t days:

$$\text{DWG Daily Weight Gain (g/day)} = \frac{Wf - Wi}{t \text{ days}}$$

$$\text{SGR Specific Growth Rate (\%BM/day)} = \frac{[\ln(Wf) - \ln(Wi)] \times 100}{t \text{ days}}$$

$$\text{RFI Relative Feed Intake (\%BW/d)} = \frac{\text{Dry feed intake} \times 100}{[(Wi + Wf)/2] \times t \text{ days}}$$

$$\text{FCR Feed Conversion Ratio} = \frac{\text{Dry feed intake (g)}}{\text{Wet mass gain (g)}}$$

$$\text{Survival (\%)} = \frac{\text{No. fish alive (at time of sampling)} \times 100}{\text{No. fish initially stocked (n = 31)}}$$

Statistical analyses were performed on the data with the software package Statistica, version 13.2 (StatSoft, Inc.), using an alpha value of p=0.05 as the significance criterion for all statistical tests. A complex general linear model was used to describe the relationship between the treatment and the response variables. This model included the following aspects of the experimental design: it was a completely randomised design with tanks randomly assigned to treatments, tanks were nested within treatments, tank means measured over time were not treated as independent, but as repeated measures, time and treatments were fixed variables with (time nested in treatments).

In the experimental design and the statistical model used to describe the relationship between the treatment and response variables, it is important to account for all possible sources of variability so that the error component is minimised (Clewer & Scarisbrick, 2013). Apart from the CRD, which was reflected in the way that the four different treatments were assigned to the experimental units (tanks) in the RAS, the experimental design used in this trial also included an element of a nested design (tanks were nested in the treatments), as well as a repeated measures element (Figure 4.3).

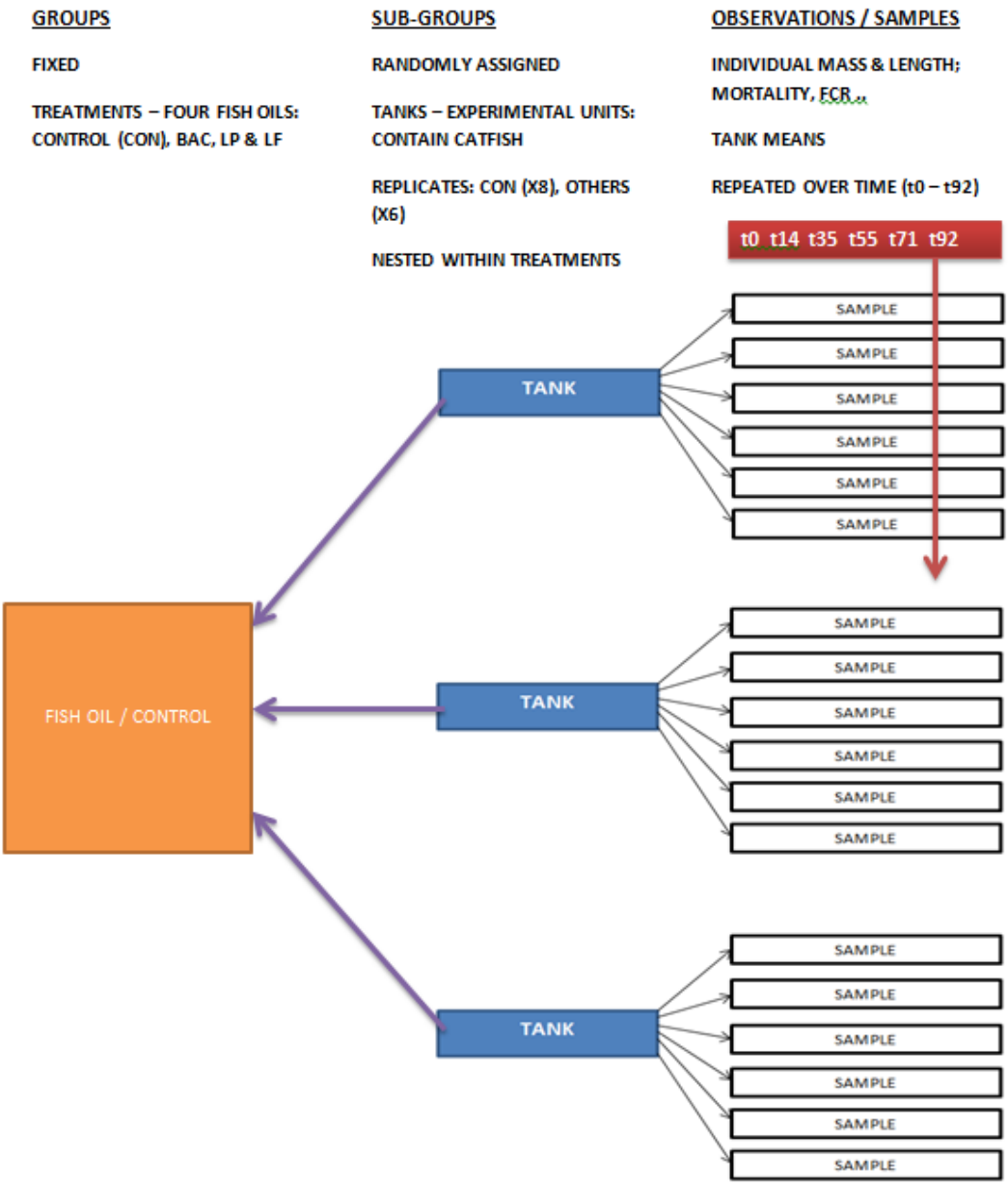


Figure 4.3 Showing a subset of the experimental design with its nested and repeated measures elements

The nested design takes into account that each of the 26 tanks (experimental units) is unique to a treatment i.e. each tank will only have one treatment administered to it, while a treatment is not unique to tanks, since any one treatment is administered to at least six replicate tanks. When this element is included in the analysis of variance (ANOVA) it accommodates for the 'tank effect', that is the probability that the difference between treatments is actually due to an effect of the tank (chemical, physical or locational) on the response parameters being measured (McDonald, 2014). This would reduce the chance of one committing a

type II error, that is, accepting a false Null hypothesis (false negative) when there is in fact a significant difference between treatments on the response variable (Rothman, 2010). The experiment also contains an element of repeated measures, since the same experimental unit (the tank) is sampled repeatedly over time; this means that the response variable measured at time t+1, for example the mean mass of fish per tank, is clearly related to, and therefore not independent of, the same variable measured previously at time t (Clewer & Scarisbrick, 2013).

All treatment means for the response variables SGR, RFI and FCR were compared using RMANOVA, while the treatment means for the response variables initial mass (Wi), final mass (Wf) and DWG were compared with one-way ANOVA using the data analysis pack in Microsoft Excel (Microsoft Inc.).

The series of mortalities during the trial resulted in many outliers among the data and response variable distributions that were neither normal nor homoscedastic. Since the general linear model used does not have a non-parametric alternative (Professor D. Nel, 2017, Statistician, Centre for Statistical Analysis, Stellenbosch University; personal communication) the data was transformed, by Winsorisation (Reifman & Keyton, 2010), to approximate normality and homoscedasticity. This method does not remove outliers, which would have resulted in the loss of replicates, but pulls the outlying values to within the limits of the distribution, which has the effect of adjusting the means accordingly (Smith, 2015).

4.4 Results

4.4.1 Production performance parameters

4.4.1.1 Mortalities

The rate of mortality started off gradually but culminated in a precipitous loss of fish between days 55 and 71 (Figure 4.4). The abnormally large total mortality mass (g) summed across all treatments is shown in parenthesis, next to the time periods when the mortalities occurred: days 27 to 31 (720 g), days 50 to 55 (2200g) and day 62 (6500 g). Overall there is no difference between percentage survival among treatments ($p=0.34$). The percentage survival is very low by the end of the trial with all treatments below 30 % survival.

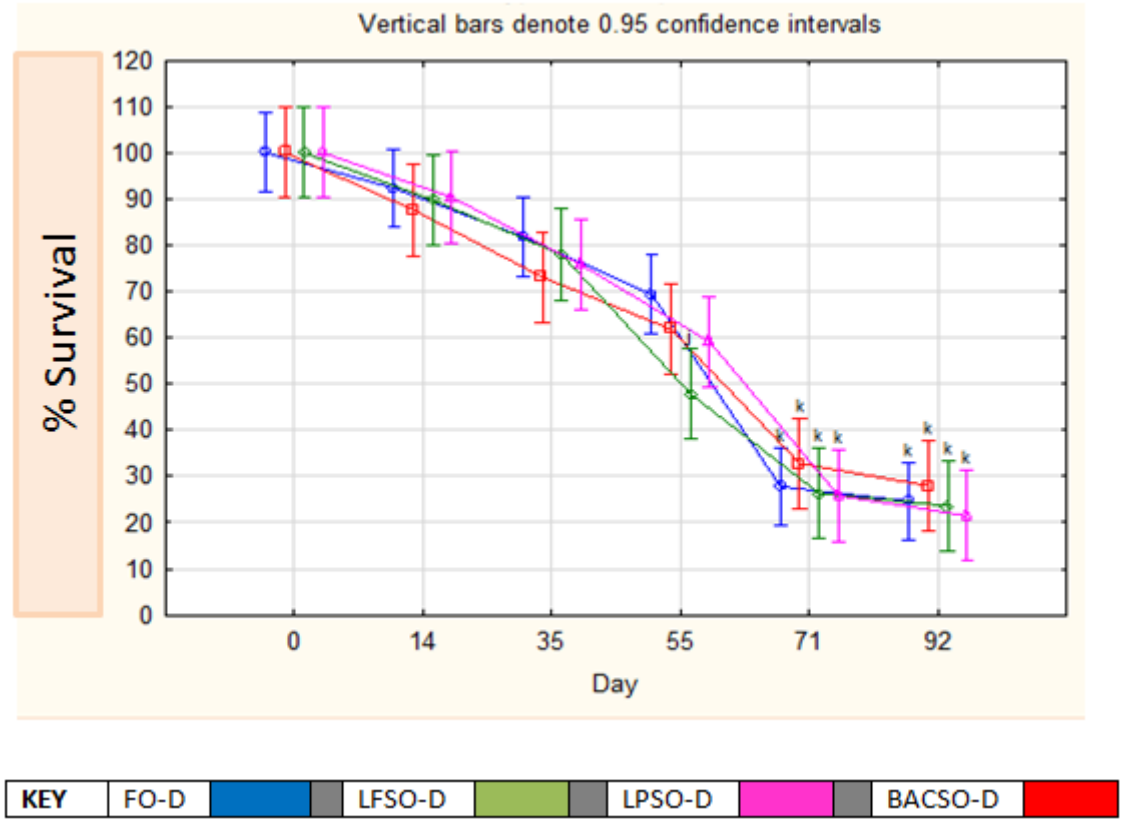


Figure 4.4 Percentage survival for each treatment over the duration of the feeding trial

4.4.1.2 Fish disease

During the initial two-week acclimatisation period fish mortalities increased with the appearance of white growths on the barbels and fins and they were treated by the application of Methylene Blue (0.5 g/1000 L) and Acriflavine HCl (3.0g/1000 L). These reagents were accidentally allowed to circulate through the biofilter, which was then treated with 25 L of a commercial water conditioning mixture (Bactomune®, by NutritionHub) containing a bacterial biofilter inoculum, in order to restore the biofilter before the start of the feeding trial.

Around Day 27 the fish started to present with symptoms of fin rot and distended stomachs, which was diagnosed by the consulting veterinarian as a likely bacterial infection. Since it would take a week to have conclusive laboratory results and mortalities increased daily, oxytetracycline HCl was administered in the system water (36 g/1000 L), with the biofilter shut off. However, mortalities increased as

fish continued to present with ulcerations and distended stomachs (Figure 4.5).

Figure 4.5 Juvenile catfish with typical symptoms (Dropsy) of a bacterial infection

The detailed antibiogram (Appendix I) revealed that the pathogenic bacteria, *Aeromonas spp*. and *Flavobacterium spp*., were resistant to a broad range of antibiotic treatments. *Aeromonas spp*. (resistant to 13 antibiotics) and *Flavobacterium spp*. (5 antibiotics), were also resistant to Oxytetracycline but susceptible to Florphenicol. Florphenicol was subsequently administered in the feed (10mg/kg body mass) over a period of 10 days, under prescription of a veterinarian.

4.4.1.3 Growth performance and feed utilisation

The results of ANOVA on the effect of the treatment diets on final body mass ($p=0.42$) and DWG ($p=0.38$) show no significant differences between the treatment means, due to the fish oil or silage oil diets, when taken over the duration of the trial (Table 4.5). A RMANOVA on the effect of diet on relative feed intake ($p=0.19$), SGR ($p=0.80$) and FCR ($p=0.45$) over the full length of the trial shows no significant differences due to treatments.

Table 4.5 Results of One–way ANOVA and RMANOVA over the full length of the trial

Parameters	Treatments				p-value
	FO-D	LFSO-D	LPSO-D	BACSO-D	
Growth performance					
Body mass (g)					
Initial mass (W_0)	1.4 ± 0.13	1.31 ± 0.11	1.44 ± 0.13	1.31 ± 0.17	0.27
Final mass (W_{92})	48.2 ± 10.4	61.8 ± 19.3	47.8 ± 17.3	55.5 ± 21.5	0.42
$AGR_{0-92;}$ DWG (g/day)	0.5 ± 0.01	0.7 ± 0.04	0.5 ± 0.03	0.6 ± 0.05	0.38
SGR (%BM/day)	4.1 ± 3.6	4.6 ± 3.7	4.2 ± 3.8	4.4 ± 3.9	0.8
Feed utilisation					
RFI	3.4 ± 1.5	4 ± 1.6	4 ± 1.4	3.8 ± 1.5	0.19
FCR	1.5 ± 1.5	1.7 ± 1.4	2.1 ± 1.8	1.7 ± 1.5	0.45

Results of One–way ANOVA of effect of treatment diets on body mass (initial mass, final mass & DWG) and RMANOVA of SGR, RFI & FCR over the full length of the trial. Results are given as means ± SD. Significant differences were evaluated at $p<0.05$.

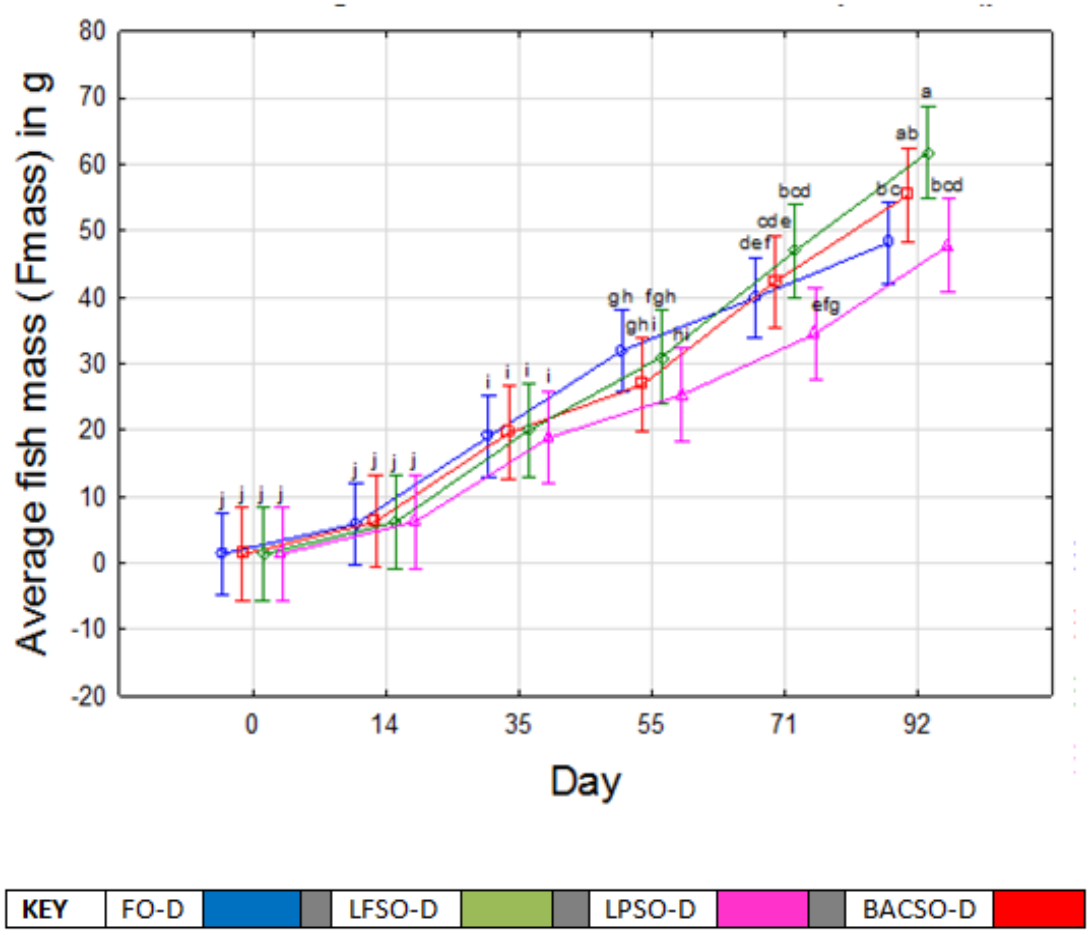


Figure 4.6 Average fish mass (± 95% confidence interval) per treatment showing no significant interactions among treatments over the entire period (p=0.40)

Since the interactions of Fmass between Diet and Time is not significant (p=0.4), the main effects Time and Diet can be interpreted separately. Fmass means do not differ between diets (p=0.28) but differ over time (p<0.001). However, the multiple comparisons of the interactions show that at times t = 71 and t = 92 significant differences appear according to the LSD multiple comparisons test. On day 71 the average fish mass for the LFSO-D diet is significantly different (p= 0.01) to the LPSO-D diet, and on day 91 the response to the LFSO-D diet differs significantly (p= 0.01) from both the LPSO-D diet and the control diet FO-D (p< 0.001).

4.4.1.4 Haematocrit

The haematocrit values are very similar across treatments, all representing about 25 % of the total blood volume (Table 4.6). There is no difference among treatments on haematocrit (p=0.96).

Table 4.6 Haematocrit values (PCV %) taken from one fish per treatment, given as the mean ± SD. Haematocrit values do not differ significantly among treatments (p=0.96)

	Treatments			
	FO-D	**LFSO-D**	**LPSO-D**	**BACSO-D**
PCV (%)	23.0 ± 5.8	24.7 ± 6.2	23.7 ± 6.1	23.7 ± 6.0

4.5 Discussion

There were numerous factors that could have impacted the production performance of the catfish during this trial. These include factors inherent to catfish behaviour such as cannibalism and rapid differential growth, as well as unforseen factors such as RAS repairs, water quality and disease. However, all the treatments were subjected to the same degree of influence by these factors and comparisons between the treatments therefore remain valid.

4.5.1 Fish size and cannibalism

The trial started with an average catfish fingerling of approximately 1.4 grams body mass. This was not desirable since small catfish are susceptible to a higher incidence of cannibalism (Naumowicz, Pajdak, Terech-Majewska & Szarek, 2017; Sallehudin, Yussof, Tan, Saad & Mukai, 2017), which negatively impacts survival. In the latter regard Swanepoel (2017), who also started with small (approximately 1 g) fish, reported 64 % survival after 10 weeks with most deaths ascribed to cannibalism.

Hecht and Appelbaum (1988) indicated that cannibalism stopped having a significant impact on mortality when juvenile catfish reached a total length of about 80 mm, which translates to about 5 g body mass (Luckhoff, 2005). However, even though our catfish reached this average mass by the first sampling (Day 14) there were still many mortalities due to cannibalism after this point.

Apart from a minimum size requirement, this intraspecific aggression is also positively correlated with stocking density and negatively associated with feed availability (Hecht & Appelbaum, 1988), with the optimum feeding rate for improving survival, growth rate and reducing the FCR reported to be 6 % BM/day (Al-Hafedh & Ali, 2004). The feeding rate was kept to 3-4 % BM/day because of poor feeding response of the fish and deteriorating water quality when the higher feeding rate regime was followed. Furthermore, it was shown (Britz & Pienaar, 1992; Mukai, Sanudin, Firdaus & Saad, 2013) that juvenile catfish exhibit faster growth rates, are less territorial and less aggressive, have a lower rate of cannibalism and a higher survival rate when cultured in low-light conditions or total darkness than in high light or continuous light. In this regard, the tops of the tanks were covered in black plastic sheets.

By dividing all the surviving juvenile catfish between three 100 L tanks (and therefore a high stocking density of eight fish/L or 8000 fish/m^3) to fatten them up for the feeding trial they may have, inadvertently, been subjected to enhanced stress and intraspecific aggression, which is positively correlated with stocking density. This could help to explain in part why they presented with skin and fin disease during the acclimation period, in addition to possible transport stress and handling damage.

In the first half of this study type 1 cannibalism was rife among all the treatments, which is where a fish is caught by the tail and consumed headwards until just behind the head (Hossain, Beveridge & Haylor, 1998). This was evident by the daily observations of head skeletons in the tanks during the morning feeding. The cannibalism led to larger than average individuals in some tanks that either continued to prey on the other fish in the tank which reduced the number of fish or consumed most of the food and were aggressive that caused the other fish to hide behind the stand pipe and they consequently grew abnormally slowly. The skin abrasions and nipped fins, resulting from aggressive interactions, were prime sites for the establishment of opportunistic diseases among the fish. The tops of the tanks were covered with 250µ black plastic sheets to reduce the light intensity and aggressive behaviour. When feed was administered at 6 % BM per day it worsened

the water quality, which was already compromised, by turning it a dark brown and leaving a thick layer of organic sediment in the tanks.

The immediate reaction to this poor water quality, and because the fish feeding response was not visible, was to reduce the quantity of feed administered. Also, our province was in the grip of a three-year drought, and in an attempt to conserve water, the tanks were not siphoned frequently enough to reduce the organic load on the system. Through all of this, the fish may have been subjected to intermittent periods of underfeeding.

4.5.2 Production performance

4.5.2.1 Mortalities

There was no significant difference in mortalities between treatments (p=0.34). Since the fish were challenged by a sustained outbreak of *Aeromonas spp*. and *Flavobacterium spp*. pathogens during the trial one might have expected that the silage oils in the diets would offer some level of protection against the bacterial disease. This expectation is supported by research that demonstrated substantial enhancement of the cellular immune function in the abalone, *Haliotis midae*, (Goosen, de Wet and Gorgens, 2014) and Mozambique tilapia, *Oreochromis mossambicus*, (Goosen et al, 2014) when rainbow trout silage oil was included in the diets. Also if some of the colony-forming units and yeasts detected in the fermented silage oil were benevolent species such as *Lactobacilli spp.*, they are generally known to have a beneficial impact on cultured aquaculture species when included in the water or diet (Wang, et al., 2008; Hoseinifar, Sun & Caipang, 2017). Notably the presence of probiotic microorganisms has been shown to inactivate pathogens (Mohapatra, Chakraborty, Kumar, DeBoeck & Mohanta, 2013), enhance survival (Al-Dohail et al., 2009) in catfish, and increase survival rates among *Labeo rohita* when challenged with an *Aeromonas sp*. infection (Giri, Sukumaran & Oviya, 2013).

It was also expected that residues of the organic acids used to produce the organic acid silages would be present in the organic acid silage oils in sufficient concentration to infer a protective advantage to the catfish in those treatments. This is because the protective role of organic acids is widely reported to protect

against pathogenic bacteria in fish intestines (de Wet, 2005; Vázquez, González & Murado, 2005), and lead to improved survival among catfish challenged by Aeromonas sp. (Omosowone, et al., 2015).

Since no benefit to survival resulted from the addition of organic acid and fermented silage oil, one could conclude that the concentration of organic acids and probiotic microorganisms in the silage oil were insufficient to have an effect on mortality.

4.5.2.2 Growth and feed utilisation

Over the duration of the trial there was no difference in growth performance between treatment diets ($p=0.28$). This is a positive result for sustainable practices in aquaculture since the silage oil, derived from fish visceral waste, produced the same amount of growth in the catfish as marine fish oil. Furthermore, post hoc multiple comparisons tests revealed that at the end of the trial (day 92) the mean final mass (61.8g) of fish on the formic acid silage oil enriched diet (LFSO-D) was significantly greater than the mean final mass of fish on the propionic acid silage oil enriched diet (LPSO-D; 47.8 g; $p=0.01$) and the marine fish oil enriched diet (FO-D; 48.2 g; $p<0.001$), but did not differ from fish on the fermented silage oil enriched diet (BACSO-D; 55.5 g). Here, the influence of the organic formic acid in the LFSO-D diet and the possible inclusion of probiotic strains in the BACSO-D diet could have enhanced growth in these treatments. The better growth achieved with the formic acid treatment is supported by the work of de Wet (2005) who found that a blend of sorbic and formic acid (up to 1.5% inclusion) produced significantly better SGR and FCR in Rainbow trout than in a control group, while Christiansen and Luckstadt (2008) also demonstrated improved growth and feed efficiency among Atlantic salmon on potassium diformate. However, Goosen, de Wet and Gorgens (2018), in work with Mozambique tilapia, found that formic acid inclusion had no effect on growth. It is noteworthy that these significant differences between treatments appeared towards the end of the trial, especially over the last 21 days of the feeding trial. One could argue that after the spate of mass mortalities, when the fish numbers were very low, the bacterial diseases appeared to be under control and the water quality was consistently of a better quality that this allowed the fish to grow in response to the effects of their treatment. But, an alternative and

more cautious hypothesis would be that the differences noted could simply be the result of differential mortality among the treatments, where quite by chance certain treatments lost more large fish thus ending up with lower average fish masses, while other treatments lost smaller fish and ended up with higher average fish masses.

The feed utilisation calculations depend on a careful measurement of feed intake by fish in the various treatments (Ruohonen, Kettunen & King, 2008). The intermittent poor water quality and the two outbreaks of disease during the trial made it difficult to deliver optimum quantities of feed and probably also had a negative effect on feed intake (Pillay & Kutty, 2005). As a result, there may have been times when more feed was fed than was consumed. This combination of suboptimal feed intake and therefore growth and the overestimation of feed intake would lead to an inflation of the FCR values obtained. According to Hecht (2013) the expected FCR for juvenile catfish reared intensively ranged from 1 – 1.2, whereas in this study the FCR was considerably higher ranging from 1.5 – 2.0 with a mean of 1.7 ± 0.3. However, for the sake of valid comparisons one could argue that the fish across all the treatments were exposed to the same conditions and therefore that the same biases influenced all the outcomes.

The take-away result for juvenile catfish production on silage oil diets versus marine fish oil diets, taken over the duration of the trial, is that there is no difference in production parameters between treatments This outcome could also be linked to a number of reasons that relate to the nutrient compositions of the competing diets and the ability of the African catfish to convert between certain fatty acid series. The four diets were all formulated to be isonitrogenous and isocalorific (Table 4.4) and an analysis of their fatty acid compositions (Table 4.3) shows that the main difference is that the marine fish oil diet (FO-D) has a higher concentration of omega-3 FAs than the silage oil diets. However, the silage oil diets all have sufficient quantities of Linoleic acid (LA; C18:2n-6) and Alpha-Linolenic Acid (ALA; C18:3n-3), which are the essential fatty acids that it requires to be able to make all the LC-PUFAs, such as EPA and DHA, that it needs for normal metabolic and physiological processes (Sargent, Tocher and Bell, 2002). The African catfish is genetically predisposed to synthesise desaturation and

elongation enzymes that can convert the carbon-18 fatty acids (LA and ALA) into the required LC-PUFAs (Oboh et al 2016, 2017). Resulting from this, the catfish will grow normally and be healthy on either the silage-based or the marine fish oil-based diets; but consumers have another important criterion that the omega-3 fatty acids should be well represented in the fish flesh and not be overwhelmed by omega-6 FAs (Tacon & Metian, 2013). However, flesh fatty acid analyses were not included in this trial and would have to be done in a follow-up study.

The importance of the results on production performance achieved during this trial for the commercial aquaculture farmer is that as the availability of marine fish oil decreases due to increased demand from other sectors and the price increase, a farmer could use silage oil as an alternative to fish oil and achieve the same production results with juvenile catfish. This may have cost-saving benefits for the farmer especially if he produces his own silage oil onsite. Furthermore, since there are preliminary indications that formic acid silage oil demonstrates additional benefits for production, that should be the preferred silage oil to trial.

4.5.3 Haematocrit

The haematocrit value, which is the percentage that the packed cell volume represents of the total blood volume, is one of a suite of haematological parameters that is used as a tool to monitor the health of fish and their response to stressful environments (Jawad, Al-Muktar & Ahmed, 2004; Musa et al, 2013). Blood haematocrit values increase or decrease depending on the physiological or environmental stressor experienced by the fish. Numerous studies show a significant decrease in haematocrit values when catfish are exposed to various environmental toxins, such as potassium permanganate, tobacco leaf dust and the antibiotic chloramphenicol (Korie-Siakperie, Ogbe & Ikomi, 2009; Musa et al, 2013; Nwani et al, 2014). In order to evaluate the haematocrit values obtained in this study to determine to what extent the fish physiology was affected by the water quality and disease outbreaks, a standard value for our size catfish was required. This proved difficult since the literature only had normal haematocrit values for African catfish at sizes that were vastly different to the fish in this study (Afia & David, 2017; Okori-Kanu & Unakalamba, 2015; Erhunmwunse & Ainerua, 2013; Nwani et al, 2014; Musa et al, 2013; Gbemi, M., & Bemigho, R., 2009). The normal

haematocrit values from these studies were therefore plotted against the mean body mass to obtain a curve (Figure 4.7; y = 3.3581ln(x) + 13.783) from which the normal haematocrit values matching the mean catfish sizes in the present study were obtained. The mean haematocrit values derived in this way (FO-D (27 %), LFSO-D (28 %), LPSO-D (27 %) and BACSO-D (27 %)) only differed very slightly from the empirically determined mean values (Table 4.6) but would be found within one standard deviation from the actual means, and do not therefore differ significantly from them.

The haematocrit values of the catfish in each treatment are therefore considered as normal values, albeit slightly lower. This leaning to the lower end of the range could possibly be attributed to the mass mortalities that occurred due to infectious disease and poor water quality episodes throughout the trial; thus reflecting the depressed values associated with disease and compromised health (Nwani et al, 2014). However, the factors that contributed to the PCV being located within the normal range could be a combination of the following: after the last mass mortality on Day 62 there were few fish (<30 %) left in the trial, but still three weeks of the feeding trial left, which meant that the organic loading resulting from leftover feed and faeces was greatly reduced. Furthermore, the bacterial disease appeared to be under control over this last period of 21 days as mortalities were very low. Therefore, either because the fish were not challenged by poor water quality and disease over an extended period or simply because those that survived probably had the more robust immune systems, their haematocrit values fell within the normal range for juvenile African catfish of their size. The conclusion that the PCV values do not differ significantly from the normal range of values for juvenile catfish and because there are no differences between treatments implies that the silage oil diets did not have a negative effect on fish health; a finding supported by Goosen et al, (2014b)

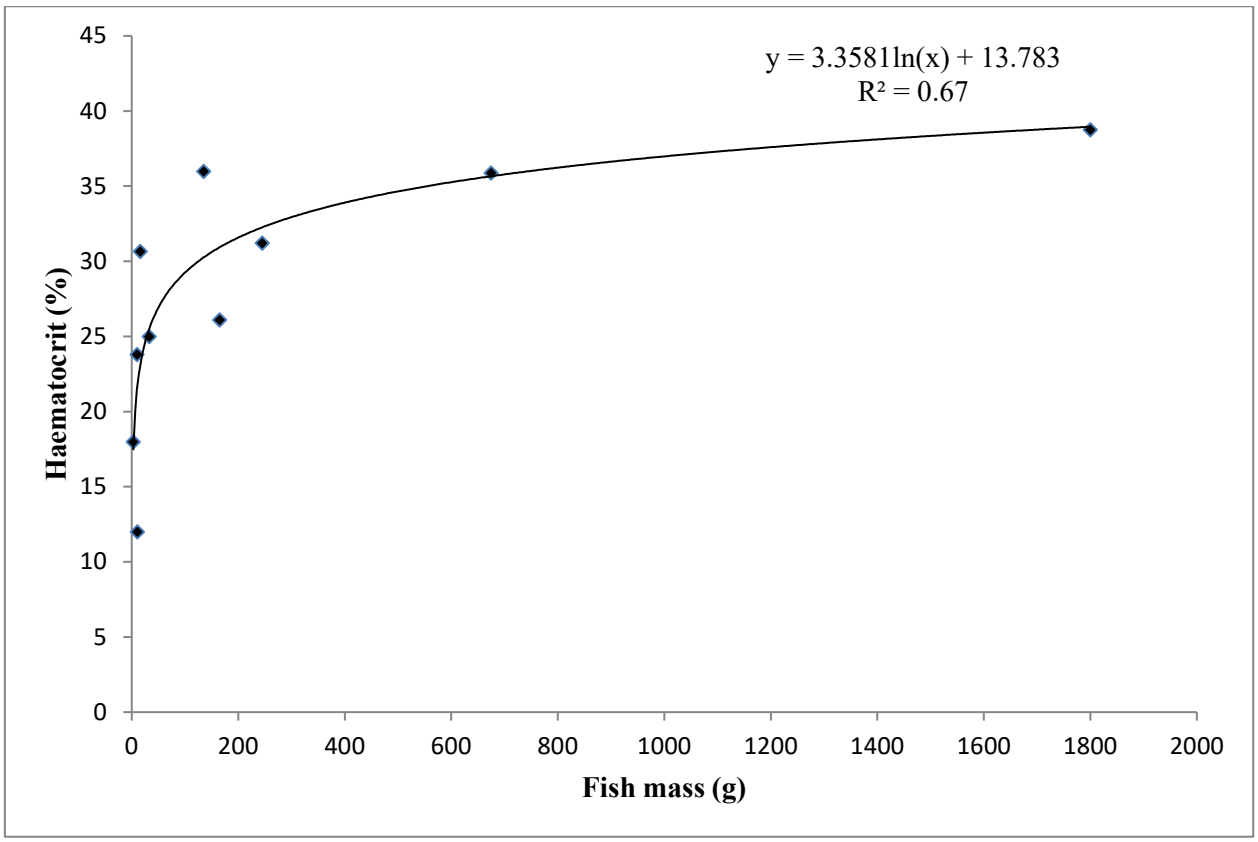


Figure 4.7 Relationship between body mass and haematocrit, in *Clarias gariepinus*

4.6 Conclusion

In conclusion it has been shown over the duration of the trial that there was no difference in growth performance parameters nor feed utilisation of juvenile African catfish on diets containing Rainbow trout silage oil or marine fish oil. This is a positive result for sustainable practices in aquaculture since silage oil derived from fish waste produced equivalent growth as the highly valued marine fish oil. There was no difference in percentage survival among treatments, even though an abnormally high mortality rate occurred resulting from challenges by antibiotic-resistant strains of pathogenic bacteria. Haematocrit (PCV) values do not differ between treatments and are found within the normal range for juvenile catfish, which indicates no negative impact of the silage oils on fish health. Further investigation with older cohorts of fish under conditions of greater biosecurity should also be undertaken, as well as the effect of silage oils on the FA profile of catfish fillets.

4.7 References

Afia, O. E., & David, G. S. (2017). Haematological profile and growth response of african sharptooth catfish (*Clarias gariepinus*, Burchell 1822) fingerlings to locally formulated and commercial pelleted diets in tarpaulin tanks. Am. J. innov. *Res. Appl. Sci., 4(6):*213-222.

Al-Dohail, M. A., Hashim, R., & Aliyu-Paiko, M. (2009). Effects of the probiotic, Lactobacillus acidophilus, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (Clarias gariepinus, Burchell 1822) fingerling. *Aquaculture Research*, *40*(14), 1642-1652.

Al-Hafedh, Y. S., & Ali, S. A. (2004). Effects of feeding on survival, cannibalism, growth and feed conversion of African catfish, Clarias gariepinus (Burchell) in concrete tanks. *Journal of Applied Ichthyology*, *20*(3), 225-227.

Ayinla, O. A., & Akande, G. R. (1988). Growth response of Clarias gariepinus (Burchell 1822) on silage-based diets. NIOMR Technical Paper No. 37. *Nigerian Institute for Oceanography and Marine Research, Lagos. 19pp*.

AOCS (2002). AOCS Official Method Ce 1b-89. Official method for marine oil fatty acid composition by GLC. *Official methods and recommended practices of the American Oil Chemists' Society*.

Borghesi, R., Portz, L., Oetterer, M., & Cyrino, J. E. P. (2008). Apparent digestibility coefficient of protein and amino acids of acid, biological and enzymatic silage for Nile tilapia (Oreochromis niloticus). *Aquaculture Nutrition, 14(3)*, 242-248.

Britz, P. J., & Pienaar, A. G. (1992). Laboratory experiments on the effect of light and cover on the behaviour and growth of African catfish, Clarias gariepinus (Pisces: Clariidae). *Journal of Zoology*, *227*(1), 43-62.

Britz, P.J. & Hecht, T. (1988). Artificial propagation and fry production. In T. Hecht, W. Uys and P. J. Britz, (Eds.), The *culture of sharptooth catfish, Clarias gariepinus in southern Africa*. *South African national scientific programmes, report No. 153, Foundation for Research Development:* CSIR.

Christiansen, R., & Lückstädt, C. (2008). Effects of different dosages of potassium diformate in fishmeal on the performance of Atlantic salmon Salmo salar. Abstract CD-Rom. *World Aquaculture Society* WAS Conference, Busan, Korea, May 2008. Accessed online: http://www.wfish.de/fulltext/WAS08_Potassium-diformate-in-salmon-feed_Lueckstaedt.pdf.

Clewer, A. G., & Scarisbrick, D. H. (2013). *Practical statistics and experimental design for plant and crop science*. John Wiley & Sons

De Wet, L.F. (2005). Organic acids as performance enhancers. Aqua Feeds: Formulation Beyond 2: 2-14.

Erhunmwunse, N., & Ainerua, M. (2013). Characterization of some blood parameters of African Catfish (Clarias gariepinus). *Am Eurasian J Toxicol Sci*, *5*, 72-76.

Fagbenro, O. A., & Fasakin, E. A. (1996). Citric-acid-ensiled poultry viscera as protein supplement for catfish (Clarias gariepinus). *Bioresource technology*, *58*(1), 13-16.

Fagbenro, O., & Jauncey, K. (1994). Growth and protein utilization by juvenile catfish (Clarias gariepinus) fed moist diets containing autolysed protein from stored lactic-acid-fermented fish-silage. *Bioresource Technology*, *48*(1), 43-48.

Fagbenro, O., & Jauncey, K. (1995). Growth and protein utilization by juvenile catfish (Clarias gariepinus) fed dry diets containing co-dried lactic-acid-fermented fish-silage and protein feedstuffs. *Bioresource Technology, 51(1),* 29-35.

Fagbenro, O. A., & Jauncey, K. (1998). Physical and nutritional properties of moist fermented fish silage pellets as a protein supplement for tilapia (Oreochromis niloticus). *Animal feed science and technology*, *71*(1-2), 11-18.

Fagbenro, O., Jauncey, K., & Haylor, G. (1994). Nutritive value of diet containing dried lactic acid fermented fish silage and soybean meal for juvenile Oreochromis niloticus and Clarias gariepinus. *Aquatic Living Resources, 7(2),* 79-85.

Fagbenro, O., Jauncey, K., & Krueger, R. (1997). Nutritive value of dried lactic acid fermented fish silage and soybean meal in dry diets for juvenile catfish, Clarias gariepinus (Burchell, 1822). *Journal of Applied Ichthyology, 13(1),* 27-30.

FAO (2003). Food energy - methods of analysis and conversion factors. FAO FOOD AND NUTRITION PAPER 77. Report of a Technical Workshop, Rome, 3-6 December 2002.

FAO. (2018). The state of world fisheries and aquaculture: meeting the sustainable development goals. Rome. Licence: CCBY-NC-SA 3.0 IGO.

Gbemi, M., & Bemigho, R. (2009). Haematological response of the African catfish: Clarias gariepinus (Burchell, 1822) to sublethal concentrations of potassium permanganate. *Scientific Research and Essays*, *4*(5), 457-466.

Giri, S. S., Sukumaran, V., & Oviya, M. (2013). Potential probiotic Lactobacillus plantarum VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, Labeo rohita. *Fish & shellfish immunology*, *34*(2), 660-666.

Goosen, N. J., de Wet, L. F., & Görgens, J. F. (2014a). Rainbow trout silage oil as immunity enhancing feed ingredient in formulated diets for South African abalone Haliotis midae. *Aquaculture*, *430*, 28-33.

Goosen, N. J., De Wet, L. F., & Görgens, J. F. (2018). Effects of formic acid in abalone diets that contain ingredients derived from fish processing by-products. *Aquaculture International*, *26*(3), 857-868.

Goosen, N. J., de Wet, L. F., Görgens, J. F., Jacobs, K., & de Bruyn, A. (2014b). Fish silage oil from rainbow trout processing waste as alternative to conventional fish oil in formulated diets for Mozambique tilapia Oreochromis mossambicus. *Animal Feed Science and Technology*, *188*, 74-84.

Hecht, T. (2013). A review of on-farm feed management practices for North African catfish (Clarias gariepinus) in sub-Saharan Africa. *On-farm feeding and feed management in aquaculture*, 463-479.

Hecht, T., & Appelbaum, S. (1988). Observations on intraspecific aggression and coeval sibling cannibalism by larval and juvenile Claias gariepinus (Clariidae: Pisces) under controlled conditions. *Journal of Zoology*, *214*(1), 21-44.

Henning, S. S., & Hoffman, L. C. (2017). Proximate and fatty acid composition of cooked South African Cape snoek (Thyrsites atun). *South African Journal of Science*, *113*(5-6), 1-4.

Hoseinifar, S. H., Sun, Y. Z., & Caipang, C. M. (2017). Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view. *Aquaculture Research*, *48*(4), 1380-1391.

Hossain, M. A., Beveridge, M. C., & Haylor, G. S. (1998). The effects of density, light and shelter on the growth and survival of African catfish (Clarias gariepinus Burchell, 1822) fingerlings. *Aquaculture*, *160*(3-4), 251-258.

Huyben, D., Bevan, D., Stevenson, R., Zhou, H., & Moccia, R. (2018). Evaluation of membrane filtration and UV irradiation to control bacterial loads in recirculation aquaculture systems. *Aquaculture International*, *26*(6), 1531-1540.

Isyagi, N.A., Veverica, K.L., Asiimwe, R. & Daniels, W.H. (2009). Manual for the Commercial Pond Production of the African Catfish in Uganda. Cooperative Agreement: 617-A-00-05-00003-00. Department of Fisheries and Allied Aquacultures; Auburn University, Alabama, USA.

Jackson, A. & Newton R.W. (2016). Project to model the use of fisheries by-products in the production of marine ingredients, with special reference to the omega 3 fatty acids EPA and DHA. Institute of aquaculture, University of Stirling and IFFO, the marine ingredients organisation.

Jawad, L. A., Al-Mukhtar, M. A., & Ahmed, H. K. (2004). The relationship between haematocrit and some biological parameters of the Indian shad, Tenualosa ilisha (Family Clupeidae). *Animal Biodiversity and Conservation*, *27*(2), 47-52.

Jenkins, D. J., Sievenpiper, J. L., Pauly, D., Sumaila, U. R., Kendall, C. W., & Mowat, F. M. (2009). Are dietary recommendations for the use of fish oils sustainable?. *Canadian Medical Association Journal*, *180*(6), 633-637.

Kirimi, J. G., Musalia, L. M., Magana, A., & Munguti, J. M. (2016). Performance of Nile tilapia (Oreochromis niloticus) fed diets containing blood meal as a replacement of fish meal. *Journal of Agricultural Science*, *8*(8), 79.

Korie-Siakperie, O., Ogbe, M.G. & Ikomi, R.B. (2009). Haematological response of the African catfish: Clarias gariepinus (Burchell, 1822) to sublethal concentrations of potassium permanganate. *Scientific Research and Essays*, *4*(5), 457-466.

Luckhoff, P.D. (2005). Application of the condition factor in the production of African Sharptooth Catfish (Clarias gariepinus). Master of philosophy thesis in aquaculture, University of Stellenbosch, South Africa.

Lugert, V., Thaller, G., Tetens, J., Schulz, C., & Krieter, J. (2016). A review on fish growth calculation: multiple functions in fish production and their specific application. *Reviews in Aquaculture*, *8*(1), 30-42.

Madage, S. S. K., Medis, W. U. D., & Sultanbawa, Y. (2015). Fish silage as replacement of fishmeal in red tilapia feeds. *Journal of Applied Aquaculture*, *27*(2), 95-106.

McDonald, J.H. (2014). Handbook of Biological Statistics, Third edition. Sparky House Publishing, Baltimore, Maryland.

Miller M.R., Nichols P.D., & Carter C.G. (2011). New Alternative n-3 Long-Chain Polyunsaturated Fatty Acid-Rich Oil Sources. In Turchini, G.M., Ng, W.-K., Tocher, D.R. (Eds.), Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. Taylor & Francis, CRC Press, Boca Raton, pp. 267-324.

Mohapatra, S., Chakraborty, T., Kumar, V., DeBoeck, G., & Mohanta, K. N. (2013). Aquaculture and stress management: a review of probiotic intervention. *Journal of animal physiology and animal nutrition*, *97*(3), 405-430.

Mukai, Y., Sanudin, N., Firdaus, R. F., & Saad, S. (2013). Reduced cannibalistic behavior of African catfish, Clarias gariepinus, larvae under dark and dim conditions. *Zoological science*, *30*(6), 421-424.

Musa, S. M., Aura, C. M., Ogello, E. O., Omondi, R., Charo-Karisa, H., & Munguti, J. M. (2013). Haematological Response of African Catfish (Clarias gariepinus Burchell 1822) Fingerlings Exposed to Different Concentrations of Tobacco (Nicotiana tobaccum) Leaf Dust. *ISRN Zoology*, *2013*.

National Research Council. (2011). *Nutrient requirements of fish and shrimp*. National academies press.

Naumowicz, K., Pajdak, J., Terech-Majewska, E., & Szarek, J. (2017). Intracohort cannibalism and methods for its mitigation in cultured freshwater fish. *Reviews in Fish Biology and Fisheries*, *27*(1), 193-208.

Nwani, C. D., Mkpadobi, B. N., Onyishi, G., Echi, P. C., Chukwuka, C. O., Oluah, S. N., & Ivoke, N. (2014). Changes in behavior and hematological parameters of freshwater African catfish Clarias gariepinus (Burchell 1822) following sublethal exposure to chloramphenicol. *Drug and chemical toxicology*, *37*(1), 107-113.

Oboh, A., Betancor, M. B., Tocher, D. R., & Monroig, O. (2016). Biosynthesis of long-chain polyunsaturated fatty acids in the African catfish Clarias gariepinus: Molecular cloning and functional characterisation of fatty acyl desaturase (fads2) and elongase (elovl2) cDNAs7. *Aquaculture*, *462*, 70-79.

Oboh, A., Navarro, J. C., Tocher, D. R., & Monroig, O. (2017). Elongation of very long-chain (> C24) fatty acids in Clarias gariepinus: Cloning, functional characterization and tissue expression of elovl4 elongases. *Lipids*, *52*(10), 837-848.

Ojogbo, A. I. (2016). Blood Parameters Reference Intervals in African Catfish Clarias gariepinus (Buchell, 1822) in Ibadan, Nigeria. *International Journal of Innovative Research and Development*, *5*(4).

Okorie-Kanu, C. O., & Unakalamba, N. J. (2015). Normal haematological and blood biochemistry values of cultured Clarias gariepinus in Southeast, Nigeria. *Comparative Clinical Pathology*, *24*(6), 1445-1450.

Olsen, R.E., Waagbø, R., Melle, W., Ringø, E., Lall, S.P., 2011. Alternative marine resources, in: Turchini, G.M., Ng, W.-K., Tocher, D.R. (Eds.), Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. Taylor & Francis, CRC Press, Boca Raton, pp. 267-324.

Omosowone, O., Dada, A., & Adeparusi, E. (2015). Effects of dietary supplementation of fumaric acid on growth performance of African catfish *Clarias gariepinus* and *Aeromonas sobria* challenge. *Croatian Journal of Fisheries*, *73*(1), 13-19.

Pauly, D., & Zeller, D. (2016). Catch reconstructions reveal that global marine fisheries catches are higher than reported and declining. *Nature communications*, *7*, ncomms10244.

Pike, I. H. (1990). The Role of Fish Oil in Feeds for Farmed Fish: Estimated Current and Potential Use. International Association of Fish Meal Manufacturers.

Pillay, T. V. R., & Kutty, M. N. (2005). *Aquaculture: principles and practices* (No. Ed. 2). Blackwell publishing.

Ramírez, J. C. R., Ibarra, J. I., Romero, F. A., Ulloa, P. R., Ulloa, J. A., Matsumoto, K. S., ... & Manzano, M. Á. M. (2013). Preparation of biological fish silage and its effect on the performance and meat quality characteristics of quails (Coturnix coturnix japonica). *Brazilian Archives of Biology and Technology, 56(6)*, 1002-1010.

Reifman, A., & Keyton, K. (2010). Winsorize. In N.J. Salkind (Ed.), *Encyclopedia of research design*, *3*, 1636-1638. Thousand Oaks, CA: Sage.

Rothman, K. J. (2010). Curbing type I and type II errors. *European journal of epidemiology*, *25*(4), 223-224.

Ruohonen, K., Kettunen, J. & King, J. (2008). Experimental Design in Feeding Experiments. In Houlihan, D., Boujard, T., & Jobling, M. (Eds.), *Food intake in fish*. John Wiley & Sons.

Sallehudin, M.F., Yusoff, N.A., Tan, N.H., Saad, S. & Mukai, Y. (2017). Optimum light wavelength and light intensity for rearing juvenile African catfish (Clarias gariepinus). *International Journal of Aquatic Science,* 8(2), 107-112.

Sargent, J.R., Tocher, D.R. & Bell, J.G. (2002). The lipids. In J.E. Halver & R.W. Hardy (Eds.), *Fish nutrition* (pp. 182-246). London, U.K.: Academic press.

Sharrer, M. J., Summerfelt, S. T., Bullock, G. L., Gleason, L. E., & Taeuber, J. (2005). Inactivation of bacteria using ultraviolet irradiation in a recirculating salmonid culture system. *Aquacultural Engineering*, *33*(2), 135-149.

Schram, E., Roques, J. A., Abbink, W., Spanings, T., De Vries, P., Bierman, S., ... & Flik, G. (2010). The impact of elevated water ammonia concentration on physiology, growth and feed intake of African catfish (Clarias gariepinus). *Aquaculture*, *306*(1-4), 108-115.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & pharmacotherapy*, *56*(8), 365-379.

Smith, G. (2015). Essential statistics, regression, and econometrics. Academic press.

Soltan, M. A., Hanafy, M. A., & Wafa, M. I. A. (2008). An evaluation of fermented silage made from fish by-products as a feed ingredient for African catfish (Clarias gariepinus). *Global veterinaria*, *2*(2), 80-86.

Soltan, M. A., Fouad, I. M., El-Zyat, A. M., & Zead, M. A. (2017). Possibility of Using Fermented Fish Silage as Feed Ingredient in the Diets of Nile Tilapia, Oreochromis niloticus. *Global veterinaria*, 18(1), 59-67.

Swanepoel, J.C. (2017). Determination of bioavailability and bioactivity of enzymatically hydrolysed fish protein and phosphates in the African catfish Clarias gariepinus. Masters thesis in chemical engineering, University of Stellenbosch, South Africa.

Tacon, A. G., & Metian, M. (2013). Fish matters: importance of aquatic foods in human nutrition and global food supply. *Reviews in Fisheries Science*, *21*(1), 22-38.

Timmons, M. B., & Ebeling, J. M. (2010). *Recirculating aquaculture* (Vol. 126). Ithaca, USA: Cayuga Aqua Ventures.

United Nations, Department of economic and social affairs, population division. (2017). World population prospects: the 2017 revison, key findings and advance tables. ESA/P/WP/248.

Vázquez, J. A., González, M., & Murado, M. A. (2005). Effects of lactic acid bacteria cultures on pathogenic microbiota from fish. *Aquaculture*, *245*(1-4), 149-161.

Vidotti, R. M., Viegas, E. M. M., & Carneiro, D. J. (2003). Amino acid composition of processed fish silage using different raw materials. *Animal Feed Science and Technology*, *105*(1-4), 199-204.

Wang, Y. B., Li, J. R., & Lin, J. (2008). Probiotics in aquaculture: challenges and outlook. *Aquaculture*, *281*(1-4), 1-4.

Woitel, F. R., Trushenski, J. T., Schwarz, M. H., & Jahncke, M. L. (2014). More judicious use of fish oil in cobia feeds: II. Effects of graded fish oil sparing and finishing. *North American Journal of Aquaculture*, *76*(3), 232-241.

Ytrestøyl, T., Aas, T. S., & Åsgård, T. (2015). Utilisation of feed resources in production of Atlantic salmon (Salmo salar) in Norway. *Aquaculture*, *448*, 365-374.

Chapter 5: Conclusion and recommendations

This thesis entered into the debate on sustainability by examining the possibility of recovering fish oil from fish processing waste. The specific aims and objectives of this thesis related to two empirical trials, which involved the removal of fish oil from rainbow trout processing waste by different ensiling processes, and the subsequent evaluation of this recovered fish oil (then referred to as silage oil) in a feeding trial on juvenile African catfish.

The first major aim was to find a sustainable alternative for the Three Streams Trout Smokehouse, a trout processing facility in Franschoek, for dealing with their rainbow trout processing waste; specifically, the trout visceral waste.

The specific challenge was to scale up the production of each rainbow trout viscera silage to approximately 1000L in an intermediate bulk containers (IBC), which would remain stable for at least 30 days while standing outside exposed to ambient weather conditions. The success of the silages would prove the feasibility of the methodology and recipes of reagents used.

All three silage treatments were successfully scaled up to about 1000 L in IBCs. This was in spite of using a fairly small-scale mincer and hand-held mixers, and it proves that this process could easily be the basis of a backyard enterprise. However, the pH of the fermented silage increased above the desired pH 4.5, which is a cause for concern especially since the first batch of fermented silage failed by putrefaction. The recommendation is to increase the percentage inclusion of sugar molasses to between 5 to 10% in order to spike the production of lactic acid early on in the ensiling process. The other imperative was to use only fresh viscera, a condition that is not negotiable since the reagents for a large volume of silage is costly and it is not worth proceeding with viscera that has stood out of refrigeration for a day or overnight. It would be better rather to wait for another fresh batch, and for this reason, the reagents could be added on a proportional basis as fresh minced viscera becomes available. And lastly the need to mix the minced viscera thoroughly with the reagents, as often as possible cannot be over-emphasised, especially initially when fresh material is added daily or hourly. It is also recommended to add sufficient antioxidant to the viscera, right from the start;

in this trial the manufacturer's required dosage was doubled, just to be sure and because rainbow trout viscera is rich in fats and oils. Since fungi and yeasts exceeded the minimum permitted quantities for food grade oil, in the fermented silage, the use of 1 % potassium sorbate is recommended for future silages.

Appreciable volumes of silage oil were produced by all three methods with an average volume of 291 L. However, the simplistic calculation of oil volume, using oil depth x width x breadth, leaves much room for error and would have to be verified in another similar trial, using more acceptable standard methods. Sufficient replicates should be included to establish the variability within and between treatments. In order to differentiate the oil volumes between the ensiling methods it is recommended that several smaller batches of silage are prepared in order to arrive at a reliable and statistically sound answer.

The three silage oils proved to be of a very high quality. Overall the quality of the acid silage oils were marginally better than the fermented silage oil, and it is recommended that they be used preferentially in future trials of this nature. The total plate count and yeast counts of the fermented silage oil are above the permitted quality criteria but they may simply be beneficial microorganisms that formed part of the silage inoculum; further tests to reveal their identity would have to be performed to rule out the possibility that they may be pathogens.

The fatty acid compositions of the three silage oils are virtually identical, which is hardly surprising, since they are all rainbow trout fish oil that has simply been extracted by slightly different means. However, by comparing the formic acid silage oil (LFSO) from this trial with silage oils also derived from rainbow trout viscera, using the same extraction methods in two previous studies, it was found to differ greatly in the percentages for linoleic acid (LA), ecosapentanoic acid (EPA) and docosahexanoic acid (DHA). Silage oils from the other studies had much lower LA and much higher EPA and DHA, which probably points to the slaughtered rainbow trout from the other studies having been fed a diet rich in EPA and DHA (characteristic of a high fish oil inclusion) and in this study the low EPA and DHA and high LA being characteristic of fish diets rich in vegetable oils. It is recommended that researchers entering in this field be aware that even when comparing raw material from the same fish species and using the same chemical

reagents for extraction, additional information, such as diet and gender must be known before comparisons can be made between trials. It is further recommended that any of the three silage oils, when combined with a standard formulated catfish diet, will provide sufficient LA, alpha-linolenic acid (ALA) and long chain polyunsaturated fatty acids (LC-PUFAs) for normal growth and and health. However, the catfish are unlikely to produce flesh with a high omega-3 fatty acid content.

The second major aim was to determine how the three silage oils, when fed as feed oil in a commercial catfish diet, would compare with regular marine fish oil, on the production parameters of juvenile African catfish.

There was no difference in the percentage survival between treatments. The percentage survival was low mainly due to mass mortality caused by antibiotic resistant pathogenic bacterial infections towards the end of the trial. It is recommended that, since the pathogens were probably already system before the trial began, that the recirculating aquaculture system be thoroughly disinfected between consecutive trials.

When taken over the entire duration of the trial, the mean growth performance parameters do not differ significantly between treatments. This is a positive result for sustainable aquaculture since it means that for juvenile African catfish, silage oil derived from rainbow trout visceral waste, could replace marine fish oil in their diets with no negative effect on growth. This finding therefore opens the door for the commercial production of silage oil from rainbow trout viscera and its inclusion in commercial catfish diet formulations.

It is recommended that the extraction of silage oil from the viscera of other fish species, especially marine fish because of their higher LC-PUFAs, is investigated. These could then be trialled on the growth performance of the most commonly farmed fish in South Africa, such as rainbow trout, tilapia and catfish. However, the result of this trial only really applies to juvenile catfish in the size range between 1.4 to 53.5 g that are raised in a temperature-controlled recirculation aquaculture system. To extrapolate these results with confidence, it is recommended that similar feeding trials that use diets containing these silage oils are conducted with

larger sized catfish and in different commercial rearing systems, such as earthen dams and tanks, at commercial stocking densities.

The dietary treatment prepared with formic acid (LFSO-D) showed significantly better growth (average fish mass; Fmass) on day 71 than the propionic acid diet (LPSO-D), and at the last sample (day 92) it performed significantly better than both the propionic acid (LPSO-D) and the marine fish oil control (FO-D) diets. Since organic acid inclusion in fish diets have shown positive growth effects in the literature, it is therefore recommended that further feeding trials with catfish are conducted mainly with the LFSO-D diet. However, caution must be exercised in making too much of these significant results, especially since they appear after a series of extreme mass mortality events, and only at these specific points in time but are not significant over the full duration of the trial.

When taken over the entire duration of the trial, the mean feed conversion ratio does not differ significantly between treatments. This is once again a good result for sustainable aquaculture since it means that African catfish could be raised on aquafeeds where silage oil derived from rainbow trout visceral waste has replaced marine fish oil, with no negative effect on the feed conversion ratio. However, the bacterial infections that challenged the trial led to the sick fish probably not eating as they normally would. As a result, the feed fed was unlikely to equal the feed taken in by the fish. So, a combination of poor growth due to illness and an inflated feed intake would result in inflated FCRs.

Once again it is recommended that the feeding trial is repeated under disease-free conditions to arrive at a more reliable result.

Future research and additional recommendations

While it was not a specific objective of this thesis, the potential of using the by-products of ensiling, namely the silage oil and fish protein hydrolysate, to establish small businesses has been a constant thought, which fits into the sustainable aquaculture theme. Good quality oil has numerous uses, such as a biofeed which was already demonstrated, and the production of biofuels and various soap-based products. The fish protein hydrolysate has a niche market mainly for use as a biofertilser, soil and compost conditioner in the horticulture and aquaponics

enterprises. Entrepreneurship and enterprises are only successful when they are based on sound knowledge and business principles. Therefore, there is a need to develop a full business plan to test the viability and sustainability of silage-based enterprises; these could use either fish waste, or poultry, or other slaughter waste as raw materials. Various business plans should be drafted and tested to satisfy different scales of operation; for example, an individual may buy 1000 L of trout viscera from Three Streams, hire a small industrial mincer, buy the organic acids and make the silage in an IBC in his backyard; while an NPO in Saldanha Bay may apply for millions of rand in grant funding, and start an industrial scale enterprise that requires large mincers that can work through 1000s of litres of waste per hour.

The recommended silage treatments for further research, including sufficient replicates and tests for FA profiles of fish fillets are: the lactic x formic acid for its quality, and its use as a feed oil because it showed a better growth performance on days 71 and 92 of the trial. For volume of silage oil, the fermented silage would be chosen because it produced the greatest volume of oil but it also performed better than the lactic x propionic acid silage on days 71 and 92 of the growth trials.

Even when 1000L volumes of silage treatments are made, additional replicates of a smaller volume, for example three 25L containers, of the same raw materials and proportion of reagents, should always be made. This will ensure that the results obtained can be backed by statistical rigor.

Key message

Rainbow trout visceral waste, especially where it is available in remote areas, in relatively small and irregular supply, can be treated by ensiling to preserve it against putrefaction and convert it into useful by-products.

Large volumes, up to 1000 L, of organic acid (lactic x formic; lactic x propionic) or fermented (*Lactobacilli spp.* x molasses) silages, using minced rainbow trout viscera as raw material, can be made successfully, using readily obtainable local apparatus.

These silages produce large volumes of silage oil and fish protein hydrolysates that are valuable by-products that could form the basis of various small businesses.

The silage oil produced is of a very good quality (the acid silage oil more so than the fermented silage oil), it is rich in polyunsaturated fatty acids and can successfully replace marine fish oil as a feed oil in aquafeeds for African catfish.

Juvenile African catfish grow equally well on diets using silage oils as feed oil as they do on marine fish oil.

The findings of this study support the goals of sustainable aquaculture in as far as it promotes environmental sustainability, and suggests small-scale enterprises for socio-economic sustainability.

Appendix 1

Antibiogram of antibiotic resistant bacteria in Welgevallen RAS

Wemmershoek Diagnostic Laboratory Culture and Antibiogram Results

Date: **17 June 2017**
Case no: **WDL171610 (submitted on 13 June 2017)**
Sample type: **Whole, juvenile catfish (morbid)**
Patient name: ***Clarias* sp. (juvenile)** Owner: **Dep. Animal Science** Company/farm: **Stellenbosch University** Contact person: **Henk Stander**
Contact details: Tel. Office **021 808 2544** Cell. **082 331 8761** Fax. e-mail: **hbs@sun.ac.za**
History:

Catfish 'fingerlings' displaying symptoms of disease – abnormal mortality observed in the recirculating system.

Bacterial ID: Organism A – *Aeromonas* spp. +++
Organism B – *Flavobacterium* sp. +++

	A Sensitivity	**B Sensitivity**	**Sensitivity**	**Sensitivity**	**Sensitivity**
Penicillin G (PG 10 U)	R	R			
Cephalexin (CFX 30 µg)	R	R			
Cefoxitin (FOX 30 µg)	S	S			
Cephalothin (KF 30 µg)	S	S			
Ceftiofur (CPZ 30 µg)	R	R			
Florphenicol (FFC 30 µg)	S	S			
Chloramphenicol (CHL 30 µg)	S	S			
Doxycycline (DXT 30 µg)	R	S			
Oxytetracycline (OT 30 µg)	R	R			
Erythromycin (E 15 µg)	R	S			
Clindamycin (CD 2 µg)	R	R			
Enrofloxacin (ENF 5 µg)	S	S			
Marbofloxacin (MAR 5 µg)	S	S			
Trimethoprim-sulfam ethoxazol e	R	S			

Amoxicillin (A 25 μg)	R	I			
Amoxicillin-clavulanic acid (AMC 20-10 μg)	S	S			
Gentamicin (GM 30 μg)	S	S			
Neomycin (NE 30 μg)	R	S			
Amikacin (AK 30 μg)	R	S			
Imipenem (IMI 10 μg)	S	S			
Azithromycin ATH (15 μg)	S	S			
Clarithromycin (CLA 15 μg)	R	S			
Rifampin RF (5 μg)	R	S			
Polymyxin B (PB 300 U)	S	S			

R = Resistant
S = Sensitive
I = Inhibitory/NOT recommended
NA = Not Available

Recommended antibiotic(s): the *Aeromonas* spp. and Flavobacterium sp. displayed mutual sensitivity to only Florphenicol as a relevant and approved antibiotic for use in aquaculture (FDA standards & DAFF regulations). Florphenicol is the drug of choice.

Comments and recommendations: *Aeromonas* spp.and a *Flavobacterium* sp.isolate were obtained in very high numbers from gill and abdominal cavity samples. The organisms were considered significant in this case due to their abundance in culture as well as dropsy symptoms (associated with especially *Aeromonas hydrophila*), and fin and gill erosion (associated with *Flavobacterium* spp. fish diseases) observed in the fish. Florphenicol is recommended for treatment in this case.

Tested by Dr. Leonard Flemming at the Wemmershoek Diagnostic Laboratory
(PhD Microbiology; SAVC authorisation no. AR14/13083)

Dr. Andrew Gray
(BVSc, veterinarian at the Wemmershoek Diagnostic Laboratory)