Optimisation of nutrient input to integrated aquaponics systems through mineral supplementation by way of fish feed additives

by

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Declaration

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Abstract

Aquaponics is an integrated production system with the primary goal of sustainable food production in the form of fish and vegetables. The main challenge in aquaponics is the imbalance of nutrients between the fish and plants grown in the system as each has different nutritional requirements. The requirements of fish are met through fish feed and those of plants by supplementing nutrients, especially trace elements, through nutrient solutions, which adds extra costs to the production system. Therefore, the aim of this study is to design a feed to fulfil a dual role: provide optimal nutrition to the fish and optimise plant production in integrated aquaponics systems. The aims were met by i) determining whether dietary supplementation of minerals through different feed additives in a recirculating aquaculture system can benefit the production performance and haematological profile of the African catfish, ii) determining whether dietary supplementation of minerals through different feed additives in a recirculating aquaculture system can enhance the excretion of iron in wastewater for ultimate use in aquaponics systems, and ultimately iii) evaluating the performance of the feed additives using the African catfish in combination with lettuce in an integrated aquaponics system.

The inclusion of feed additives, potassium, and iron in the diet of the African catfish improved its haematological profile and excreted wastewater with high concentrations of potassium and iron in a recirculating aquaculture system. Fish production and non-specific immunity were not affected by the inclusion of different additives. Further investigations into an integrated aquaponics system revealed that the inclusion of these feed additives at the right inclusion level in the diet of the African catfish improved lettuce growth. The high concentrations of potassium and iron excreted from the supplemented feed were absorbed by the lettuce in the aquaponics system, resulting in improved growth when compared to the control treatment.

From these results, it can be concluded that the addition of minerals through fish feed additives can reduce or even eliminate the need to supplement plants with nutrients in the form of nutrient solutions. The improvement of plant growth through dietary feed additives of fish in aquaponics systems can improve the efficiency of integrated aquaponics production systems. These results contribute to the improvement of the overall performance of the aquaponics system and the production of the African catfish in recirculating systems.

Opsomming

Akwaponika is ’n geïntegreerde produksiestelsel met die primêre doel van volhoubare voedselproduksie in die vorm van vis en groente. Die hoof uitdaging in akwaponika is die wanbalans van nutriënte tussen die visse en plante wat in die stelsel groei omdat elkeen verskillende voedingsvereistes het. Die vereistes van visse word bevredig deur visvoer en dié van die plante deur supplementêre nutriënte, veral spoorelemente, deur nutriëntoplossings wat ekstra kostes by die produksiestelsel bydra. Daarom is die doel van hierdie studie om ’n voer te ontwerp wat ’n dubbele doel dien: om optimale nutriënte aan die visse te verskaf en plantproduksie te optimeer in geïntegreerde akwaponikastelsels. Die doelwitte is bereik deur i) te bepaal of dieetkundige aanvulling van minerale deur verskillende voerbymiddels in ’n hersirkulerende akwakultuurstelsel die produksiedoeltreffendheid en hematologiese profiel van die Afrika-baber kan bevoordeel, ii) om te bepaal of dieetkundige aanvulling van minerale deur verskillende voerbymiddels in ’n hersirkulerende akwakultuurstelsel die ekskresie van yster in afvalwater, vir die uiteindelike gebruik in akwaponikastelsels, kan verbeter, en eindelik iii) die doeltreffendheid van die voerbymiddels te evalueer deur die Afrika-baber in kombinasie met blaarslaai te gebruik in ’n geïntegreerde akwaponikastelsel.

Die insluiting van voerbymiddels, kalium en yster in die dieet van die Afrika-baber het sy hematologiese profiel en uitgeskeide afvalwater met hoë konsentrasies van kalium en yster in ’n hersirkulerende akwakultuurstelsel verbeter. Visproduksie en nie-spesifieke immuniteit is nie geaffekteer deur die insluiting van verskillende bymiddels nie. Verdere ondersoeke in ’n geïntegreerde akwaponikastelsel het gewys dat die insluiting van hierdie voerbymiddels by die regte insluitingsvlak in die dieet van die Afrika-baber die blaarslaai se groei verbeter het. Die hoë konsentrasies kalium en yster uitgeskei van die gesupplementeerde voer is geabsorbeer deur die blaarslaai in die akwaponikastelsel, wat verbeterde groei tot gevolg het as dit met die gekontroleerde behandeling vergelyk word.

Uit hierdie resultate kan dit afgelei word dat die byvoeging van minerale deur visvoerbymiddels die benodigheid om plante met nutriënte te supplementeer in die vorm van nutriëntoplossings, te verminder of selfs te elimineer. Die verbetering van plantegroei deur dieetkundige voerbymiddels van visse in akwaponikastelsels kan die doeltreffendheid van geïntegreerde akwaponikaproduksiestelsels verbeter. Hierdie resultate dra by tot die

verbetering van die algehele doeltreffendheid en die produksie van die Afrika-baber in hersirkulerende stelsels.

Dedication

This dissertation is dedicated to my dearly departed aunts and uncle, Nonzelakhe Siqwepu, Lulama Siqwepu and Inathi Thobi.

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List of Abbreviations

ADC	Apparent digestibility coefficient
ANOVA	Analysis of variance
B	Boron
Ca	Calcium
$C_2H_3KO_4$	Potassium diformate
Cr_2O_3	Chromium (III) oxide
Cl	Chlorine
Cu	Copper
DO	Dissolved oxygen
DWC	Deep water culture
DTPA	Diethylenetriamine pentaacetic acid
EC	Electric conductivity
EDTA	Ethylene diamine tetra acetate
f	Final
fl	Femolitre
FCR	Feed conversion ratio
Fe	Iron
FeAA	Amino acid chelated iron
$FeSO_4$	Iron sulphate
g	Gram
Hb	Haemoglobin
HCT	Haematocrit
HSI	Hepatosomatic index
i	Initial
K	Potassium
KCl	Potassium chloride
KDF	Potassium diformate
Kg	Kilogram
KW	Kilowatt
L	Litre
LLDPE	Linear low-density polyethylene
LSD	Least significant differences
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular and haemoglobin concentration
MCV	Mean corpuscular volume
MDCP	Monodicalcium phosphate

Mg	Magnesium
mg	Milligram
mm	Millimetre
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
NFT	Nutrient filter technique
NH_3 –N	Ammonia nitrogen
NH_4	Ammonium
NO_2-N	Nitrite-Nitrogen
NO_3-N	Nitrate-Nitrogen
P	Phosphorus
PEG	Poly-ethylene glycol
pg	Picogram
PO_4^{-3}-P	Phosphate-phosphorus
RAS	Recirculating aquaculture system
RBC	Red blood cells
rpm	Revolutions per minute
S	Sulphur
SE	Standard error
SGR	Specific growth rate
TAN	Total ammonia nitrogen
TDS	Total dissolved solids
TSS	Total suspended solids
v	Volume
µ	Micro
WBC	White blood cells
w	Weight
Zn	Zinc

Chapter 1

1.1 Introduction

Aquaponics is an integrated production system combining aquaculture, the fastest growing food production sector in the world (Endut et al., 2010), and hydroponics, the cultivation of plants in soilless media (Palm et al., 2014a; FAO, 2016). Previously, these two systems of production have been practised separately. The sole practise of either of the production systems has been characterised and yielded several benefits, with aquaculture contributing to the total food production for the increasing global population, which has been projected to reach 9.6 billion by 2050 (FAO, 2016). Conversely, it has been shown that the combination of the two systems may be more beneficial, contrary to the practice of production in separate systems of production (Blidariu and Grozea, 2011). Aquaponics offers an integrated system in which a symbiotic relationship exists between the organisms being produced (Goddek et al., 2015).

The advantage of aquaponics over conventional production systems is that this integrated system uses waste nutrients from the fish production as input to the plant production system. It has the potential further to lessen freshwater stresses resulting from the excess abstraction of water resources for agriculture through irrigation. Uneaten feed, metabolites, and faecal matter released by fish produce nutrient-rich water containing NH_3 –N, NO_2-N, NO_3-N, and PO_4^{-3}-P (Saufie et al., 2015) that promotes plant growth (Endut et al., 2010; Liang and Chien, 2013).

Aquaponics has ecological advantages over aquaculture and hydroponics, which include the shared infrastructural costs of growing fish and plants, water is saved because it is reused, waste produced from aquaculture is managed well, it saves on artificial fertilizers for plants grown on the hydroponics system, and the economic benefits from producing two types of products from a single system, i.e. fish and plants (Blidariu and Grozea, 2011; Liang and Chien, 2013; Palm et al., 2014a). Aquaponics systems have further advantages in that they provide versatility in production where certain vegetables can be grown in uncommon locations, such as urban areas and areas where the soil is poor (FAO, 2016; Van Woensel and Acher, 2015). The transport distance of produce from the production site to final consumption can be

reduced, providing customers with fresher products (Goddek et al., 2015). Aquaponics systems provide an alternative solution to the conventional management of water quality in recirculating aquaculture systems (RAS) (Endut et al., 2010). Ammonia from fish waste and gill excretion can accumulate and reach toxic levels if the water is not changed frequently in the system (Somerville et al, 2014) and this problem is addressed through biofiltration in conventional RAS. In aquaponics, the water quality management is achieved by plants that are eventually harvested as a crop, thereby providing additional income to the operation. This is contrary to conventional RAS, where water quality management is seen solely as an unavoidable expense (Endut et al., 2010).

Aquaponics offers the opportunity to enhance food production at low fertilizer and water usage, especially in environments with freshwater depletion (Pantanella et al., 2012; FAO, 2016). This system has significant social advantages because it has the potential to enhance food security for increasing populations by meeting the requirements for animal protein and vegetables simultaneously (Goddek et al., 2015; FAO, 2016). It can also secure food and income for poor and landless households (Somerville et al., 2014).

Aquaponics systems face several challenges that make it difficult for the system to be viable and profitable. For example, aquaponics carries the risks of both aquaculture and hydroponics, and there may also be difficulty in obtaining an optimum nutrient balance between fish and plants in the system (Somerville et al., 2014).

One of the main challenges experienced in aquaponics systems is to optimise nutrient levels in the wastewater to achieve maximum plant production rates. Fish feed is designed to provide optimal nutrition to the fish in aquaculture systems, but not to provide optimal plant nutrients when excreted. Fish excreta can therefore limit plant growth, causing the need for nutrient supplementation. For example, small quantities of nutrients like potassium, sulphur, magnesium, and iron are added to an aquaponics system to increase the electric conductivity of the circulating water (EC) and to obtain a balanced nutrient profile sufficient for good plant growth (Pantanella et al., 2012). A high EC is indicative of increased nutrients available in the system for plants, generally a high EC is better for plant growth (Somerville et al., 2014). It is important to have a balance between the waste produced by fish and the mineral requirements of plants in an aquaponics system (Nichols and Savidov, 2012).

Adjusting nutrients or minerals to optimise plant growth is a complex subject. Nutrients (especially micronutrients) made available to plants either by fertilizer supplementation or through fish excrement (and by extension, through the fish feed), need to be balanced and studied in relation to other nutrients, as the abundance of one may affect the uptake of another (Voogt, 2002; Rakocy et al., 2006; Goddek et al., 2015). Therefore, it is not only the quality of single nutrients that are important in aquaponics systems, but also the presence and/or levels of other nutrients in the system. For example, if potassium is in excess in plants it will affect the uptake of magnesium or calcium, while either of these nutrients can affect the uptake of potassium (Voogt, 2002; Goddek et al., 2015). Similarly, excess amounts of phosphorous in plants decrease the availability of iron and zinc (Bugbee, 2004).

The feed provided to fish in aquaponics systems represents the primary nutrient input into this integrated system. Therefore, the macro and micronutrients that are excreted by the fish as a result of the particular composition of the feed need to be understood to determine whether these will meet the needs of plants that are cultivated. The need to adjust ratios or supplement additional nutrients may result in additional costs to aquaponics (Goddek et al., 2015). If the nutritional demands of plants are not met, nutrient deficiencies, leading to poor plant production result. Symptoms revealing susceptibility to a range of diseases, including chlorosis of the leaves in leafy plants (Rakocy et al., 2004) or blossom-end rot in fruiting plants appear (Sonneveld and Welles, 1988).

In an ideal aquaponic system, the fish feed needs to fulfil a dual role by providing optimal nutrition to both fish and plants once it has been digested and excreted by the fish. The main nutrients provided to fish via feed are proteins, amino acids, lipids, carbohydrates, minerals, and vitamins. When the feed has been digested and metabolised by the fish, the nutrients that can be utilised by plants such as ammonia (NH_3) and phosphorus (P) are excreted in the form of faeces and urine. While trace elements are also excreted, they are not excreted in sufficient levels for plant production (Somerville et al., 2014).

To summarise, aquaponics is an integrated production system with the main goal of sustainable food production in the form of fish and vegetables (Goddek et al., 2019). This system has many advantages and represents a solution to conventional management of water quality in RAS by way of wastewater uptake by plants (Endut et al., 2010). However, it faces several challenges, mainly the imbalance of nutrient requirements between fish and plants

grown in the system. Therefore, there is a need to optimise nutrient levels in wastewater to maximise plant production because fish feed only provides nutrients that optimise fish production. There is, therefore, a need to design feeds that will meet the nutritional needs and optimise the production of both fish and plants. The addition of such elements to fish feed could reduce or even eliminate the need to supplement plants with these nutrients in the form of nutrient solutions.

This dissertation is divided into 4 experimental chapters (chapters 4 – 7). Chapter 1 covers the introduction to the study. Chapter 2 details the literature survey and the conclusions thereof. The aim and objectives of this study are discussed in chapter 3, along with the design of the study. Chapter 4 presents the novel contributions of this study. The fifth and sixth chapters detail the evaluation of potassium diformate and potassium chloride and the evaluation of chelated iron and iron sulphate in the diets of the African catfish in a recirculating aquaculture system, respectively. Chapter 7 discusses potassium diformate and chapter 8 details iron sulphate supplemented as mineral sources in the diet of the African catfish for production in aquaponics systems in combination with lettuce. Two of the chapters (Chapter 5 and 6) are written in manuscript format as they are already published and the remainder will be submitted for publication (Chapter 7 and 8). The lists of tables, figures, and abbreviations are presented at the beginning of the dissertation. Appendices A and B present the ethical clearance for use of the African catfish in the experiments and the temperature, pH, and DO data collected during the aquaponics trials, respectively.

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Chapter 2 Literature survey

2.1 General description of aquaponics systems

Integrating aquaculture with hydroponics results in the use of nutrient-rich effluent that is excreted during fish production (Figure 2.1). In aquaculture, a large portion (up to 36 %) of the nutrients in fish feed remain unused and are excreted in the form of organic waste. Approximately 75 % of the feed nitrogen and phosphorus remain unused and excreted as waste products in the water, thereby producing nutrient-rich effluent that can be used for plant production in aquaponics (Endut et al., 2011).

Poor nutrient utilisation from aquaculture feeds result in economic loss, along with high-nutrient effluent. Protein losses are expensive, as protein is the most expensive nutrient in formulated aquafeeds. Feed makes up to 40 – 60 % of the costs in aquaculture production, which are expected to increase (Fagbenro, 1998; Sofia, 2015; FAO, 2016). Therefore, the integration of aquaculture with hydroponics where the effluent of the aquaculture acts as input into the hydroponic plant production makes environmental sense.

Aquaponics systems consist of either single-loop (coupled) (Figure 2.1) or multiple-loop (decoupled) systems. Most aquaponics systems are single-loop systems, which circulate water between the aquaculture and hydroponic components in the system in a single loop (Goddek et al., 2015; Goddek et al., 2016; Monsees et al., 2017; Goddek and Körner, 2019). However, plants and fish have different biological and nutritional requirements. Because these systems share the same water, a compromise must be reached in terms of pH, temperature, and nutrients (Goddek et al., 2015; Monsees et al., 2017). In contrast, decoupled multi-looped systems separate the aquaculture and hydroponic components. The separation of components in decoupled systems allows for better and more independent control of the system for fish and plants, allowing the specific requirements of fish and plants to be met (Monsees et al., 2017; Goddek and Körner, 2019). These requirements include optimum growth conditions such as pH, temperature, and nutrient concentrations (Goddek and Körner, 2019). The use of single-loop systems has limited the diversity in aquaponics production, whereas multiple-loop systems allow for a variety of fish species and plants to be produced (Monsees et al., 2017). Increased plant harvests have been observed in multi-loop decoupled

systems compared to single-loop systems even when similar nutrient fertilizers were used. This is because the pH in a single-loop system is not always optimum for the uptake of nutrients by plants (Monsees et al., 2017).

Although multi-loop decoupled systems provide the advantage of optimal fish and plant production, single-loop systems are still used because multi-loop decoupled systems require an additional level of technological sophistication which is expensive and complex. Moreover, a multi-loop decoupled system may have considerably higher labour requirements compared to a single-loop system (Monsees et al., 2017). Single-loop recirculating aquaculture systems (RAS) reuse water in the system, resulting in less than 10 % water volume replacement per day (Blidariu and Grozea, 2011). Moreover, it can be conducted at varying fish stocking densities and plant growing areas, depending on how much water is recirculated in the system (Bregnballe, 2015).

In integrated systems, different types of aquaponics system designs are used to ensure that nutrient-enriched water from fish reaches the plants (Palm et al., 2014b). The three most frequently used growth systems are media-based beds, deep water culture (DWC) beds, and nutrient film technique (NFT). Media-based beds generally use gravel, sand, expanded clay, perlite, and pumice as support systems, a filtration unit, and a surface for microbial growth. DWC and rafts are generally used to grow a diverse number of plants (Palm et al., 2014b). In some growth media, roots can either be periodically surrounded by water (ebb and flow) or constantly surrounded by nutrients (aggregate system) (Palm et al., 2014a; b).

Each of the growth systems used in aquaponics has advantages and disadvantages. For example, gravel is good because it provides aeration for plant roots, however, it is heavy and requires a strong support system and clogs regularly, the roots and microbial growth may remain after harvesting, and it is difficult to clean (Rakocy et al., 2006; Goddek et al., 2015). Coarse sand is commonly a good substrate for growth and reduces the potential for clogging. Sand may also act as a substrate for nitrifying bacteria, therefore eliminating the need for a separate bio-filter (Lennard and Leonard, 2006). In an experiment by Sikawa and Yakupitiyage (2010), sand growth media resulted in high yields of plants compared to gravel and Styrofoam growth media. In raft hydroponics (floating), plant roots are submerged directly in the nutrient solution. Its advantage is that it is easy to install and manage, however, there are higher chances of diseases because the roots are entirely submerged in water (Saufie et al., 2015).

Generally, there is no optimal aquaponics system, because each system must be adjusted to environmental conditions (Goddek et al., 2015).

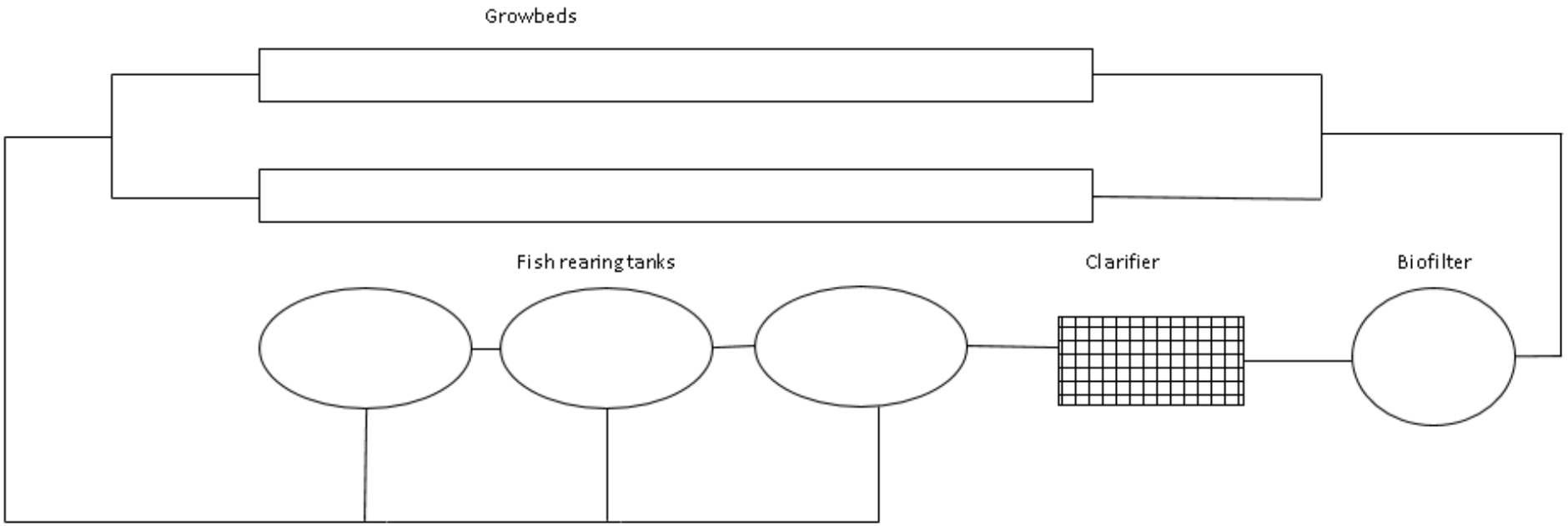

Figure 2-1 Schematic diagram of an integrated recirculating aquaponics system: fish rearing tanks, a clarifier, and a biofilter connected to hydroponic plant growing beds.

2.2 Nitrogen transformation in aquaponics systems

In aquaponics systems, the main source of nitrogen is through fish feed, which is excreted by fish as waste in the form of ammonia nitrogen (TAN, i.e., NH_3 and NH_4^+) (Hu et al., 2015; Wongkiew et al., 2017). Plants absorb ammonium (NH_4^+) and the unionised ammonia (NH_3) is used during nitrification (Tyson et al., 2011). Ammonia is converted through nitrification into nitrate that can be assimilated by plants in the hydroponic component of the system. In most systems, biofilters are used to convert ammonia to nitrite and subsequently nitrate by aerobic bacteria. Biofilters are surfaces or filter media that can be colonised by nitrifying bacteria. However, biofiltration can occur in various places in the system, which includes pipes and tank walls (Tyson et al., 2011). Nitrifying bacteria have also been found on the surface of plant roots, suggesting that nitrification occurs in the plant root area when dissolved oxygen levels are sufficient (Hu et al., 2015; Wongkiew et al., 2017). Nitrifying bacteria play a significant role in the nitrogen cycle in aquaponics systems (Hu et al., 2015). During nitrification, ammonia oxidising bacteria, mainly *Nitrosomonas* sp., convert ammonia to nitrite, then nitrite oxidising bacteria, while *Nitrobacter* sp. converts nitrite to nitrate in the presence of oxygen (Blidariu and Grozea, 2011, Wongkiew et al., 2017). Ammonia and nitrite need to be maintained at low concentrations as they can be toxic to both fish in plants at high levels. If nitrification is insufficient in the system, which may result at pH levels below 6.4 and above 9.4, ammonia can build up to toxic levels (Tyson et al., 2004, 2007; Wongkiew et al., 2017).

The uptake of nitrogen by plants is influenced by light intensity, temperature, pH, dissolved oxygen, and nutrient concentrations (Seawright et al., 1998; Wongkiew et al., 2017). In aquaponics systems, pH is the main factor that affects the availability of nitrogen and nutrients required by plants. The bioavailability of nutrients such as potassium, calcium, and phosphorus also depends on the pH of water in the root zone (Tyson et al., 2011, 2007; Zou et al., 2016; Wongkiew et al., 2017). Dissolved oxygen levels and high temperatures may also affect nitrogen loss through denitrification and nutrient uptake. As a result, it is suggested that DO levels are maintained above 5 mgl^{-1} in fish-rearing tanks and plant-growing beds (Rakocy, 2007; Graber and Junge, 2009). Dissolved oxygen levels decrease mainly in the biofilter and root zone, resulting in nitrogen loss and root rot, especially at high temperatures (Rakocy, 2007). Air blowers are used in the aquaponics system to minimise anoxic regions, decrease root rot, and optimise DO levels (Wongkiew et al., 2017).

When anoxic regions occur due to the feed and organic waste not being completely broken down, they accumulate and some of these dissolved organic metabolites may change the water colour to brown or tea colour (Tidwell, 2012). These dissolved metabolites contain organic acids such as tannic acid, humic acid and other organic acids. Humic compounds have the ability to form metalo-organic complexes with minerals such as Fe, Zn and Mn. This complex increased the availability of these minerals to plants in the system (Tidwell, 2012). In this systems, mineral transport is facilitated by the presence of organic acids and slighty acid pH.

Nitrifying bacteria are the most studied microorganisms in aquaponics systems. However, nitrifying bacteria co-exist with heterotrophic aerobic bacteria in aquaponics systems. (Schmautz et al., 2017; Goddek et al., 2019). Heterotrophic bacteria increase with increasing concentrations of organic carbon or C:N ratio. They contribute to the major quantity of microbial biomass production in aquaponics systems (Wongkiew et al., 2017; Goddek et al., 2019). Along with organic carbon, these bacteria use NH_4^+ and NO_3^- for growth. The accumulation of organic matter in the system may result in the presence of these highly competitive heterotrophic bacteria. These include species such as *Pseudomonas*, *Bacillus*, *Enterobacter*, *Streptomyces* and *Trichoderma* (Yep and Zheng, 2019).

These bacteria play an important role in the plants ability to absorb nutrients (Yep and Zheng, 2019). Along with their ability to affect the absorption nitrogen which would otherwise be absorbed by plants (Wongkiew et al., 2017), hydroponic rhizobacteria have beneficial effects on plant growth, they can enhance the availability of P to plants and have a potential for biocontrol of pathogens to plant roots (Schmautz et al., 2017; Cerozi and Fitzsimmons, 2017; Goddek et al., 2019). When different types of bacteria exists in aquaponics systems, they may have a synergetic effect on plant growth (Yep and Zheng, 2019).

2.3 Role of plants in aquaponics

In aquaponics, plants act as biological filters and absorb nutrients from the water, which are used by the plants for growth and development (Endut et al., 2011; Saufie et al., 2015). Nitrification (a two-step process mediated by bacteria) is when ammonia is converted to nitrite mostly by *Nitrosomonas* sp. and subsequently nitrate by *Nitrobacter* sp. Nitrate, obtained from nitrification, is non-toxic at low concentrations and can be absorbed by plants (Blidariu and Grozea, 2011). The uptake of nutrients by plants is generally faster in aquaponics

systems than in soil, likely because of the direct contact of plant roots and nutrients and that less energy is required to extract the nutrients from water than from soil (Saufie et al., 2015). Horticultural plants like tomatoes and cucumbers produced by aquaponics surpass the typical yields of vegetables produced by organic soil-based technology (Savidov et al., 2007). Nutrient absorption by plants improves water quality, pH, and maintains dissolved oxygen in the water (Endut et al., 2010). Plant absorption has also been suggested as the best contaminant removal in the treatment of wastewater (Blidariu and Grozea, 2011; Endut et al., 2011). Generally, if the number of plants increases, the nutrient concentration in the water decreases due to absorption (Blidariu and Grozea, 2011).

For the best growth, plants grown in aquaponics systems require 16 essential nutrients. Some are needed in larger quantities (macronutrients) while others are required in smaller quantities (micronutrients) (Rakocy et al., 2006; Bittsanszky et al., 2016). Macronutrients include nitrogen (N), potassium (K), phosphorus (P), sulphur (S), magnesium (Mg), and calcium (Ca). Micronutrients include chlorine (Cl), iron (Fe), manganese (Mn), boron (B), zinc (Zn), copper (Cu), and molybdenum (Mo) (Rakocy et al., 2006; Bittsanszky et al., 2016). The requirements and uptake of nutrients are different for different plants and change as the plants grow with different growth conditions and the needs of the plant (Voogt, 2002; Bugbee, 2004).

Although there is extensive knowledge about plant nutritional requirements in hydroponics, in aquaponics, the current knowledge on optimum nutrient levels of leafy and fruiting plants in aquaponics remains tentative because plants differ in their ability to extract nutrients (Goddek et al., 2015). Additionally, plants differ in their tolerance to the concentrations of these nutrients e.g. nitrogen (Savidov, et al., 2007; Graber and Junge 2009; Endut et al., 2011, Knaus and Palm, 2017b). In plants, the different absorption capacities are due to differing fertilizer needs (Graber and Junge, 2009). Some of the nutrients taken up by plants are easily absorbed while others undergo complex biodegradation to make them available to plants. For example, lettuce, herbs, spinach, chives, and basil have low to medium nutritional requirements while fruiting plants like tomatoes, peppers, and cucumbers have a higher nutritional demand and grow better in an aquaponics system with high stocking-density fish cultures to match their high nutritional demands (Diver, 2000; Goddek et al., 2015). Aquaponics farmers generally favour plants that can reach a harvestable size at a faster rate

such as basil, salad greens, non-basil herbs, head lettuce, and kale, as they can be planted multiple times a year and therefore produce multiple harvests annually (Love et al., 2015).

2.4 Role of fish species and fish feed in aquaponics

Different species of aquatic animals have been raised in aquaponics systems; tilapia (*Tilapia* spp.), ornamental fish, catfish (*Ictalurus* spp., *Clarias* spp.,), trout (*Onchorynchus* spp.), bass (*Micropterus* spp., *Morone* spp.,), crayfish (*Astacoidea* and *Parastacoidea* families), and prawns (Love et al., 2015). However, the most popular fish species cultured in most aquaponics systems is tilapia (Nichols and Savidov, 2012). The African catfish (*Clarias gariepinus*) has also been used in several studies as a research species with various leafy plants (Palm et al., 2014a; Knaus and Palm, 2017a). Each species of fish has particular needs in terms of feed composition, feeding rate, growth rate, stocking density, and various other parameters, all of which affect the nutrient excretion in the system (Goddek et al., 2019).

The protein content of the diet will affect the nutrient composition of the water, such as ammonia, nitrite, nitrate, and phosphorus content (Endut et al., 2010; Palm et al., 2014a). Along with the feeding strategy and stocking density, the protein content of the feed also affects the assimilation of nutrients by fish and the production of nutrients from fish feed and waste (Endut et al., 2010; Blidariu and Grozea, 2011). Therefore, nutrient quality and balance in fish feed directly affect plant production (Savidov et al., 2007). Nutrients from unconsumed food and faecal matter need to be solubilised into ionic mineral form to be assimilated by plants (Goddek et al., 2015).

Plants and fish, however, have different nutrient requirements, for example, they have different potassium requirements (Savidov et al., 2007; Graber and Junge, 2009). Fish feed may not be rich in certain nutrients, such as potassium and iron that are required by plants. These may need to be supplemented to meet the needs of plants (Savidov et al., 2007; Graber and Junge, 2009). In an experiment by Graber and Junge (2009), circulating water in an aquaponics system had low concentrations of potassium compared to a hydroponics system. In most systems, the ratio of nutrients excreted by fish does not reflect the ratio of nutrients absorbed and required by plants because nutrients do not accumulate in the circulating water at equal rates and they are not extracted from the circulating water at equal rates (Endut et al., 2010; Endut et al., 2011). However, Rakocy et al. (2004) propose that if an optimum ratio

between daily feed input and plant growing area is sustained, nutrient accumulation or deficiency can be kept constant.

In aquaponics, one of the difficulties is attaining a feed that has the correct balance of nutrients that will benefit both the plants and fish (Goddek et al., 2015). The fish feed that is selected should be aimed at minimising the addition of supplemental nutrients that may be necessary for plants and which may add extra costs (Goddek et al., 2015).

2.5 Water quality in aquaponics systems

Water quality parameters in an aquaponics system such as temperature, dissolved oxygen (DO), pH, and mineral concentration need to be reconciled for plants, fish, and nitrifying bacteria (Tyson et al., 2004, 2007). One of the challenges in aquaponics is that it is difficult to obtain optimum water quality conditions for the survival and growth of plants and fish and the optimum performance of nitrifying bacteria (Tyson et al., 2004; Palm et al., 2014b). Because minerals have different solubilisation rates, their concentrations in the water differ (Seawright et al., 1998), as does their uptake by plants (Endut et al., 2011).

One of the parameters making it difficult to obtain an optimum match for plants, fish, and nitrifying bacteria is pH, because each of these have different optimum pH values (Goddek et al., 2015). Nitrification occurs efficiently at a pH of 7.5 – 8.0. This may be too high for plant growth (Savidov et al., 2007), which generally requires a pH of 6.0 – 6.5 for optimal nutrient uptake (Goddek et al., 2015). The nutrient uptake of elements such as Mn, Cu, Zn, and Fe are reduced at a higher pH (Bugbee, 2004). In most systems, pH is adjusted by the addition of carbonate, bicarbonate, or hydroxide using a buffer based on calcium, potassium, or magnesium which may also be a nutritional supplement for plants in the system (Rakocy et al., 2006; Goddek et al., 2015).

Dissolved oxygen concentration in aquaponics systems should be fixed to meet the minimum requirements of the specific fish species being cultured (Lennard and Leonard, 2006). Generally, deteriorating water quality and mineral toxicity are the two factors that inhibit fish growth in aquaponics (Endut et al., 2011). These may result from plants' inefficiency to absorb minerals from water (Liang and Chien, 2013). Therefore, in most systems, the quality of water depends mainly on the plants' ability to remove nutrients from water (Liang and Chien, 2013). Other systems utilise a solid removal component, and the faecal matter and solid uneaten food are removed as soon as possible to reduce ammonium build-up and clogging of plant

roots (Rakocy et al., 2006; Graber and Junge, 2009). In most aquaponics systems, there is a dedicated biofiltration unit through which nitrification occurs (Goddek et al., 2015).

2.6 Macro and micronutrients in aquaponics

Copper (Cu) is involved in respiration and formation of chlorophyll (Ru et al., 2017), while in fish it is an essential mineral that participates in energy production, collagen synthesis, and melanin production (Yildiz et al., 2017). Low Cu levels in fish may result in poor growth and feed efficiency (Yildiz et al., 2017). Fish feed generally has sufficient Cu to meet the demands of fish (Watanabe et al., 1997). In plants, Cu deficiency causes chlorosis of the leaves (Somerville et al., 2014).

Zinc is an important nutrient for both fish and plants. In fish, it is important in immunity and forms part of the structural components of bones, scales, and skin (Watanabe et al., 1997; Yildiz et al., 2017). In plants, Zn is involved in the synthesis of auxin, which maximises photosynthesis (Ru et al., 2017).

Manganese is important in photosynthesis, resulting in reduced growth when deficient in plants (Somerville et al., 2014). In fish, Mn is a cofactor of metalloenzymes involved in the bone development of fish (Watanabe et al., 1997; Yildiz et al., 2017). In aquaponics systems, its optimum absorption by plants occurs at pH levels below 8.

Iron is involved in cellular respiration and lipid oxidation and its deficiency may induce anaemia in certain species of fish (Watanabe et al., 1997). In plants, iron is important in photosynthesis and is one of the nutrients that is generally supplemented in aquaponics systems because it is not excreted in sufficient quantities to be available to plants (Goddek et al., 2019)

Nitrogen is the most important macronutrient in aquaponics. It enters the system mainly through fish feed and is assimilated by fish. Plants use it in the form of NH_4 and NO_3^- for growth after it is excreted (Somerville et al., 2014). Its deficiency in plants results in chlorosis of the leaves (Goddek et al., 2019). Because of its importance, the nitrogen cycle is discussed in section 2.2.

Phosphorus is required by fish because it is the main constituent of skeletal tissues and is necessary for optimum growth and metabolism (Sarker and Satoh, 2007). Fish feed supplies dietary phosphorus to fish mainly through fishmeal (Sarker and Satoh, 2007). Phosphorus in

plants is important in photosynthesis, respiration, and regulation of enzymes in plants. For adequate growth, most plants require 1.9 – 2.8 mgl^{-1} (Cerozi and Fitzsimmons, 2016a). In fish feed, it is generally available in sufficient quantities to meet the needs of fish and not those of plants (Goddek et al., 2019). At pH levels above 7, P can precipitate and become insoluble, making it unavailable for absorption by plants (Tyson et al., 2011; Cerozi and Fitzsimmons, 2016b). Its deficiency in plants can cause poor root development (Somerville et al., 2014). The absorption of P in plants is affected by pH and can only occur when it is an orthophosphate ion HPO_4^{2-} $H_2PO_4^-$ and PO_4^3 (Goddek et al., 2015; Cerozi and Fitzsimmons, 2016b, Goddek et al., 2019). Microorganisms from the *Bacillus* spp. play a critical role in the availability of P to plants by mineralising organic P and solubilising precipitated phosphates (Cerozi and Fitzsimmons, 2016a).

Calcium is important for plant root development and strengthening of the stem. In plants, Ca deficiency results in stunted growth (Somerville et al., 2014; Goddek et al., 2019). Fish require calcium because it functions as a structural component of bones, scales, and the exoskeleton (NRC, 1993). In some aquaponics systems, Ca is supplemented through nutrient supplementation or the buffering method (Rakocy et al., 2006).

Sulphur is important in protein production and photosynthetic enzyme production. Sulphur deficiencies in plants are rare and it is not usually supplemented in aquaponics systems (Somerville et al., 2014).

Magnesium is important in photosynthesis and its deficiency results in the yellowing of plant leaves (Somerville et al., 2014). When it is lacking in aquaponics systems, it is generally supplemented as [$(CaMg(CO_3)_2$] (Rakocy et al., 2006).

Potassium is required by both fish and plants; however, the requirements differ between them as generally, plants require potassium in higher quantities than fish (Savidov et al., 2007, Somerville et al., 2014). Potassium is a key cellular cation in fish; in plants, it is required for water uptake and photosynthesis (Graber and Junge, 2009, Wang et al., 2013).

2.7 Role of potassium and iron in fish and plants

Two important candidate plant nutrients that have been identified for addition to fish feed are potassium and iron. These elements are often supplemented in aquaponics systems because they are not present in sufficient levels in fish feed to result in sufficient excretion

into the water, therefore making them less available for plants (Seawright et al., 1998; Pantanella et al, 2012; Somerville et al, 2014; Goddek et al., 2015, Kasozi et al., 2019). These elements are important to both fish and plants, however, they should not be toxic to fish and they should be available to plants in the correct ratio if they are not retained by fish (Seawright et al., 1998).

Potassium and iron are essential elements in animals, including fish. Potassium is a primary cellular cation (Wilson and El Naggar, 1992; Shiau and Hsieh, 2001b) and iron is important in cellular respiration, oxygen transportation, and mitosis (Lim et al., 1996). Fish have a dietary requirement for both potassium and iron, and deficiencies can result in reduced growth (Wilson and El Naggar, 1992; Lim et al., 1996; Shiau and Su, 2002). Wilson and El Naggar (1992), working on the channel catfish *Ictalurus punctatus*, established that potassium requirements for fish can be met by dietary potassium. Fish feed is typically the main source of iron and potassium for fish because these minerals are available in low concentrations in natural water (NRC, 1993; Lim et al., 1996; Shiau and Hsieh 2001b; Shiau and Su, 2002). The requirement for potassium and iron differ for each species of fish. It is, however, difficult to compare the requirements of fish because different sources of potassium and iron are used in fish feed (Shiau and Su, 2002). When supplemented in the diet, weight increased with increasing levels of potassium in the diet of shrimp (Shiau and Hsieh, 2001a).

Potassium and iron are also essential nutrients that are required by plants for healthy growth (Goddek et al., 2015). Potassium plays a key role in the yield and quality of plants (Voogt, 2002; Prajapati and Modi, 2012; Wang et al., 2013). It is essential for processes like photosynthesis, activation of enzymes, protein synthesis, and controlling the uptake of other ions (Camak, 2005; Prajapati and Modi, 2012; Wang et al., 2013). It is required in relatively higher quantities than other nutrients and it is the most abundant cation in plants (Wang et al., 2013). The need for potassium changes as the plant grows, with less being required as the plant grows (Voogt, 2002). Iron is also an essential element that plays a role in plant metabolism and it is key in the optimal growth and reproduction of plants (Christ, 1974; Nenova, 2006; Hochmuch, 2011). It is involved in the synthesis of chlorophyll and is required for the functioning of certain enzymes (Hochmuch, 2001)

The best dietary source of these minerals must be investigated as their availability to fish is limited when the diets of fish contain fish meal and plant protein as sources of protein (Satoh

et al., 2001; Apines et al., 2003) and phytase phosphatase enzyme is not included in the diet (Cerozi and Fitzsimmons, 2017). Fish meal alternatives used as a major source of protein in aquafeeds have high concentrations of the antinutritional factors, phytate and tri-calcium phosphate, that reduce the availability of minerals to fish (Satoh et al., 2001).

The availability of minerals also differs depending on the source of the minerals. Therefore, it is important to use a dietary source that will provide higher availability of the minerals to fish (Apines et al., 2003). Chelates and complexes are preferred over inorganic sources because of their ability to compete with the antinutritional factors in plant protein sources, making the minerals available to the fish (Paripatanont and Lovell, 1995; Satoh et al., 2001).

Using fish feed that has been supplemented with trace elements in the form of chelated minerals or adding dietary acidifiers may be beneficial to fish (Satoh et al., 2001; Apines-Amar et al., 2004; Lückstädt et al., 2012) and subsequently to plants. They may be beneficial because the nutrient-enriched water from uneaten fish feed and faecal matter with supplemented trace elements could be used to produce plants. It is anticipated that the supplemented trace elements, if unused or excreted by fish, will be available for use as fertiliser by plants. This may benefit plants and lessen the need to add artificial fertilisers to the hydroponic system.

In aquaponics, there has been limited research into developing feed that is aimed at optimising both fish and plant growth by supplementing the fish feed with important plant nutrients. A paper by Rono et al. (2018) on aquaponics production showed that iron amino acid chelated supplemented at 30 Fe kg^{-1} in fish feed improved the growth of spinach, indicating a potential to benefit both fish and plants in aquaponics. The addition of such elements to fish feed could reduce or even eliminate the need to supplement plants with these nutrients in the form of nutrient solutions. A review by Kasozi et al. (2019) discussed the importances of iron and management in aquaponic systems.

The addition of dietary supplements to fish feed could benefit both the fish and the plants. For example, the dietary additive potassium diformate dissociates at a pH > 4 to formate (CHOO-) and potassium ions. Formate, a salt of formic acid, has been used as a feed additive and has been demonstrated to significantly improve animal growth, including fish (Partanen and Mroz, 1999; De Wet, 2005; Lückstädt and Mellor, 2011). After dissociation, the formate anion is the active part of the feed additive that can be utilised in the digestive system of fish to improve the retention of elements like Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+}. It also has an

antimicrobial effect and ability to improve pepsin activity, subsequently improving protein and amino acid digestibility (De Wet, 2005; Lückstädt, 2006) while the potassium ions may be beneficial to plants if they are not absorbed by the cultured fish, but excreted into the water recirculating through the aquaponics system.

In a commercial catfish diet, when KDF was added at a dose of 0.2 % for eight weeks, the fish had a significantly higher weight and a better feed conversion ratio compared to their counterparts fed a diet with 0 % KDF (Lückstädt et al., 2013). The addition of KDF in fish diets has led to significantly higher weight gain, reduced mortality, and a good feed conversion ratio (Lückstädt, et al., 2012). The typical inclusion level of KDF in fish diets ranges from 0.2 – 1.4 % (Lückstädt and Christiansen, 2008; Zhou et al., 2009; Lückstädt et al., 2012; Abu Elala and Ragga 2015).

Chelates and complexes have been proven to compete with mineral inhibitors, making minerals more available to animals (Apines et al., 2003). Amino acid chelation provides more stability to minerals, allowing it to increase absorption and inhibit the formation of insoluble complexes when the mineral has been ingested (Apines et al., 2001). Amino acids act as transfer molecules to ensure that the mineral reaches the tissues and is readily available for uptake (Apines et al., 2001, Satoh et al., 2001). The benefits of amino acid chelated minerals have been tested on aquaponics systems by Rono et al. (2018) and shown to improve plant growth in aquaponics systems. Although there are limited studies regarding the inclusion of amino acid chelated minerals for aquaponics production, it is anticipated that if the minerals are excreted through faeces, it is because they were not retained by the fish or were made available through uneaten feed. The minerals will be available in ionic form for uptake by plants in the aquaponics system.

2.8 Commercial aquaponics

Commercial aquaponics is the newest sector of agriculture, thus far. The success of this field depends on its profitability; plant and fish growth are the benchmark on which to correlate its efficacy and sustainability (Endut et al., 2011). Because aquaponics is a new research area, there is limited information, making it difficult to compare, especially since there are different system designs and unlimited fish-plant combinations (Palm et al., 2014b; Saufie et al., 2015).

There have been investigations into the technical systems, among others system design and their effects on chemo-physical parameters of fish and plants (Palm et al., 2014b), evaluation

of plant ratios in terms of daily feed input to plant growth area (Rakocy et al., 2004), and the hydraulic loading rate for obtaining a balance between fish and plant growth (Endut et al., 2010). There have also been investigations into the efficacy of plants to absorb nutrients from wastewater in aquaculture (Endut et al., 2011). Most scientific literature in aquaponics highlights technical aspects and there is limited information on the economic viability of aquaponics (Goddek et al., 2015).

Aquaponics entail significant start-up costs compared to soil vegetable production or hydroponics (Somerville et al., 2014). However, the combined income from fish and plants may be able to offset these costs if the operation is run well (Somerville et al., 2014).

There have been a few papers that discuss the commercial viability of aquaponics (Adler et al., 2000; Rakocy et al., 2004; Endut et al., 2011; Love et al., 2015). In most of the literature (Adler et al., 2000; Rakocy et al., 2004; Endut et al., 2011; Love et al., 2015), there are certain costs that have not been considered, making the commercial viability of aquaponics difficult to determine or assess (Goddek et al., 2015).

Authors like Savidov et al. (2007) have proposed that aquaponics is economically feasible when growing high-value plants. Goddek et al. (2015) suggest that it may also be feasible if product manufacturing costs are low (i.e. feed manufacturing). In aquaculture, feed is one of the main cost drivers, which can amount to more than half the total cost of production (Goddek et al., 2019). Tokunanga et al. (2015), researching different aquaponics systems in Hawaii, cite fish feed as one of the main costs components. It would therefore be financially beneficial for a commercial aquaponics system to have a feed that optimises fish and plant production, reducing the need to purchase nutrient fertilizers.

2.9 Literature summary

From the above literature review, it is apparent that the food production sector has identified a need for a sustainable and reliable food production system that uses resources efficiently. Consequently, the integrated aquaponics system meets those needs. Its role in reducing inputs, pollution, and waste, and efficient resource use is particularly relevant because it uses a single input (fish feed) and pollution and waste are avoided by using wastewater from fish production to cultivate plants and use water efficiently.

Fish and plants are cultivated in aquaponics systems, however, they have different nutritional requirements. The nutritional requirements of fish are met through fish feed. The major nutritional needs of the plants are met by the nutrients in the fish effluent. Some of the minerals, especially trace elements may be added. The addition of nutrient solutions to aquaponics systems to meet the mineral requirements of plants can potentially increase the costs of production. This warrants the need to investigate alternative ways to meet the nutritional requirements of both fish and plants simultaneously without additional supplementation in the form of nutrient solutions.

Aquaponics system technology has improved to optimise and independently control the production of fish and plants through the development of decoupled systems. The specific requirements of plants are met by manipulating the water before it reaches the plants. However, these decoupled systems are very expensive, technologically sophisticated, and may require special skills to operate. Therefore, most systems used are still single-loop systems that supplement the minerals required by plants.

The important nutrients required by plants in aquaponics systems to optimally grow are both micro and macro nutrients. These nutrients are required in different quantities by plants, depending on the plant species, growth stage, and specific requirements of plants. Major macronutrients P, K, Ca and Mg also contribute to the EC levels in the aquaponics water.

Nitrogen is one of the most important nutrients in aquaponics systems. Nitrogen transformation plays a crucial role in the efficiency and functioning of the aquaponics system. Bacteria converts ammonia to nitrate in the nitrification process and makes it available for plant uptake and growth.

There is currently limited knowledge or literature that has investigated whether the mineral requirements of plants and fish in integrated aquaponics systems can be met through mineral supplementation using fish feed additives. The only research that has been conducted using mineral supplementation through feed additives has been to investigate fish growth and production only (Gatlin and Wilson, 1986; Lim et al., 1996; Baker et al., 1997; Satoh et al., 2001; Apine-Amar et al., 2004). There is limited research about attempting to use fish feed to optimise the nutrient levels of plants.

Different inclusion levels of minerals supplemented in fish feed for the production of fish have been tested (Lim et al., 1996; Baker et al., 1997). However, there has been limited investigation of minerals supplemented in fish feed using feed additives for the production of both fish and plants in integrated aquaponics systems. Additionally, different dietary sources supplemented with minerals have been tested to produce fish only (Apines et al., 2003; Apines –Amar et al., 2004). There has been little or no pubished research into the use of different dietary sources of minerals supplemented to fish feed for aquaponics production of fish and plants. The inclusion levels and dietary sources are expected to affect both fish and plant production, therefore, it is important that they are investigated.

Fish feed makes up over 50 % of the production costs in aquaculture and is one of the major cost drivers in aquaponics, along with start-up costs. To optimise commercial aquaponics, feed needs to be used more efficiently and optimised specifically for aquaponics systems.

This study focuses on developing a fish feed unique to integrated aquaponics systems. It is developed for the optimisation of both fish and plant production in an integrated aquaponics system.

2.10 Conclusions from the literature

I. Aquaponics is an integrated food production system that is environmentally sustainable because of its water saving approach and low environmental waste discharge. It is an important component in hunger alleviation and food security. By using fish feed as the main nutrient input, aquaponics has the potential to reduce economic loss as feed makes up most aquaculture costs (40 – 60 %).

II. There have been improvements in aquaponics systems to enhance fish and plant production in the form of decoupled systems, however, these systems are expensive

and sophisticated, resulting in the continued use of coupled systems. Coupled systems need nutrient fertilizer supplementation to meet the nutrient requirements of plants.

III. Information regarding the optimum nutrient production by fish and uptake by plants in aquaponics systems is tentative, especially the minimum and maximum nutrient requirements of leafy and fruiting plants. It is important that the correct balance of nutrients is provided to optimise the growth of fish and plants in the system.

IV. There is limited information regarding the supplementation of fish feed with nutrients required by plants for optimum production in aquaponics systems.

V. Aquaponics systems produce low mineral concentrations compared to hydroponic systems where nutrient solutions are regularly added. Plants in aquaponics systems need supplementation of minerals to meet their nutritional requirements. No research to investigate supplementation through manipulation of fish feed is available.

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Chapter 3 Aim, objectives, and design of the study

3.1 Aim and objectives

The study aims to determine whether dietary supplementation of minerals through fish feed additives can improve the nutrient input to the plant component of the aquaponics system. To achieve this aim, the following objectives were set:

I. **Evaluate potassium and iron as dietary additives in feeding trials to test their effect on fish performance, haematological profile, and water quality in a recirculating aquaculture system (RAS).** Dietary feed additives differ in availability to fish depending on their source. Organic sources of minerals are thought to be highly available for absorption by fish compared to inorganic sources. The effect of different mineral sources was evaluated in a recirculating aquaculture system on various production and haematological parameters of the African catfish. The effect of different dietary sources of potassium are discussed in Chapter 5 and the effect of different dietary sources of iron are discussed in Chapter 6 where each chapter is written as a paper.

II. **Investigate the effect of potassium and iron as dietary feed additives supplemented at different inclusion levels on fish production, haematological profile, and water quality in RAS.**

In this study, the inclusion level of minerals in fish feed was evaluated to ensure that the minerals are available in sufficient quantities for fish production. The wastewater excreted by fish was characterised to investigate the best inclusion level to excrete minerals in fish wastewater for plant production. The inclusion levels of potassium and iron to the diet of the African catfish are investigated in Chapters 5 and 6.

III. **Use the information obtained from objectives I and II, regarding the optimal feed additive and inclusion levels, to validate the results by cultivating fish and plants in an integrated aquaponics system.**

Dietary sources and inclusion levels of minerals are known to affect fish production and health and possibly the wastewater excreted by fish. The best source and inclusion level for African catfish production and plant production are evaluated in an integrated aquaponics system based on the production of catfish, its haematological profile, and the effect of the feed additives on the wastewater produced. The effect of these additives on fish production and plant growth are discussed in Chapters 7 and 8.

3.2 Design of the study

The study focused on developing feed for aquaponics systems that would be beneficial to both plant and fish production. Specially selected feed additives were used to achieve this by conducting several experiments at the Welgevallen Experimental Farm, Stellenbosch University. The first phase was a feeding trial that met objectives I and II, that is, to measure the effect of the selected feed additives on water quality parameters, fish production, and fish haematology in a recirculating aquaculture system (RAS). Furthermore, the different dietary sources were added at three different inclusion levels. The best dietary source and inclusion level were selected based on the water quality results, fish production, and haematological profile of the fish. The information from the first phase, objectives I and II, was used in objective III, the second phase, where the best dietary source of each of potassium and iron and the best dietary inclusion level were used in the production of fish and plants in an integrated aquaponics system.

The first experiment (Phase I to meet objective I and II) was a feeding trial conducted using a basal diet without any supplementation of potassium (Siqwepu et al., 2020a) and iron (Siqwepu et al., 2020b) and comparing it to a feed that has been supplemented with the minerals potassium and iron, at different inclusion levels. These two chapters are reported in the form of scientific articles and have been published in peer reviewed journals. The fish species that was used in RAS was the African catfish, *Clarias gariepinus,* which is known to perform well in recirculating systems (Akinwole and Faturoti, 2007). The trial was run for 96 days using feed that was formulated specifically for this experiment. The trials for both potassium and iron were conducted at the same time but are reported in separate chapters (Chapters 5 and 6). Potassium and iron are added because they are not excreted in sufficient quantities for plant production in aquaponics systems; therefore they need supplementation (Seawright et al., 1998). The minerals were added using different dietary sources: iron from an amino acid-chelated source of iron and iron sulphate (ferrous sulphate heptahydrate), while potassium was provided in the fish feed through an organic acid salt, potassium diformate (KDF) and potassium chloride (KCl) (Figure 3.1).

The second experiment, forming part of phase one, was a two-week trial in RAS assessing the apparent digestibility coefficient (ADC) of potassium and iron. Fish were fed diets supplemented with potassium and iron additives. The digestibility of these minerals was

evaluated by measuring their excretion in faeces. These results are presented and discussed in Chapters 5 and 6.

The last component was a separate two-day trial, also forming part of the first phase. Nutrients in wastewater excreted by fish as they were fed diets with potassium and iron additives at different inclusion levels were evaluated. This experiment offered insight into which nutrients are released by fish when they are fed the control treatment and the experimental treatments, respectively. This was to measure the concentration of minerals excreted when the additives were included in the diet compared to the control. The produced nutrients through wastewater can affect plant growth (Buzby et al., 2016) and therefore needed to be investigated.

The second experiment (Phase II to meet objective III) was to grow plants and fish in an integrated aquaponics system using the nutrient enriched water produced by fish fed the control and experimental diets. The second phase was investigated over 3 months, from November 2018 to March 2019. The fish and plants used in this experiment are the African catfish, *C. gariepinus* and lettuce *Lactuca sativa*. The growth of the fish and plants were evaluated along with the water quality. Water samples from the influent and effluent of the hydroponic component were collected once a week for water quality determination. There was no replication for this experiment, because space was limited for the integrated aquaponics system and it is difficult to manage more than one system simultaneously (Figure 3.2). The trials were performed simultaneously and are reported separately in Chapters 7 and 8.

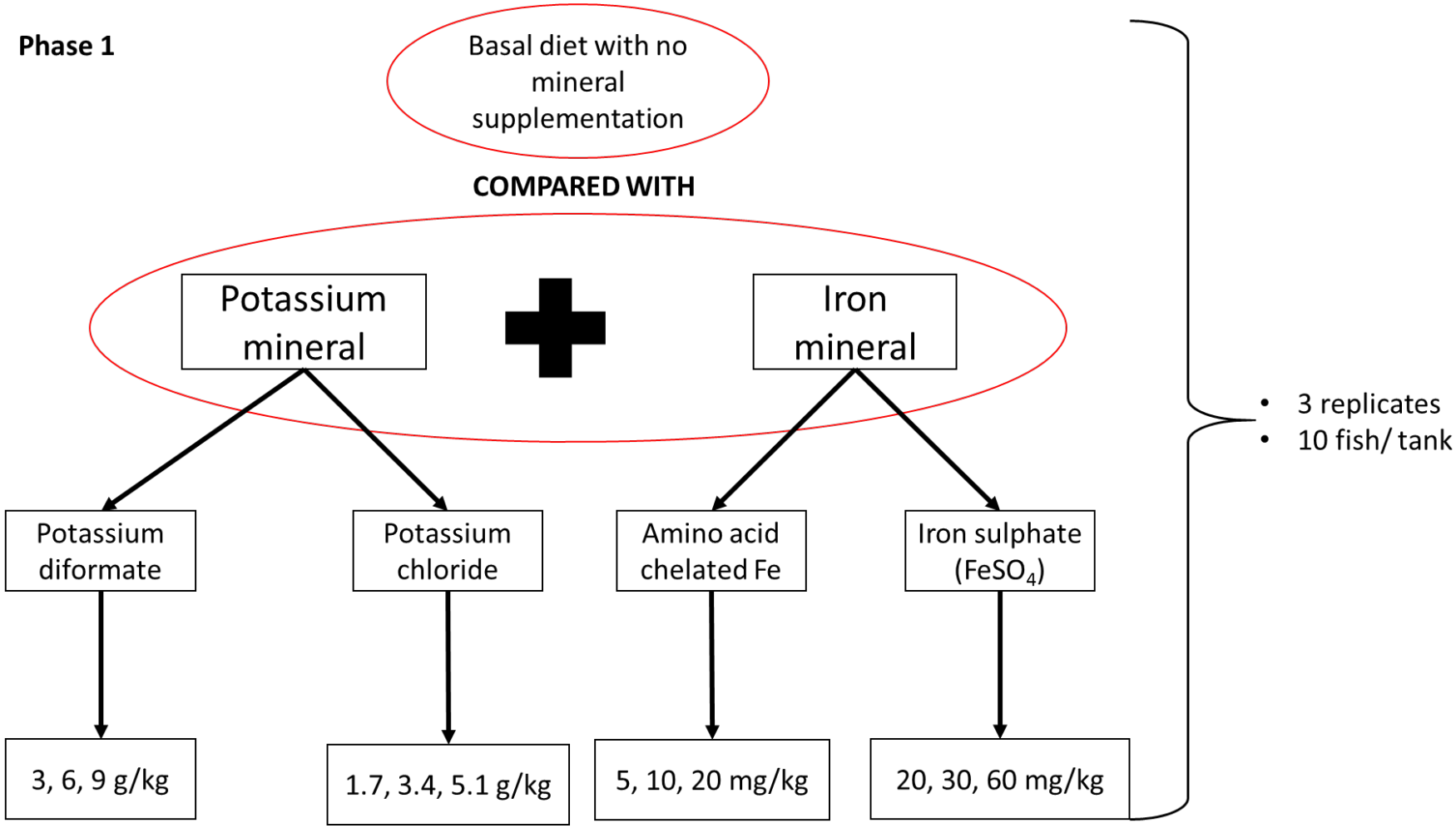

Figure 3-1 Summary of dietary treatments with different sources and inclusion levels of potassium (Chapter 5) and iron (Chapter 6) that were fed to *C. gariepinus* in a recirculating aquaculture system (RAS) over a 96-day trial period.

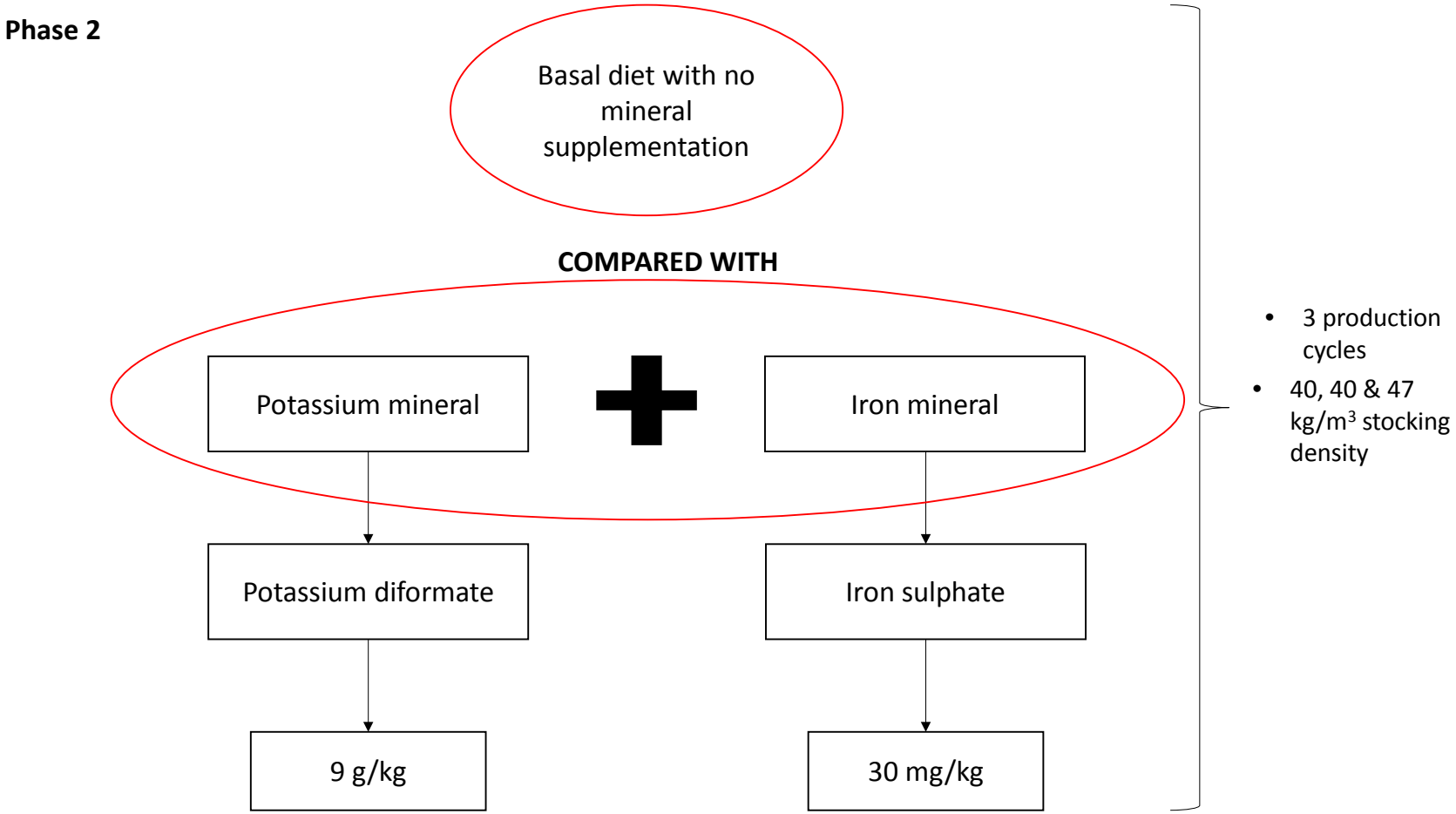

Figure 3.2 Summary of dietary treatments and inclusion levels of potassium diformate (Chapter 7) and iron sulphate (Chapter 8) that were fed to *C. gariepinus* in an integrated aquaponics system over three consecutive 31-day trial periods.

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Chapter 4 Novel contributions of the study

The work that was performed during the study has made the following contributions:

I. The effect of different dietary feed additives on the production and haematological profile of the African catfish, *C. gariepinus* in RAS was established. This study provided information regarding which source of potassium (Chapter 5) and iron (Chapter 6) can provide the most favourable haematological profile as each source was expected to affect productivity and haematology in different ways.

II. The effect of different inclusion levels of potassium and iron in the feed of African catfish was established. The best inclusion levels allowed for successful production of the African catfish in RAS while improving the haematological profile. The wastewater produced had higher concentrations of the desired minerals, potassium (Chapter 5) and iron (Chapter 6).

III. A combination of the best dietary feed additive and best inclusion level for optimum fish and plant production was established and improved the integrated production of the African catfish and lettuce (Chapter 7 and 8).

IV. It was shown that the inclusion of potassium and iron feed additives in the feed of the African catfish increased the concentration of potassium (Chapter 7) and iron (Chapter 8) in the wastewater, which translated to increased concentrations of the minerals in lettuce leaves.

V. The study showed that improvement of lettuce growth through inclusion of the dietary fish feed additives, potassium diformate (KDF) (Chapter 7) and iron sulphate ($FeSO_4$) (Chapter 8) is possible at the right environmental conditions. This study developed feed that significantly improved fish and plant production in integrated aquaponics systems, reducing or possibly eliminating the use of fertilizer nutrient solutions for the addition of potassium and iron.

Author contributions

Declaration by candidate

With regards to chapter 5: Evaluation of potassium diformate and potassium chloride in the diet of the African catfish, *Clarias gariepinus* in a recirculating aquaculture system, the nature and scope of my contribution were as follows:

Chapter	Pages	Nature of contribution	Extent of contribution (%)
5	40 - 81	Planned and designed the experiments. Formulated the fish feed, procured the ingredients and prepared the feed. Carried out the daily feeding, water measurements, the sampling of fish for production performance, performed the proximate analysis on feed and fish. Responsible for collection of blood samples and performing the haematology and non-specific immunity experiments. Responsible for tissue fileting for mineral analysis, collected faecal samples for the apparent digestibility coefficient trial. Collected and performed the analysis on the water quality trial. Prepared and analysed data. Wrote the manuscript and collated all the comments from the co-authors.	80

The following co-authors have contributed to chapter 5:

Name	Email address	Nature of contribution	Extent of contribution (%)
Neill Goosen	njgoosen@sun.ac.za	Contributed in data interpretation, proof reading and substantially improving the manuscript.	15
Khalid Salie	Ks1@sun.ac.za	Assisted with proof reading the manuscript and general	5

		discussions around the manuscript.	

Signature of candidate……..

Date……

Declaration by co-authors

The undersigned hereby confirm that

1. The declaration above accurately reflects the nature and extent of the contribution of the candidate and the co-authors to chapter 5, in the dissertation,
2. No other author contributed to chapter 5 and
3. Potential conflict of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in chapter 5 of this dissertation.

Signature	Institutional affiliation	Date
	Department of Process Engineering, Stellenbosch University	
	Department of Animal Sciences, Stellenbosch University	

Chapter 5 Evaluation of potassium diformate and potassium chloride in the diet of the African catfish, *Clarias gariepinus* in a recirculating aquaculture system

5.1 Abstract

This study focused on developing feed for aquaponics that would be beneficial to plant and fish production. Aquaponics is an integrated production system combining aquaculture and hydroponics. However, it is difficult to maintain synchrony between fish and plants because they have different nutritional requirements. Therefore, there is a need to develop feed uniquely for aquaponics systems, to meet the demands of both fish and plants. The results presented are the first phase of a two-phase study. The first phase was a standard feeding trial in a recirculating aquaculture system using the African catfish. Potassium, an essential mineral for fish and plant production, was added to fish feed from different mineral sources to evaluate it as a dietary feed additive. Potassium diformate (KDF) at 3, 6, and 9 gkg^{-1} and potassium chloride (KCl) at 1.7, 3.4, and 5.1 gkg^{-1} were used. The trial used three replicates per treatment of 10 fish with an average weight of ±112 g per 100 l tank for 96 days. Samples were taken after 96 days and one fish per tank was sampled to measure proximate composition, non-specific immunity parameters, tissue mineral analysis, and haematology. To measure production parameters, fish were weighed at the start of the trial and every four weeks subsequently. In separate trials, the effects of the feed additives on water quality and apparent digestibility coefficient of potassium were evaluated. Proximate body composition of the fish was significantly affected by the feed additives, except for the ash content ($p>0.0.5$). Moisture content significantly differed between the control diet (79% ±1.16) and diets containing KCl 1.7 and KCl 5.1(76% ±1.44; 76% ±0.37). Haematocrit (HCT) levels differed significantly from 36.7% ± 1.84 for KDF 9 to 32.11% ± 2.30 for KCl 1.7, KDF 9 had the highest haemoglobin (Hb) level, while there were no differences in red and white blood cell counts. The water quality parameters tested were also significantly affected by the different dietary treatments. Wastewater from KDF diatry treatments showed improved potassium concentration compared to the control. The study showed that the inclusion of potassium from KDF as a dietary source in fish feed can improve the haematological profile of the African

catfish compared to the control, based on this, it has the potential for use in integrated aquaponics systems.

Keywords: Potassium supplementation, Aquafeeds, Aquaponics, Haematologcal profile

5.2 Introduction

Due to the integrated nature of aquaponics production systems, where both fish and plants are produced simultaneously, it is difficult to balance nutrient input such that optimal growth is obtained for both fish and plants (Goddek et al., 2015; Suhl et al., 2016). Aquaponics has shown potential as a food-producing system, providing an alternative solution to conventional management of water quality in recirculating aquaculture systems (RAS) (Endut et al., 2010, Goddek et al., 2019), and using significantly less water than aquaculture and other agricultural production systems (Somerville et al., 2014; FAO, 2016).

Nutrient imbalances in aquaponics systems can lead to poor plant performance, nutrient deficiencies, increased disease susceptibility, and subsequently, poor economic returns (Rakocy et al., 2004; Nichols and Savidov, 2012). Currently, fish feed is the main nutrient supplied to aquaponics systems and needs to supply nutrients to fish and plants through wastewater excreted by the fish. However, conventional fish feeds are formulated solely on the nutritional requirements of the fish, which could lead to insufficient plant nutrients in the aquaponics system.Nutrients such as nitrate are excreted in sufficient concentrations for good growth, however other important macronutrients such as potassium and phosphorus may not be excreted in the required concentrations (Goddek et al., 2019). Nutrients excreted by the fish can therefore be limiting for optimal plant growth and require nutrient supplementation. Therefore, the macro and micronutrients that are excreted by the fish because of the particular composition of the feed need to be understood to determine if they will meet the needs of cultivated plants. Nutrients must be supplied with consideration as the availability of one nutrient may affect the uptake of another (Voogt, 2002; Goddek et al., 2015). Additionally, the nutrient needs to be in a form that can be assimilated easily by plants. The need to adjust ratios or supplement additional nutrients may result in additional costs to aquaponics (Goddek et al., 2015).

Potassium is a macronutrient for plant growth and is the most abundant cation in plants (Wang et al., 2013). The need for potassium changes as the plant grows, with less being

required at later life stages (Voogt, 2002). Apart from potassium, other macronutrients are nitrogen (N), phosphorus (P), sulphur (S), magnesium (Mg), and calcium (Ca). Micronutrients include chlorine (Cl), iron (Fe), manganese (Mn), boron (B), zinc (Zn), copper (Cu), and molybdenum (Mo) (Rakocy et al., 2006). In aquaponics systems, potassium is supplemented through nutrient fertilisers because conventional fish feeds do not contain sufficient concentrations to result in levels required by plants when excreted in fish wastewater (Seawright et al., 1998; Pantanella et al., 2012; Somerville et al., 2014; Goddek et al., 2015).

Ideally, when potassium is supplemented in fish feed for aquaponics, it is to benefit both fish and plants. It is therefore important to find the best dietary source of minerals as the source could affect their availability to animals (Satoh et al., 2001; Apines et al., 2003). Chelates and organic sources of minerals are preferred over inorganic sources because of their ability to compete with antinutritional factors in fish feed, making them highly available for absorption by fish (Paripatananot and Lovell, 1995; Satoh et al., 2001). The use of dietary acidifiers consisting of organic acids and their salts has been beneficial to fish (Hassan et al., 2014; Lückstädt, 2006; Lückstädt, 2008; Ng et al., 2009). Potassium diformate (KDF), the first source of potassium in this study, is a salt of formic acid that has been used as a feed additive and demonstrated significant improvement in growth in pigs (Partanen and Mroz, 1999; Lückstädt and Mellor, 2011) and fish such as *Oncorhynchus mykiss* (De Wet, 2005), *Oreochromis* sp. (Ng et al., 2009) and *Oreochromis niloticus ×O. aureus* (Zhou et al., 2008). KDF dissociates to formate ($CHOO^-$) and potassium ions (EFSA, 2012; Lückstädt et al., 2012). After dissociation, the formate anion is the active part of the feed additive that can be utilised in the digestive system of fish to improve the retention of elements like Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, and Zn^{2+} and if the potassiumion is not absorbed it may be excreted in wastewater. KDF also has an antimicrobial effect and ability to improve pepsin activity, subsequently improving protein and amino acid digestibility (De Wet, 2005; Lückstädt, 2006). Conversely, the potassium cation may also be beneficial to plants if they are not absorbed by fish. Potassium chloride (KCl) is a further potential dietary source of potassium, and has been used in aquaculture studies both as a dietary potassium source and to quantify the potassium requirements of fish and shrimp (Shiau and Hsieh, 2001a; Shiau and Hsieh, 2001b; Zhu et al., 2014; Booth and Fielder, 2016).

The African catfish, *Clarias gariepinus* is an emerging aquaculture species in South Africa and is a commercially important fish in Africa, due to its ease of culture, hardiness, ability to withstand deteriorating water conditions, and high stocking density (Akinwole and Faturoti,

2007; Omosowone et al., 2015). The African catfish performs well in aquaculture recirculating systems (Akinwole and Faturoti, 2007) and is suitable for use in integrated aquaponics systems (Endut et al., 2010; 2011).

The study aims to determine whether dietary supplementation of potassium feed additives can benefit African catfish performance in a recirculating aquaculture system and simultaneously enhance the excretion of potassium in wastewater for ultimate use in aquaponics systems. The aim was met by measuring the impact of feeding potassium diformate and potassium chloride at different dietary inclusion levels on production parameters, proximate body composition, haematological indices, non-specific immunity, tissue mineral composition, apparent digestibility coefficient of potassium, and water quality using the African catfish as an experimental species.

5.3 Materials and methods

5.3.1 Experimental fish

630 African catfish obtained from the Agricultural and Technology Demonstration Centre at Gariep Dam, Free State, South Africa were used for the experiments. The fish were randomly distributed, taking stocking density into consideration. Each 100 l glass tank was randomly allocated 10 mixed-sex fish with initial weights ± standard error (SE) of 112 g ± 6.30. However, for the apparent digestibility coefficient of K and water quality trials, 6 fish per 100 l tank were used. The fish were hand-fed to apparent satiation three times a day (08:00, 12:00, and 16:00), with equal amounts of feed in each tank. The fish were observed through glass tanks during feeding to minimise feed waste. Fish were starved for 24 hours prior to handling. Handling was done under anaesthesia (400 mgl^{-1} of tricaine methanesulfonate, MS-222, (Sigma-Aldrich, Johannesburg, South Africa) using 800 mgl^{-1} of sodium bicarbonate as a buffer. The procedures used in this experiment were approved by the Research Ethics Committee: Animal Care and Use (REC: ACU) of Stellenbosch University (Protocol number: SU-ACDU17- 00015).

5.3.2 Experimental unit

The experiments were conducted at Welgevallen Experimental Farm in Stellenbosch University, South Africa. The fish were stocked in 100 l glass tanks in a recirculating

aquaculture system with aeration provided to each tank. The water temperature and dissolved oxygen were measured with YSI Pro Plus Multi Parameter Water Quality Meter (YSI Incorporated, Ohio, USA). The temperature was maintained at ±27° C and dissolved oxygen ranged from 5.06 – 7.81mgl^{-1} throughout the trial. The pH was measured with a Hanna pH 211 microprocessor (Hanna Instruments, Sarmeola di Rubano, Italy) and maintained at 5.6 –7.3 throughout. These conditions are deemed to be conducive for African catfish production (Eding and Kamstra, 2001). Total system water volume was reglated automatically using a float valve in the sump of the recirculating system. Fresh water addition to replace system losses due to evaporation, splashingand leakages was not measured, but in the absence of tank washing operations and due to the system being indoors, daily water replacement was expected to be low.

5.3.3 Experimental diet

The six experimental diets containing various levels of potassium were from both an organic and inorganic source. The organic source was potassium diformate (KDF) (ADDCON, NordicAS, Porsgrunn, Norway) and the inorganic source was potassium chloride (KCl) (Sigma-Aldrich, Johannesburg, South Africa). The control diet was formulated to meet the nutritional demands of a catfish (NRC, 1993). All the diets were formulated to be (10063 kJkg^{-1}) and isonitrogenous (35% crude protein). Fishmeal was from a local supplier (65% crude protein, Concentra Ltd., Cape Town, South Africa) and soya (46% crude protein, FeedPharm, Cape Town, South Africa). The mineral contents among the six treatments were similar except for potassium. The first source of potassium, KDF (ADDCON, NordicAS, Porsgrunn, Norway), was included at 3, 6, and 9 gkg^{-1}. The inclusion levels of KDF were selected because at 2 and 3 gkg^{-1}, KDF significantly affected the growth of *Oreochromis niloticus* (Abu Elala and Ragaa, 2015). At inclusion levels of 3 and 6 gkg^{-1}, the hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) showed improved growth and food conversion ratio and had higher weight gain and specific growth rate when compared to treatments with no KDF (Zhou et al., 2009). The second source, KCl (Sigma-Aldrich, Johannesburg, South Africa) was added at 1.7, 3.4, and 5.1 gkg^{-1} to match the potassium concentration in KDF. These concentration levels of KCl are similar to those that have been investigated for other fish and shrimp species (Shiau and Hsieh, 2001a; Shiau and Hsieh, 2001b). Cellulose, a non-nutritive binder, was included as an inert filler material in the

control treatment and the potassium additives replaced an equivalent amount of cellulose in the experimental diets (Table 5.1).

Table 5.1 Feed formulation and proximate composition of feed of experimental diets fed to *C. gariepinus* (gkg^{-1}) during a 96-day trial period.

Ingredients (gkg^{-1})	Treatment						
	KDF3	KDF6	KDF9	KCl1.7	KCl3.4	KCl5.1	Control
Fish meal	120	120	120	120	120	120	120
Soya	570	570	570	570	570	570	570
Maize	200	200	200	200	200	200	200
Cellulose	12	9	6	13.3	11.6	9.9	15
Vit/Min premix[a]	15	15	15	15	15	15	15
MDCP[b]	20	20	20	20	20	20	20
Fish oil	30	30	30	30	30	30	30
Sunflower oil	30	30	30	30	30	30	30
KDF	3	6	9	--	--	--	--
KCl	--	--	--	1.7	3.4	5.1	--
Proximate composition (gkg^{-1})							
Moisture	50	50	47	32	45	49	66
Ash	107	98	113	109	97	99	86
Crude Fat	97	111	103	107	108	108	103
Crude Protein	370	370	380	380	370	380	380
Crude Fibre	22	16	21	25	23	24	23
Carbohydrates[b]	354	355	336	347	357	340	342

[a]Vit/Min premix -Vitamins: Vitamin A, 12 500 000 IU; Vitamin D3,2 500 000 IU; Vitamin E, 150 000; Vitamin K3, 8g; Vitamin B1, 15g; Vitamin B2, 20g; Vitamin B6,15g; Vitamin B12,0.035g; Niacin, 80g Cal Pnth, 50g; Folic Acid, 2.50g; Biotin, 0.350g; Iodine, 2.50g; Cobalt, 0.55g; Selenium, 0.25g; Vitamin C (Stay 35), 300g.
Minerals: Manganese, 60g; Zinc, 60g; Copper, 6g; Choline, 1000g.
[b]Determined by difference as: 1000 – Moisture - Crude Protein - Crude Lipids – Ash.
MDCP: Monodicalcium phosphate. KDF: Potassium diformate. KCl: Potassium chlorid

5.3.4 Diet preparation

The diet was prepared by mixing the dry ingredients and adding water and oils. The ingredients were then mixed in a commercial dough mixer (MacAdams, SM 401) (McAdams International, Cape Town, South Africa). Subsequently, 4 mm pellets were extruded from a single-screw extruder (custom model, Reomach Engineering, South Africa) and dried overnight at 55° C in a convection oven (Envirowatch, Cape Town, South Africa). The feed was packed in airtight bags until use.

5.3.5 Production parameters

Individual fish weights were measured at the start of the trial and every 4 weeks thereafter. After the 96-day trial period, all the fish were measured and weighed. The production parameters that were evaluated were initial weight, final weight, weight gain, feed conversion ratio (FCR), specific growth rate (SGR %), and survival rate (%). The parameters were calculated as follows: Weight gain = ($W_2 - W_1$)

$$\text{Specific growth rate (SGR)} = \frac{\ln W_2 - \ln W_1}{T_1 \times 100}$$

$$\text{Survival rate (\%)} = \frac{\text{No. of fish at end of experiment}}{\text{No. of fish at start of experiment} \times 100}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Dry weight of feed (g)}}{\text{Weight gain (g)}}$$

Where:

W_1 = Initial mean weight (g)

W_2 = Final mean weight (g)

T_1 = Duration of the experiment

ln = Natural log

5.3.6 Proximate analysis

Whole-fish samples were ground individually using a Hobart meat grinder (Hobart Food Equipment, Troy, OH, USA) and homogenised. Each treatment was replicated three times and one fish per tank was randomly selected for proximate composition. Fish feed samples were mixed and ground with a hammer mill (Centrotec, Cape Town, South Africa) with a 1.5 mm sieve. The whole-fish samples were analysed in duplicate while the feed samples were analysed in triplicate for proximate composition following the standard methods (AOAC, 2002a, 2002b). Moisture content for fish and feed was determined by drying samples in an oven at 100° C for 24 hours (AOAC, 2002a). Fish and feed samples were then incinerated overnight in a muffle furnace at 600° C for measurement of ash content (AOAC, 2002b). Protein for both samples was measured by the combustion Dumas method with a LECO FP 528 (AOAC, 2002c). The total fat content of the fish samples was determined by chloroform–methanol extraction (1: 2) (Lee et al., 1996) and for the feed samples using the ether extraction method (AOAC, 2002d).

5.3.7 Haematology and non-specific immunity

At least 2 ml of blood was collected from the caudal vein of each fish while under anaesthesia (400 mgl^{-1} of tricaine methanesulfonate, MS-222, (Sigma-Aldrich, Johannesburg, South Africa), using 800 mgl^{-1} of sodium bicarbonate as a buffer. Blood was drawn from one fish per tank using a syringe and the blood was placed into sample bottles containing an anticoagulant, ethylene diamine tetra acetate (EDTA), for haematological parameters. Haematocrit values were determined directly after sampling by centrifuging samples in glass capillary tubes for five minutes in a microhaematocrit centrifuge and read with a Graphic Reader. The Cell Dyn 3700 haematology analyser at the Department of Physiology, Stellenbosch University was used for haematological analysis. Cell Dyn is a multi-parameter blood analyser, measuring EDTA-anticoagulated whole blood. It utilises volumetric impedance and optical detection and is able to generate white blood cells (WBC), red blood cells (RBC), and haemoglobin (Hb) directly while haematological measurements such as haematocrit levels (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular and haemoglobin concentration (MCHC) are calculated from these measured parameters.

Haemoglobin is measured by a spectrophotometer at a wavelength of 540 nm in the analyser. The haematocrit values measured with a microhaematocrit centrifuge were compared with values obtained from the Cell Dyne 3700 blood analyser to validate the results. The same values were obtained with both methods and are presented in the results section. The ratio of liver weight to fish weight was measured to determine the hepatosomatic index (HSI). The fish that were used to draw blood were weighed and dissected. The liver was weighed and the HSI was measured according to the equation:

$$\text{HSI}\ (\%) = \frac{\text{Liver weight}}{\text{Fish weight}\ \times 100}$$

To measure non-specific immunity parameters, serum lysozyme activity, total protein, and immunoglobulin, blood was drawn from the caudal vein of the fish while under anaesthesia (400 mgl^{-1} of tricaine methanesulfonate, MS-222, (Sigma-Aldrich, Johannesburg, South Africa), using 800 mgl^{-1} of sodium bicarbonate as a buffer, using a syringe. The blood was centrifuged at 500 rpm (rpm) for 5 minutes at a relative centrifugal force of 1400 g and the serum was collected. To determine lysozyme activity in blood serum, the method from Sankaran and Gurnani (1972) was used. A phosphate buffer (0.05 M, KH_2PO_4 and Na_2HPO_4) was prepared at a pH of 6.2 along with a bacteria solution (*Micrococcus lysodeiticus*) (0.0075%, w/v) that was maintained at 25° C. A lysozyme standard (0.85% w/v) was also prepared; 50 µl of the lysozyme standard or blood serum was added to 250 µl of the bacteria solution in a 96-well microplate. The microplate was shaken for two minutes and the absorbance was measured at 490 nm. After shaking for 20 minutes, the absorbance was measured again. A standard curve of change in absorbance was plotted to determine the serum lysozyme concentration. The total serum protein was determined according to methods by Zor and Selinger (1996) using the linearised Bradford assay. For total protein serum, 20 µl of blood serum was diluted with 1500 µl dilution agent in an Eppendorf tube. The mixture was added in triplicate into 96-well microplates at 50 µl along with 200 µl of the Bradford dye reagent. An absorbance ratio of 630 nm/ 450 nm was used to plot a standard curve and measure the total serum concentration. Immunoglobulin was determined according to Ardó et al. (2008) using a 12% w/v poly-ethylene glycol (PEG) solution (Sigma- Aldrich, Average molecular weight 10000 Dalton, Sigma). The blood serum was diluted with 12% PEG (1:1 dilution) and allowed to stand at room

temperature for two hours before being centrifuged for 10 minutes at 1400 rpm. The supernatant after PEG precipitation was used to measure immunoglobulin by taking the difference between the total serum protein and the supernatant, while taking the dilution into consideration.

5.3.8 Tissue mineral analysis

At the conclusion of the 96-day feeding trial, one fish from each tank was selected and killed by overexposure to anaesthetic (800 mgl^{-1} of tricaine methanesulfonate, MS-222, (Sigma-Aldrich, Johannesburg, South Africa,) using 800 mgl^{-1} as a buffer. The filet, liver, and vertebrae were harvested and frozen at -20° C until further analysis. The filet and the liver were ground in a Hobart meat grinder (Hobart Food Equipment, Troy, OH, USA). The samples for minerals were digested in nitric acid and hydrogen peroxide using the CEM MAR microwave digester. The Thermo iCAP6000 series, ICP Emission Spectrometer was employed for determining the mineral concentrations at Stellenbosch University by the Central Analytical Facility (ASTM D 4239; ASTM D 5373). To perform mineral analysis, the vertebrae were treated with an enzyme (*Alcalase® 2.4L FG, Novozyme,* Denmark) at 60° C for 1 hour to remove any protein, then rinsed with distilled water, dried in an oven at 80° C for 6 hours, and subsequently ground.

5.3.9 Apparent digestibility coefficient

A two-week apparent digestibility coefficient trial was conducted to determine the apparent digestibility coefficient (ADC) of potassium. The trial used six fish per 100 l tank with an average weight of 400 g. Each treatment was replicated three times. The fish were fed at 3 % of their body weight, 120 minutes before faeces were collected. The ADC was evaluated by use of an inert marker (Chromium (III) oxide, Cr_2O_3) (Sigma-Aldrich, Johannesburg, South Africa) included at 5 gkg^{-1}. The concentration of the inert marker was measured in the feed and the faeces, and ADC of potassium was calculated according to Harter et al., (2015) with the equation:

$$\mathrm{ADC} = \left(\frac{1 - \left(\frac{\mathrm{N_f}}{\mathrm{M_f}}\right)}{\left(\frac{\mathrm{N_d}}{\mathrm{M_d}}\right)} \right) \times 100$$

where:

ADC = the apparent digestibility coefficient (%)

M= marker

N= nutrient

M_d = marker concentration in the diet

M_f = marker concentration in the faeces

N_d = nutrient concentration in the diet

N_f = nutrient concentration in the faeces

5.3.10 Faecal collection method

During a 14-day trial period, the fish were stripped twice, first at week one and then at week two to obtain faeces. Stripping is a non-lethal way of collecting faeces where the area above the anus of the fish is squeezed gently until faeces is produced. Forceful stripping was avoided to not contaminate the faeces with urine, sperm, or eggs (Ramsay et al., 2000). The animals were stripped in the morning, 120 minutes after feeding. The animals were anaesthetised during the stripping and faeces were collected in a sampling bottle. The faeces samples were pooled and stored at -20° C until further analysis was done. Because the samples were pooled, they could not be statistically analysed.

5.3.11 Water quality analysis

The experiment was performed over 2 days using a static system with three replicates per treatment, this is because a period longer than two days would adversely affect fish welfare. Feed water to the tank was closed, allowing no water exchange while each individual tank was fitted with an aerator to provide oxygen. This was due to the lack of a system with a collection tank or sump that would separate wastewater from different treatments. The effluent produced affects the type of plants and rate that the plants can be produced in an aquaponics

system (Buzby et al., 2016) therefore, it is important to measure the nutrients excreted by the fish.

All the treatments were replicated three times in 100 l glass tanks. Six fish per tank with an average weight of ±477 g were used. The fish were fed at 3 % of their body weight, three times a day. Equal amounts of feed were given to each tank. The fish were observed visually through the glass tanks during the feeding and were fed until apparent satiation to avoid feed loss. A randomised experimental set-up was used.

The measured parameters are water temperature, pH, dissolved oxygen, total dissolved solids, total suspended solids, total ammonia nitrogen, reactive phosphorus, potassium, and iron. Samples were measured twice a day (08:00 and 16:00) and samples were taken from each tank (Table 5.2).

Table 5.1 Summary of water quality parameters measured with method and instrument applied.

Parameter	Units	Method/Instrument
Temperature	°C	YSI Pro Plus Multi-Parameter Water Quality Meter
Dissolved Oxygen	mgl^{-1}	
pH	NA	Hanna pH 211 microprocessor
Total Dissolved Solids	µS	AZ 8603 IP67 Water Quality Portable Meter
Total Suspended solids	mgl^{-1}	Photometric Method/ Hach DR 850 Colorimeter
Total Ammonia Nitrogen	mgl^{-1}	Hach DR 3900 Laboratory Spectrophotometer
Phosphorus (reactive P)	mgl^{-1}	Hach DR 3900 Laboratory Spectrophotometer
Potassium	mgl^{-1}	Hach DR 3900 Laboratory Spectrophotometer
Iron	mgl^{-1}	Hach DR 3900 Laboratory Spectrophotometer

5.3.12 Statistical analysis

A statistician from the Stellenbosch University's Centre for Statistical Consultation was consulted for all analyses performed. In this study, all continuous response variables are presented as mean± SE (standard error). Experimental results were analysed using Statistica 13 (Dell, Inc). A one-way analysis of variance (ANOVA) was used to determine whether the treatment means of continuous response variables are the same. If the null hypothesis was rejected, the means were deemed statistically significant if the ANOVA p – value was less than 0.05. Fisher's Least Significant Differences (LSD) procedure was used as a post-hoc test to determine which means were different from one another. If the variance of the treatment groups were non-homogeneous according to the Levene test, a Games-Howell multiple comparisons procedure was used to compare the treatment means instead of LSD multiple comparisons.

5.4 Results

5.4.1 Production parameters

Table 5.3 represents the summary of the production parameters evaluated in this study. The production parameters were not significantly affected by the dietary additives during the trail. The FCR ranged from 1.08 ± 0.02 for fish fed the diet containing KDF 3, while fish fed the diet containing KDF 6 had an FCR of 1.18 ± 0.13. The fish fed the diets containing KCl 3.4 had a weight gain of 337 ± 40.17 g, while KDF 3 was 307 ± 18.33 g. Fish fed the diets containing KDF 6 had a weight gain of 261 ± 29.64 g. The highest SGR was recorded in fish fed the diet containing KCl 3.4 and the lowest in fish fed KDF 6 and KCl 17. The survival rate was generally high throughout the study, ranging from 73% for KDF 3 to 97% for KCl 5.1. The mortalities observed were due to the cannibalistic nature of the fish.

Table 5.2 Mean growth performance of the African catfish *C. gariepinus* fed different dietary fish feed additives at different levels (n=3) over a 96-day trail period.

Parameter	Treatment						
	KDF 3	KDF 6	KDF 9	KCl 1.7	KCl 3.4	KCl 5.1	Control
Initial weight (g)	106±4.68	108±12.23	113±3.67	117±7.33	97±7.88	109±4.04	110±7.24
Final weight (g)	414±13.65	370±24.57	414±23.67	402±40.04	434±39.16	399±32.80	384±30.57
Weight gain (g)	307±18.33	261±29.64	301±27.21	284±38.16	337±40.17	289±28.76	273±33.26
FCR	1.08±0.02	1.18±0.13	1.15±0.06	1.16±0.14	1.09±0.10	1.15±0.09	1.11±0.10
SGR (%)	1.38±0.07	1.25±0.14	1.32±0.08	1.25±0.09	1.52±0.12	1.32±0.04	1.27±0.11
Survival (%)	73±6.66	83±8.81	87±3.33	90±5.77	77±3.33	97±3.33	90±5.77

Data presented as mean ± SE. All treatments p> 0.05. FCR: feed conversion ratio. SGR: specific growth rate.

5.4.2 Whole-body proximate composition

The whole-body proximate composition of the African catfish over the 96-day feeding trail was affected by the different dietary treatments, except for the ash content ($p > 0.05$) (Table 5.4). The moisture content of the fish fed the control diet was the highest (79% ± 1.16) and statistically similar to diets containing KDF 6, 9 and KCl 3.4. The lowest moisture content of 76%± 1.44 and 76% ± 0.37 was recorded in fish fed diets containing KCl 1.7 and KCl 5.1, respectively and these treatments were not significantly different from KDF 3 (77% ± 0.40). The crude fat content was significantly different between the dietary treatments. The highest crude fat content was observed in fish fed diets containing KCl 1.7 (4.7% ± 1.04) and KCl 5.1 (4.7% ± 0.24), however, they were similar to treatments containing KDF 3 and KDF 6. Crude fat differed significantly between the dietary treatments, ranging from 15.3% ±1.11 (control) to 17.5%±0.34 (KDF9).

Table 5.3 Whole-body proximate composition of the African catfish, *C. gariepinus* fed experimental diets and control diet (n=3).

Component (%)	Treatment						
	KDF3	KDF6	KDF9	KCl1.7	KCl3.4	KCl5.1	Control
Moisture	77±0.40^{bc}	78±0.33^{ab}	78±0.33^{ab}	76±1.44^{c}	78±0.30^{ab}	76±0.37^{c}	79±1.16^{a}
Ash	1.2±0.02	1.1±0.02	1.1±0.07	1.3±0.16	1.1±0.09	1.2±0.07	1.3±0.18
Crude Fat	3.7±0.24^{abc}	3.8±0.30^{abc}	2.7±0.10^{d}	4.7±1.04^{a}	3.5±0.14^{bcd}	4.7±0.24^{a}	3.3±0.38^{cd}
Crude Protein	17.2±0.38^{a}	16.6±0.30^{ab}	17.5±0.34^{a}	17.0±0.47^{ab}	16.3±0.43^{ab}	17.1±0.38^{a}	15.3±1.11^{b}

Data presented as mean ± SE. Different superscripts in the same row indicate significant differences, $p < 0.05$

5.4.3 Haematology and non-specific immunity

The summary of haematological indices are presented in Table 5.5. The haematological indices were affected by the different treatments, except the red blood cells (RBC) and white blood cells (WBC) ($p > 0.05$). Haematocrit levels (HCT) were significantly affected by the dietary treatments ($p < 0.05$); KDF 9 (36.7%± 1.84) significantly differed from KCl 1.7 treatment (32.1%± 2.30). The inclusion level of KDF 9 had significantly higher and different haemoglobin values (Hb) than the rest of the dietary treatments (14.55 gdl^{-1} ± 0.54). Mean corpuscular haemoglobin concentration (MCHC) values were significantly higher for KCl 1.7 treatment (41.8 gdl^{-1} ± 0.69) but did not differ from the KDF 3, KDF 6, KDF 9 treatments. They differed only from KCl 3.4, KCl 5.1, and the control treatment. The mean corpuscular haemoglobin (MCH) values of the KDF 9 treatments were significantly high although not different from the control, KDF 3, and KCl 1.7 treatment. The mean corpuscular volume (MCV) was similar for all treatments except the KCl 1.7 dietary treatment (see Table 5.5).

The non-specific immunity parameters (Table 5.6) were not affected by the different dietary treatments ($p > 0.05$). The total protein concentration ranged from 26.13 $mgml^{-1}$ ± 6.25 for the control to 36.68 $mgml^{-1}$ ± 2.12 for the fish fed the KDF 9 diet. Immunoglobulin concentrations observed in KCl 1.7 and KCl were 9, 24.39 $mgml^{-1}$ ± 0.30 and 24.12 $mgml^{-1}$ ± 0.64, respectively.

Table 5.4 Haematological indices of the African catfish, *C. gariepinus* fed different experimental diets and the control (n=3).

Indices	Treatment						
	KDF3	KDF6	KDF9	KCl1.7	KCl3.4	KCl5.1	Control
HCT (%)	33.38±1.27^{ab}	33.61±0.31^{ab}	36.76±1.84^{a}	32.11±2.30^{b}	36.18±0.87^{ab}	34.35±0.99^{ab}	33.93±0.78^{ab}
Hb (gdl^{-1})	12.86±0.45^{b}	12.85±0.11^{b}	14.55±0.54^{a}	13.15±0.42^{b}	13.31±0.24^{b}	12.86±0.34^{b}	12.86±0.18^{b}
RBC ($10^{12}l^{-1}$)	2.44±0.09	2.48±0.01	2.51±0.15	2.37±0.19	2.57±0.04	2.49±0.04	2.37±0.56
MCHC (gdl^{-1})	38.61±0.76^{ab}	38.21±0.36^{ab}	39.66±0.60^{ab}	41.80±0.69^{a}	36.81±0.52^{b}	37.41±0.47^{b}	38.03±1.08^{b}
MCH (pg)	52.78±0.84^{ab}	51.68±0.28^{b}	58.23±1.52^{a}	57.11±0.43^{ab}	51.65±0.43^{b}	51.55±0.78^{b}	54.23±0.68^{ab}
MCV (fl)	136.66±0.49^{ab}	135.33±0.76^{ab}	146.50±1.74^{a}	116.50±2.08^{b}	140.16±1.85^{ab}	137.83±2.77^{ab}	143.33±4.67^{a}
WBC ($10^{9}l^{-1}$)	7.19±2.80	9.59±5.60	11.98±5.96	7.78±4.61	5.10±2.29	6.80±4.18	11.23±4.32
HSI (%)	1.02±0.08	1.05±0.11	1.16±0.11	1.01±0.14	1.26±0.05	1.03±0.06	1.20±0.12

Data presented as mean ± SE. Different superscripts in the same row indicate significant differences, $p< 0.05$. HCT: haematocrit levels. Hb: haemoglobin. RBC: red blood cells. MCHC: mean corpuscular haemoglobin concentration. MCH: mean corpuscular haemoglobin. MCV: mean corpuscular volume. WBC: white blood cells. HSI: hepatosomatic index.

Table 5.5 Summary of non-specific immunity indicators of the African catfish, *C. gariepinus* fed experimental diets and the control (n=3).

Parameters	Treatment						
	KDF3	KDF6	KDF9	KCl1.7	KCl3.4	KCl5.1	Control
Lysozyme (μgml^{-1})	3.10±0.76^{a}	2.39±0.56^{a}	2.36±0.12^{a}	2.90±0.34^{a}	1.78±0.58^{a}	2.70±0.65^{a}	3.53±0.49^{a}
Immunoglobulin ($mgml^{-1}$)	23.64±0.34^{a}	22.95±0.75^{a}	24.12±0.64^{a}	24.39±0.30^{a}	23.57±0.82^{a}	22.69±0.44^{a}	23.97±0.36^{a}
Total protein ($mgml^{-1}$)	28.38±2.56^{a}	30.34±2.61^{a}	36.68±2.12^{a}	32.68±2.56^{a}	34.94±1.89^{a}	26.24±1.50^{a}	26.13±6.25^{a}

Data presented as mean ± SE. Different superscripts in the same row indicate significant differences, $p< 0.05$

5.4.4 Tissue mineral analysis

The results of the mineral composition analysis of the vertebrae, liver, and filet of fish fed different potassium feed additives are presented in Table 5.7. The potassium and iron concentrations in the vertebrae and filets of fish were not affected by the different treatments. There was no significant difference in the potassium levels in the liver of fish fed diets containing potassium from different sources; however, there were significant differences in the iron levels in the liver of the fish. The fish fed the control diet did not differ significantly from the fish fed diets containing KDF 3, KCl 1.7, and KCl 5.1. The lowest iron levels in the liver were observed in the fish fed diets containing KDF 6, KDF 9, and KCl 3.4, all containing 0.04 gkg^{-1} ±0.00.

Table 5.6 Summary of mineral analysis of the African catfish, *C. gariepinus* fed experimental diets and the control (n=3).

	Treatment						
	KDF 3	KDF 6	KDF 9	KCl 1.7	KCl 3.4	KCl 5.1	Control
Vertebrae							
K (gkg^{-1})	5.05±1.64	4.77±1.73	2.69±1.11	2.86±0.38	5.29±2.13	5.11±2.00	2.15±0.14
Fe (gkg^{-1})	0.05±0.01	0.06±0.03	0.03±0.01	0.04±0.00	0.03±0.00	0.04±0.01	0.03±0.01
Filet							
K (gkg^{-1})	5.70±0.51	5.59±0.60	5.97±0.14	5.03±0.13	5.26±0.27	5.68±0.14	5.23±0.41
Fe (gkg^{-1})	0.01±0.00	0.00±0.00	0.02±0.01	0.01±0.00	0.03±0.02	0.01±0.00	0.01±0.00
Liver							
K (gkg^{-1})	4.78±0.27	5.09±0.52	4.23±0.35	4.50±0.80	4.12±0.40	4.14±0.33	4.27±0.22
Fe (gkg^{-1})	$0.05±0.01^{ab}$	$0.04±0.00^{b}$	$0.04±0.00^{b}$	$0.05±0.01^{ab}$	$0.04±0.02^{b}$	$0.06±0.01^{ab}$	$0.25±0.13^{a}$

Data presented as mean ± SE. Different superscripts in the same row indicate significant differences, $p < 0.05$.

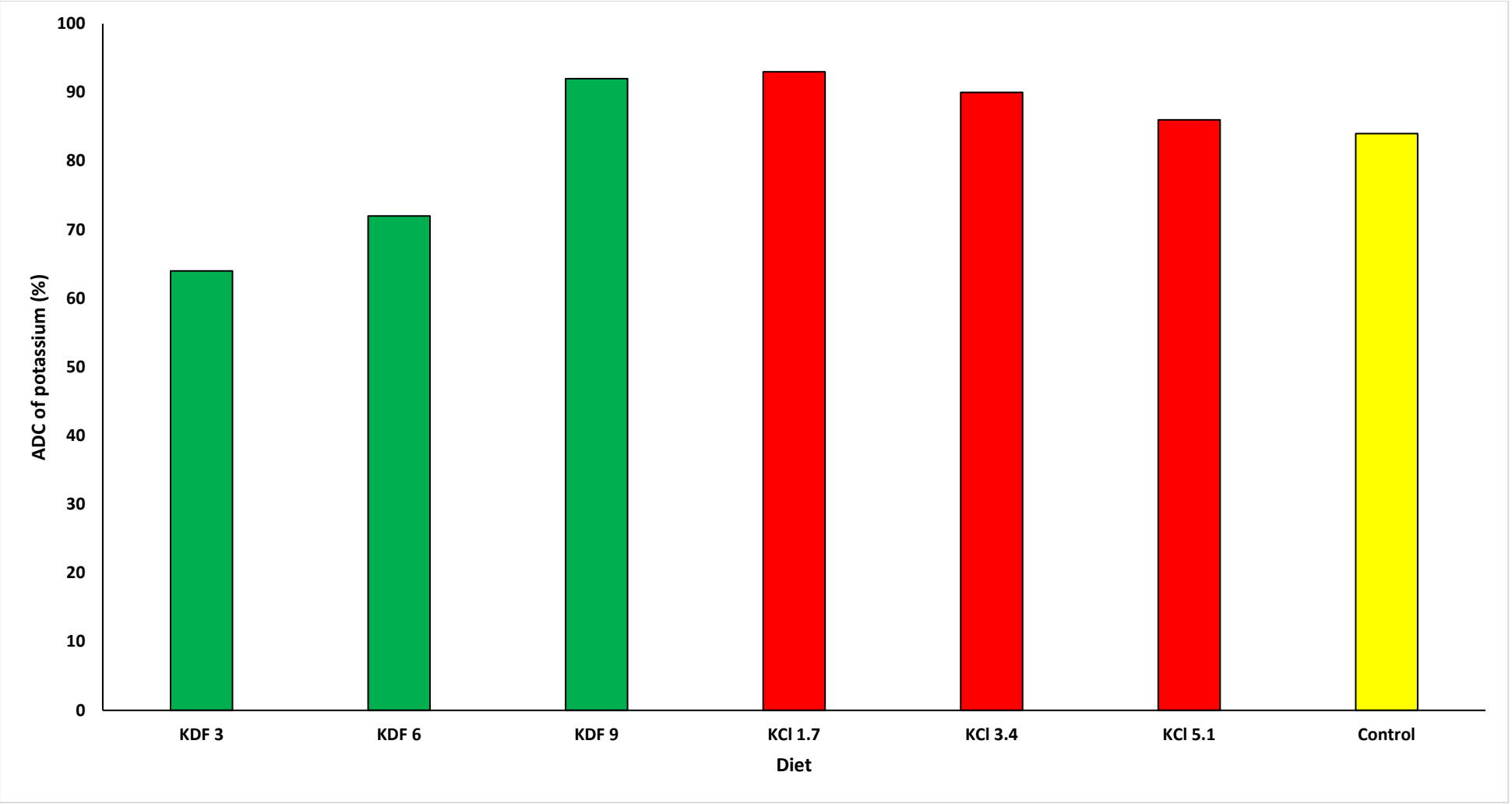

Figure 5.1 Apparent Digestibility Coefficient (ADC) of potassium from feed additives over a 14-day trial period using the African catfish ($p > 0.05$). KDF: Potassium diformate. KCl: Potassium chloride. No replicates where available due to sample pooling thus no SE bars are shown.

5.4.5 Apparent digestibility coefficient

The results of the apparent digestibility coefficient of K trial could not be replicated as the faecal samples were too small and had to be pooled. Therefore, the data presented was not statistically analysed. The ADC observed in the KCl 1.7 treatment was 93 % followed by KDF 9, and KCl 3.4 at 92, and 90%, respectively. The digestibility coefficient of the KDF 3 treatment at 64% (Figure 5.1).

5.4.6 Water quality analysis

A summary of the effects of different dietary treatments on water quality parameters in a static tank is presented in Table 5.8. The results show the changes in the parameters over two days, day one and day two. The pH was not affected by the different treatments ($p > 0.05$) and ranged from 7.70±0.05 to 7.76±0.05. In dissolved oxygen (DO) concentrations, the treatments that significantly differed were KCl 3.4 and KCl 5.1 ($p < 0.05$) on day one. The total dissolved solids (TDS) concentrations differed significantly on day one; KCl 3.4 was significantly higher than the control and KCl 1.7 ($p < 0.05$) treatment. The total suspended solids (TSS) differed significantly between treatments; KCl 3.4 was significantly higher than all other treatments, except the control and KCl 1.7. Total Ammonia Nitrogen (TAN) concentrations differed between treatments, KDF 3 (7.32 mgl^{-1} ±1.95) and KCl 3.4 (5.21 mgl^{-1} ±1.95). The phosphorus (P), potassium (K) and iron (Fe) concentrations did not differ between the treatments on the first day ($p > 0.05$). The pH, DO, TDS, and TSS did not differ between treatments on day two. The TAN concentrations of KCl 5.1, KCl 3.4, and KCl1.7 were significantly higher than KDF 3 and KDF 9 on the second day. The control had the highest P concentration and differed significantly from KCl 1.7 and KCl 3.4. The potassium concentration did not differ between treatments ($p > 0.05$), however, iron concentrations between KCl 3.4 and KCl 5.1 were significantly higher than the control.

Table 5.7 Effect of potassium supplementation through feed additives on water quality in a static aerated culture system over a two-day period (n=3).

Parameters	Treatment						
	KDF3	KDF6	KDF9	KCl1.7	KCl3.4	KCl5.1	Control
Day 1							
Temperature	27.38±0.26^{ab}	27.52±0.26^{a}	27.50±0.26^{a}	27.52±0.26^{a}	27.42±0.26^{ab}	27.47±0.26^{a}	27.25±0.26^{b}
pH	7.64±0.05^{a}	7.70±0.05^{a}	7.73±0.05^{a}	7.71±0.05^{a}	7.76±0.05^{a}	7.74±0.05^{a}	7.70±0.05^{a}
DO(mgl^{-1})	7.59±0.13^{ab}	7.52±0.13^{ab}	7.68±0.13^{ab}	7.62±0.13^{ab}	6.60±0.13^{b}	7.81±0.13^{a}	7.63±0.13^{ab}
TDS (µS)	142.91±10.8^{ab}	144.40±10.8^{ab}	145.30±10.8^{ab}	142.61±10.8^{b}	145.53±10.8^{a}	144.98±10.8^{ab}	142.80±10.8^{b}
TSS(mgl^{-1})	14.50±7.43^{b}	13.507.43±^{b}	14.66±7.43^{b}	17.83±7.43^{ab}	21.33±7.43^{a}	14.33±7.43^{b}	16.33±7.43^{ab}
TAN(mgl^{-1})	7.32±1.95^{a}	6.35±1.95^{ab}	7.04±1.95^{ab}	6.64±1.95^{ab}	5.21±1.95^{b}	5.40±1.95^{ab}	6.42±1.95^{ab}
P(mgl^{-1})	1.53±0.15^{a}	1.50±0.15^{a}	1.59±0.15^{a}	1.46±0.15^{a}	1.41±0.15^{a}	1.45±0.15^{a}	1.42±0.15^{a}
K(mgl^{-1})	6.56±4.81^{a}	5.76±4.81^{a}	6.93±4.81^{a}	5.65±4.81^{a}	6.20±4.81^{a}	6.20±4.81^{a}	5.45±4.81^{a}
Fe(mgl^{-1})	0.010±0.00^{a}	0.010±0.00^{a}	0.000±0.00^{a}	0.003±0.00^{a}	0.013±0.00^{a}	0.010±0.00^{a}	0.000±0.00^{a}
Day 2							
Temperature	23.00±0.26^{b}	23.05±0.26^{b}	23.33±0.26^{ab}	22.92±0.26^{b}	22.77±0.26^{b}	23.77±0.26^{a}	22.95±0.26^{b}
pH	7.59±0.05^{a}	7.61±0.05^{a}	7.59±0.05^{a}	7.58±0.05^{a}	7.59±0.05^{a}	7.53±0.05^{a}	7.61±0.05^{a}
DO (mgl^{-1})	5.56±0.13^{a}	5.06±0.13^{a}	5.56±0.13^{a}	5.91±0.13^{a}	6.10±0.13^{a}	7.01±0.13^{a}	6.51±0.13^{a}
TDS (µS)	135.41±10.8^{a}	236.55±10.8^{a}	212.73±10.8^{a}	222.28±10.8^{a}	233.06±10.8^{a}	243.00±10.8^{a}	211.20±10.8 ^{a}
TSS (mgl^{-1})	47.50±7.43^{a}	65.83±7.43^{a}	46.66±7.43^{a}	64.00±7.43^{a}	63.16±7.43^{a}	62.16±7.43^{a}	50.16±7.43^{a}
TAN (mgl^{-1})	19.97±1.95^{c}	22.17±1.95^{abc}	19.93±1.95^{c}	23.52±1.95^{ab}	24.03±1.95^{a}	24.97±1.95^{a}	21.05±1.95^{bc}
P (mgl^{-1})	2.23±0.15^{ab}	2.40±0.15^{ab}	2.45±0.15^{ab}	2.04±0.15^{b}	1.99±0.15^{b}	2.23±0.15^{ab}	2.57±0.15^{a}
K (mgl^{-1})	28.10±4.81^{a}	21.01±4.81^{a}	22.28±4.81^{a}	23.60±4.81^{a}	21.48±4.81^{a}	18.45±4.81^{a}	18.73±4.81^{a}
Fe (mgl^{-1})	0.006±0.00^{abc}	0.005±0.00^{bc}	0.011±0.00^{abc}	0.010±0.00^{abc}	0.013±0.00^{ab}	0.018±0.00^{a}	0.000±0.00^{c}

Data presented as mean ± SE. Different superscripts in the same row indicate means are significantly different, $p<0.05$. DO: dissolved oxygen. TDS: total dissolved solids. TSS: total suspended solids. TAN: total ammonia nitrogen. P: phosphorus. K: potassium. Fe: iron.

5.5 Discussion

In the present study, different dietary potassium sources at different inclusion levels did not significantly affect the production performance of the African catfish. Some authors have reported differences in production performance upon the addition of minerals in feed from different sources (Apines et al., 2001; Satoh et al., 2001). In this study however, the two dietary additives KDF and KCl did not impact production performances.

The lack of significance observed when KCl was included in the diet of the African catfish is not unheard of, as the inclusion of KCl in the diet of fish and shrimp species has showed variation in the past. In some studies, the inclusion of KCl as a dietary feed additive significantly improved growth (Shiau and Hsieh, 2001a, 2001b; Roy et al., 2007) while in others it did not (Booth and Fielder 2016). The study by Booth and Fielder (2016) on juvenile snapper *Pagrus auratus* did not show improved growth when KCl was added in its diet, they presume that the fish was unable to utilise KCl from the aquafeed and that K was not being absorbed, similar to this study.

Results on the use of KDF as a feed additive in literature are not always consistent. It was expected that K from an organic source such as KDF would be readily available compared to an inorganic source such as KCl, possibly resulting in improved production performance of fish (Apines et al., 2003; Lückstädt et al., 2012; Lückstädt et al., 2013). The inclusion of KDF in diets of some animals has improved growth and FCR (Partanen and Mroz, 1999; De Wet, 2005; Lückstädt and Mellor, 2011; Abu Elala and Ragaa, 2015), while in others it did not (Petkam et al., 2008; Zhou et al., 2009). It is clear from literature that the inclusion of KDF as a dietary additive does not always produce improved animal growth. These differences in the effect of KDF have been attributed to species-specific differences and the nutritional utilisation or digestive processes of fish (Hassan et al., 2014). The results of this study with the African catfish are therefore consistent with literature as they showed no significant effects as a result of KDF inclusion.

The different dietary feed additives had a significant effect on the proximate composition of the African catfish. The primary determinants of proximate composition in growing fish are size, life cycle, and energy intake (Shearer, 1994). Ofudje et al. (2014), also state that variation in proximate composition is generally attributed to the quantity and quality of food the fish

consumes. In this study, diets were formulated isonitrogenous and isocaloric, with the exception of the minerals that were added at different inclusion levels. Therefore, the differences in proximate composition in this study can be attributed to the dietary inclusion of potassium additives. Each potassium additive has different effects on different species, and literature on the effect of these additives is scarce. Ng et al. (2009) reported no adverse effects and significant differences in proximate composition when tilapia were fed blended organic acids and KDF at 2 gkg^{-1}. Similarly, the whole body proximate composition of grass carp was significantly affected when KCl was included in the diet, where the moisture content increased as the lipid content decreased (Zhu et al., 2014). Furthermore, the inclusion of KDF in the diet of Vietnamese pangasius (*Pangasianodon hypophthalmus*) promoted protein digestion (Lückstädt et al., 2012), and it also significantly improved protein efficiency ratio in tilapia (Abu Elala and Ragaa, 2015). Improved protein utilization may impact whole body protein composition through higher protein deposition (Abu Elala and Ragaa, 2015). Evidently, the addition of potassium as a dietary feed additive changed the proximate composition of the African catfish in this study and it did not have any adverse effects on its production. However, the data does not present any clear mechanisms behind the differences observed in the proximate composition. This is evident in the moisture content that is similar between KDF, KCl and the control treatments. Equally, the crude protein is similar between some KDF and KCl treatments.

The haematological parameters were affected by the different dietary treatments. Nutrient additives affect the nutritional status of fish, thereby affecting the immune system (Kiron, 2012; Khosravi et al., 2015). Therefore, when haematological values are examined, they show the general health status of the fish during a feeding trial (Harikrishna et al., 2010). In the parameters measured, KDF 9 showed the highest numerical values, even though for HCT and MCV these values did not differ statistically from the control. Existing literature on the effect of KDF and KCl on the haematological profile of the African catfish or other fish species is scarce, making it difficult to align our results to any literature. Even so, KDF 9 had a significantly higher Hb levels and, Hb plays a significant role in meeting the oxygen demands the body needs to survive. These findings point to KDF's ability to improve the haematological profile of the African catfish.

It is difficult to make a direct comparison of normal haematological profiles of the African catfish because different experimental conditions are used in different studies. However, the

results of this study are compared to studies by Al-Dohail et al. (2009) and Ayoola (2016) also working on the haematological profile of the African catfish. Although these studies used different additives and control diets to evaluate the haematological profile of the catfish, there were similarities in the parameters. The study by Al-Dohail et al. (2009) showed HCT levels for the control were 26.85% and were significantly lower than 30.01% of the supplemented diet. The HCT results of Al-Dohail et al. (2009) are comparable to this study, which ranged from 32.11% to 36.76% and to the study by Ayoola (2016), which ranged from 31.97% to 34.84%. The HCT levels in this study can be considered normal for an African catfish. Similarly, the WBC and RBC levels in the study by Al-Dohail et al. (2009) are comparable to this study, however, the WBC levels in the study by Ayoola (2016) were different and higher. The MCHC levels of the control in the study by Al-Dohail et al. (2009) were 31.51 gdl^{-1} while the supplemented diet were 32.10 gdl^{-1}, the Hb was 8.46 gdl^{-1} for the control and 9.62 gdl^{-1} for fish fed the supplemented diet. In this study, the MCHC levels ranged between 36.81 to 41.80 gdl^{-1}, while the Hb ranged from 12.85 – 14.55 gdl^{-1}. Based on the comparison of the haematological profiles of these catfish, the results obtained in this study can be considered within normal range for catfish and any statistical differences obtained entail a biological significance in the haematology of the catfish. As such, these results indicate that the inclusion of KDF as a potassium additive has positive implications for the production of the African catfish in recirculating aquaculture systems, an increase in Hb and HCT levels is associated with a better haematological profile (Al-Dohail et al., 2009). A better haematological profile in fish translates to a better immune system that responds against diseases and infections in fish.

The different dietary treatments did not have an effect on the HSI of the African catfish during this study. HSI is described as a ratio of liver weight to fish weight. The lack of differences in HSI observed between the dietary treatments in this study may be an indication that none of the potassium dietary additives affected liver activity, although liver activity was not directly measured. Ayoola (2016) who also worked on the African catfish found a higher HSI compared to this study which he attributed this to increased liver activity during digestion of diets containing bentonite.

The non-specific immunity parameters measured from blood serum of the African catfish showed no significant differences between the different dietary treatments. The possible reason may be that the non-specific immunity function observed was good for all the dietary treatments throughout the trial, and any small differences in non-specific immunity were not

large enough to be measurable (Kiron, 2012). The results of this study are different from Abu Elala and Ragaa (2015) working on *Oreochromis niloticus* who found that serum lysozyme activity was higher for fish fed KDF at 3 gkg^{-1} inclusion than the control and KDF at 1 and 2 gkg^{-1}. Investigations into the effects of non-specific immunity using KCl as a feed additive are scarce, and none could be found dealing specifically with the African catfish.

In this study we used the stripping method to obtain fish faeces. The stripping method resulted in small faeces being obtained over the two-week sampling period, therefore the samples were pooled and each of the samples was represented. This study provided a good indication of the apparent digestibility coefficients of potassium feed additives evaluated during this study. Even though the ADC values could not be statistically analysed, there were numerical differences between the percentage digestibility coefficients, indicating how the potassium in the dietary treatments were assimilated by fish. The ADC of potassium for these fish feed additives are not widely available, unlike ADC values for crude protein, energy, and dry matter using the African catfish which are well-documented (Fagbenro, 1996; 1998; Pantazis and Neofitou, 2004). Information on the apparent digestibility of K in KDF and KCl feed additives may play a role in the improvement of feed formulation for both aquaculture and aquaponics systems. The best method of faecal collection in fish digestibility studies is controversial (Cook et al., 2000). The inclusion of different potassium additives as mineral sources at different inclusion levels did not affect the K and Fe content of the filet and vertebrae of the fish. However, the Fe content of the liver was affected. Fish muscles are capable of bioaccumulation of minerals when they are present in the culture water or in their diets (Fawole et al., 2007; Ofudje et al., 2014). Therefore, the filet, liver, and vertebrae of fish had to be tested to investigate the effect of the different potassium additives and inclusion levels. The vertebrae and filet mineral composition in this study were similar for all dietary treatments. The vertebrae mineral composition in this study is comparable to the bone mineral composition in Toko et al. (2008) also working on the African catfish. The inclusion of potassium feed additives to the diet of the African catfish in this study did not result in the accumulation of minerals. The Fe levels in the filet of the fish were lower compared to results by Toko et al. (2008). The Fe levels in the liver of the fish fed the control treatment was significantly higher than all dietary treatments except for KDF 3, KCl 1.7, and KCl 3.4. The reason for this high concentration of iron in the liver of the fish fed these treatments is unclear.

There does not seem to be a discernible reason for the high concentration of iron in the liver of these treatments.

The water quality parameters in a static tanks were measured to evaluate the accumulation of nutrients resulting from excretion by the experimental animals. Mineral excretion is important in aquaponics systems because the effluent produced and its composition affects the type of plants that can be grown in aquaponics systems, and rate that the plants can be produced (Buzby et al., 2016). Furthermore, the nutrient concentrations produced by fish through wastewater must maintain plant production (Seawright et al., 1998; Pantanella et al., 2012; Endut et al., 2010). The water quality experiment showed that nutrients in the form of faeces, urine and ammonia excreted through fish gills can build up in a tank when fish are fed different dietary treatments. Although not entirely representative of the conditions in an aquaponics system, the static set-up gave a good indication of how nutrients from the different dietary treatments would accumulate, without compromising the wellbeing of the fish.

TAN, a combination of ammonium ion (NH_4^+) and un-ionised ammonia (NH_3), was significantly affected by the treatments. The significant difference of TAN concentration between the treatments may have been due to different rates of decomposing faeces and uneaten feed in the different tanks (Endut et al., 2010). In this study, however, the latter is unlikely to be a reason for the differences in the TAN as fish were observed actively consuming the feed through the glass tanks. Furthermore, the African catfish can feed at the bottom of the tank (Martins et al., 2005), and any feed left at the bottom would be consumed. The differences may also have resulted because nutrients in aquaculture systems do not accumulate at equal rates (Seawright et al., 1998; Endut et al., 2010; Endut et al., 2011). Even though TAN is pH and temperature dependent, it remained at non-toxic levels during the trial period. In this study, temperature was not observed to have any effect on the treatment, which could be as a result of the short trial period and because the decrease in temperature was gradual for all the treatments, including the control. It is expected, however, that the effects of temperature would have been significant over a longer period of study. The changes observed in TAN concentration can be attributed to the dietary treatments.

In this study potassium content of the feed was manipulated along with its inclusion level to influence its excretion and possibly reduce the need to supplement K if this feed is used in

aquaponics systems. This was done because potassium is routinely supplemented to aquaponics systems (Seawright et al., 1998; Rackocy et al., 2006; Pantanella et al., 2012). The inclusion of K feed additives to the diet of the African catfish increased the K concentration in the wastewater excreted by fish. However, the K concentration in the wastewater did not differ significantly between the treatments. Numerically, KDF 9 had a high K concentration compared to other treatments and the control on day 1, while on day 2 KDF 3 had the highest concentration. Although the differences were not significant, further investigations in an aquaponics trial are worth carrying out, especially with KDF 9 because it also showed an improvement in the haematological profile of the catfish. The addition of KDF has been proven to improve fish production parameters (De Wet, 2005; Lückstädt and Mellor, 2011), intestinal function (Abu Elala and Ragaa, 2015), and production in terrestrial animals (Partanen and Mroz, 1999). It is worth noting that if there is no dual benefit to the aquaponics sytem resulting from dietary inclusion of potassium additives (i.e, benefits to both the fish and to the plants), it might remain a more practical option to supplement nutrients only to the plants, seeing that trace element supplementation might still berequired, regardless of the possible potassium increase.

5.6 Conclusion

The results indicate that KDF as feed additive can improve the health status via improved haematological profile of the African catfish in a recirculating aquaculture system. Furthermore, the results show that the nutrient composition of a fish diet can be manipulated so that the excreted nutrients are similar to nutrients required by plants grown in aquaponics, while still maintaining optimal fish growth. Particularly the 9 gkg^{1} KDF treatment deserves further investigation in an integrated aquaponics system, as this treatment did not adversely affect the production and proximate composition of the fish, it improved haematological indices, and had similar potassium excretion than the other treatments.

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Author contributions

Declaration by candidate

With regards to chapter 6, Evaluation of chelated iron and iron sulphate in the diet of African catfish *Clarias gariepinus* to enhance iron excretion for application in integrated aquaponics systems, the nature and scope of my contribution were as follows:

Chapter	Pages	Nature of contribution	Extent of contribution (%)
6	80 - 116	Planned and designed the experiments. Formulated the fish feed, procured the ingredients and prepared the feed. Carried out the daily feeding, water measurements, the sampling of fish for production performance, performed the proximate analysis on feed and fish. Responsible for collection of blood samples and performing the haematology and non-specific immunity experiments. Responsible for tissue fileting for mineral analysis, collected faecal samples for the apparent digestibility coefficient trial. Collected and performed the analysis on the water quality trial. Prepared and analysed data. Wrote the manuscript and collated all the comments from the co-authors prior to submission.	80

The following co-authors have contributed to chapter 6:

Name	Email address	Nature of contribution	Extent of contribution (%)
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Signature of candidate……..

Date……

Declaration by co-authors

The undersigned hereby confirm that

1. The declaration above accurately reflects the nature and extent of the contribution of the candidate and the co-authors to chapter 6 in the dissertation,
2. No other author contributed to chapter 6 and
3. Potential conflict of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in chapter 6 of this dissertation.

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Chapter 6 Evaluation of chelated iron and iron sulphate in the diet of African catfish *Clarias gariepinus* to enhance iron excretion for application in integrated aquaponics systems

6.1 Abstract

The best source and inclusion level of iron from feed additives for optimising the production of African catfish was determined while simultaneously evaluating excretion of effluent that can be used to optimally grow plants in aquaponics systems. Using six experimental treatments and a control, the production parameters, proximate composition, haematological indices, non-specific immunity, tissue mineral composition, apparent digestibility coefficients and water quality trials were examined. Mineral sources were iron from an organic source, chelated amino acid and an inorganic source, iron sulphate. No significant differences were observed in the production parameters. There were differences in proximate composition. Fish fed $FeSO_4$ at 30 $mgkg^{-1}$ had significantly higher hematocrit levels and red blood cell levels compared to the control, while $FeSO_4$ 60 had the highest hemoglobin levels. Tissue mineral composition showed significantly higher iron concentrations in the liver of the control compared to all the treatments. $FeSO_4$ 30 treatment had the highest ADC (96%). Iron concentrations in the effluent of the water differed between treatments. Iron from $FeSO_4$ can improve the hematological profile of catfish compared to the control, and its effluent from culture water has the potential to minimise or reduce the use of nutrient fertilizers in integrated aquaponics systems.

Keywords: aquafeeds, catfish aquaponics, iron supplementation, recirculating systems

6.2 Introduction

There is a demand for affordable protein to feed people due to the increasing world population (Roosta, 2014). Aquaculture provides protein in the form of fish and it affords lower pressure on natural fish stocks (Roosta, 2014).To mitigate some of the challenges presented by aquaculture, it can be combined with hydroponic plant production in systems referred to as aquaponics systems (Somerville et al., 2014), where the plants utilize the waste excreted by fish as nutrients, thereby cleaning the water for re-use. Aquaponics has proven advantages, however, there is a need for compromise in aquaponics systems as they consist of three distinct types of biological organisms; bacteria, fish, and plants (Goddek et al., 2015), where each of the organisms have distinct biological and nutritional needs. More specifically, fish and plants have different nutritional requirements, for example, they have different potassium and iron requirements (Savidov et al., 2007; Graber and Junge, 2009). Fish feed used in aquaponics systems may not be rich in nutrients such as potassium and iron. These nutrients are added as nutrient fertilizers to obtain a balanced nutritional profile sufficient for optimum plant growth (Pantanella et al., 2012). If these nutritional requirements are not met, nutrient deficiencies would result in reduced productivity within the particular production section of aquaponics, which in turn will negatively impact the economic performance of the overall aquaponics system (Pantanella et al., 2012; Somerville et al., 2014). Fish feed is designed to provide optimal production for fish only and not for plants. There is a need to meet the plant's nutritional demands by supplementing fish feed with the required nutrients for plant production.

In aquaponics systems, wastewater excreted by fish needs to supply plants with the required nutrients. The nutrients needed by plants to grow optimally are required in micro and macro quantities. The micronutrients are chlorine (Cl), iron (Fe), manganese (Mn), boron (B), zinc (Zn), copper (Cu) and molybdenum (Mo) and the macronutrients are nitrogen (N), potassium (K), phosphorus (P), sulphur (S), magnesium (Mg), and calcium (Ca) (Rakocy et al., 2006). Micronutrients such as iron are currently being supplemented by way of nutrient fertilizers to aquaponics systems (Rakocy et al., 2006).

Iron needs to be supplemented because it is an essential mineral required for growth by both fish and plants, and its deficiency can result in reduced growth and lost productivity in

aquaponics systems (Lim et al., 1996; Nenova, 2006). In fish, iron is important in cellular respiration, oxygen transportation, and mitosis (Lim et al., 1996; Shiau and Su, 2003). In plants iron plays a role in metabolism, in optimal growth and reproduction (Christ, 1974; Nenova, 2006; Hochmuth, 2011), it is involved in the synthesis of chlorophyll and is required for the functioning of certain enzymes (Hochmuth, 2011).

It is important that the best dietary source of iron is investigated. Iron availability is limited when fish diets contain antinutritional factors from feed containing plant protein as protein sources (Satoh et al., 2001; Apines et al., 2003). Antinutritional factors are substances that disturb the utilization of food, either by affecting mineral and protein utilization or digestion (Francis et al., 2001). To overcome this limitation, chelates and organic sources of minerals are generally favoured for inclusion in aquafeeds as opposed to inorganic sources as the chelates compete with the antinutritional factors, making the minerals available to fish for absorption (Paripatananot and Lovell, 1995; Satoh et al., 2001). Using feed supplemented with trace minerals in the form of chelated minerals has been proven beneficial to fish (Satoh et al., 2001; Apines-Amar et al., 2004), but whether this will also result in higher excretion of nutrients for use by plants in aquaponics system is unclear. If higher excretion of minerals results from using feed that has been supplemented with iron additives in aquaponics systems, it may be beneficial and result in enriched water from the urine and faecal matter excreted by fish. This enriched water could lower or even eliminate the need for addition of nutrient fertilizers to aquaponics systems. To the best of the author's knowledge, there has been limited study investigating the effect of different iron feed additives and inclusion levels on the African catfish production performance and haematological profile, especially with the potential for use in aquaponics systems.

In this study, the twofold aim is to i) determine whether dietary supplementation of iron through different iron feed additives in a recirculating aquaculture system can benefit the production performance and haematological profile of the African catfish and ii) enhance the excretion of iron in wastewater for ultimate use in aquaponics systems. The fish species that has been selected in this study, the African catfish, *Clarias gariepinus* is an important aquaculture species in African countries such as Nigeria (Akinwole and Faturoti, 2007) and an emerging species in South Africa. The African catfish is well-adapted to high stocking density, easy to culture, and robust (Akinwole and Faturoti, 2007; Omosowone et al., 2015). Knowledge of the effects of different iron feed additives could contribute to the optimal

production of the African catfish while potentially providing a feed additive that would result in the excretion of nutrients with optimal iron concentrations for plant production in aquaponics system.

6.3 Materials and methods

6.3.1 Experimental fish

Six hundred and thirty apparently healthy *C. gariepinus* were obtained from Agricultural and Technology Demonstration Centre at Gariep Dam, Free State, South Africa. The fish were acclimated to the experimental conditions for one week and thereafter were randomly distributed into 100 l glass tanks. Taking stocking density into consideration, 10 mixed sex fish with initial weights ± standard error (*SE*) 112 g ± 6.30 were distributed into tanks except for the apparent digestibility coefficient (ADC) and water quality trial that both used six fish per 100-l tank. The fish were hand-fed 6 days a week, at 08:00 am, 12:00, and 16:00 pm to apparent satiation with equal amounts of feed. During feeding fish were visually observed through the glass tanks consuming the feed to avoid any feed loss or waste. The fish were not fed 24 hours before handling. Handling was done under anaesthesia (400 mgl^{-1} of tricane methanesulfonate, MS-222, Sigma-Aldrich, Johannesburg, South Africa using 800 mgl^{-1} of sodium bicarbonate as a buffer). The procedures used in this experiment were approved by the Research Ethics Committee: Animal Care and Use (REC: ACU) of Stellenbosch University (Protocol number: SU-ACDU17-00015).

6.3.2 Experimental unit

This study was conducted at Welgevallen Experimental Farm at Stellenbosch University, South Africa. The experimental unit consisted of 100-l glass tanks in a recirculating aquaculture system (RAS) with aeration provided to each tank. Water temperature and dissolved oxygen were measured with YSI Pro Plus Multi-Parameter Water Quality Meter (YSI Incorporated, OH, USA). The temperature was maintained at ±27 ° C and dissolved oxygen ranged from 5.06 – 7.81 mgl^{-1} throughout the trial. The pH was measured with a Hanna pH 211 microprocessor (Hanna Instruments, Sarmeola di Rubano, Italy) and maintained at 5.6 – 7.3 throughout the trials. Conditions were maintained within the tolerance limits of the African catfish (Eding and Kamstra, 2001).

6.3.3 Experimental diet

The six experimental diets contained iron feed additives from an organic and inorganic source (Table 6.1). The organic source was an amino acid chelated iron (FeAA) and the inorganic source was iron sulphate ($FeSO_4$) (Sigma-Aldrich, Johannesburg, South Africa). The control diet did not include any of the iron feed additives and was formulated to meet the nutritional needs of catfish (NRC, 1993). The diets were isocaloric (2405 $Kcalkg^{-1}$) and isonitrogenous (35% crude protein and fishmeal (65% crude protein, Concentra Ltd., Cape Town, South Africa) and soya (46% crude protein, FeedPharm, Cape Town, South Africa) were sourced locally. $FeSO_4$ was included at 20, 30, and 60 $mgkg^{-1}$ this inclusion level was chosen based on findings by Gatlin and Wilson (1986), who determined that using $FeSO_4$ at 30 $mgkg^{-1}$ met the needs of channel catfish. The additive FeAA was included at 5, 10, and 20 $mgkg^{-1}$ levels that meet catfish dietary requirements (Lim et al., 1996). In the control diet, cellulose was included as an inert filler and the iron additives replaced an equivalent amount of cellulose in the experimental diets (Table 6.1).

Table 6.1 Feed formulation and proximate composition of feed of experimental diets fed to *C. gariepinus* (gkg^{-1}) during 96-day trial period.

Ingredients (gkg^{-1})	Treatment						
	$FeSO_4$ 20	$FeSO_4$ 30	$FeSO_4$ 60	FeAA 5	FeAA 10	FeAA 20	Control
Fish meal	120	120	120	120	120	120	120
Soya	570	570	570	570	570	570	570
Maize	200	200	200	200	200	200	200
Cellulose	14.98	14.97	14.94	14.99	14.99	14.98	15
Vit/Min premix	15	15	15	15	15	15	15
MDCP*	20	20	20	20	20	20	20
Fish oil	30	30	30	30	30	30	30
Sunflower oil	30	30	30	30	30	30	30
$FeSO_4$	0.02	0.03	0.06	--	--	--	--
FeAA	--	--	--	0.005	0.01	0.02	--
Proximate composition (gkg^{-1})							
Moisture	55	48	46	57	80	80	66
Ash	94	100	93	91	95	91	86
Crude Fat	114	112	112	110	107	109	103
Crude Protein	370	370	380	370	370	370	380
Crude Fibre	24	23	26	25	26	21	23
Carbohydrates[a]	367	370	369	372	348	350	365

[a]Determined by difference as: 1000 – Moisture - Crude Protein - Crude Lipids – Ash.

*MDCP: Monodicalcium phosphate

6.3.4 Diet preparation

All the diets were prepared by mixing the dry ingredients and adding water and oils in a commercial dough mixer (MacAdams SM 401) (McAdams International, Cape Town, South Africa). The feed was extruded into 4 mm pellets using a single-screw extruder (custom model, Reomach Engineering, South Africa) and dried overnight at 55° C in a convection oven (Envirowatch, Cape Town, South Africa). The feed was stored in airtight containers until use.

6.3.5 Production parameters

The fish were weighed individually at the beginning of a 96-day trial and every 4 weeks subsequently. At the end of the trial, fish were measured and weighed. The production parameters that were measured were initial body weight, final body weight, weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR %), and rate of survival %. The parameters were calculated as follows: Weight gain = ($W_2 - W_1$)

$$\text{Specific growth rate (SGR)} = \frac{\ln W_2 - \ln W_1}{T_1 \times 100}$$

$$\text{Survival rate (\%)} = \frac{\text{No. of fish at end of experiment}}{\text{No. of fish at start of experiment} \times 100}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Dry weight of feed (g)}}{\text{Weight gain (g)}}$$

Where:

W_1 = Initial mean weight (g)

W_2 = Final mean weight (g)

t_1 = Duration of the experiment

ln = Natural log

6.3.6 Proximate analysis

On the last day of the experiment, the fish were sacrificed for proximate analysis. Each treatment was replicated three times. One fish from each tank was selected and ground individually using a Hobart meat grinder (Hobart Food Equipment, Troy OH, USA). Samples of fish feed were ground with a hammer mill (Centrotec, Cape Town, South Africa) with a 1.5 mm sieve. Fish samples were analysed in duplicate while feed samples were analysed in triplicate using the AOAC (2002) methods. Both fish and feed moisture were determined by drying the samples at 100°C in an oven for 24 hours (AOAC, 2002a). The fish and feed samples were incinerated at 600°C overnight in a muffle furnace to measure ash content (AOAC, 2002b). The crude protein of both samples was measured by the combustion Dumas method with a LECO FP 528 (AOAC, 2002c). The total fat of fish samples was determined using the methods of Lee et al., (1996) by chloroform-methanol extraction (1:2) and the feed samples were determined using the AOAC (2002d) extraction method.

6.3.7 Hematology and non-specific immunity

A minimum of 2 ml blood was collected from the caudal vein of the fish that were under anaesthesia (400 mgl^{-1} of tricane methanesulfonate, MS-222, Sigma-Aldrich, Johannesburg, South Africa, using 800 mgl^{-1} of sodium bicarbonate as a buffer) with a syringe into a bottle containing an anticoagulant, ethylene diamine tetra acetate (EDTA) to determine hematological indices. Immediately after sampling, hematocrit values were determined in a glass capillary tube for 5 minutes in a microhematocrit centrifuge and read with a Graphic Reader. Hematological analysis was conducted using a Cell Dyne 3700 hematology analyzer at the Department of Physiology, Stellenbosch University. Cell Dyne 3700 uses several parameters to analyze blood, measuring EDTA-anticoagulated whole blood. It uses volumetric impedance and optical detection and is able to generate white blood cells (WBC), red blood cells (RBC), and hemoglobin (Hb) directly whereas the hematological measurements, such as hematocrit levels (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) are calculated from WBC, RBC, and Hb. Cell Dyne 3700 measures hemoglobin spectrophotometrically at a wavelength of 540 nm. The microhematocrit centrifuge values were compared to the results obtained from the Cell Dyne 3700 blood analyzer along with results from Ayoola (2016) who used this

method with *C. gariepinus* to validate the results. Identical results were obtained from both the hematocrit centrifuge and the Cell Dyne blood analyser, and the results are presented in the results section. The ratio of liver weight to fish weight was measured to determine the hepatosomatic index (HSI). The fish that were used to draw blood were weighed and dissected. The liver was weighed and the HSI was measured according to the equation:

$$\text{HSI}\ (\%) = \frac{\text{Liver weight}}{\text{Fish weight}\ \times 100}$$

The non-specific immunity parameters such as serum lysozyme activity, total protein, and immunoglobulin were measured from blood drawn at the caudal vein of the fish that were under anaesthesia (400 mgl^{-1} of tricane methanesulfonate, MS-222, Sigma-Aldrich, Johannesburg, South Africa, using 800 mgl^{-1} of sodium bicarbonate as a buffer) with a syringe. Serum from blood samples was collected after blood was centrifuged for 5 minutes at 500 rpm at a relative centrifugal force of 1400 g. Lysozyme activity was determined by methods from Sankara and Gurnani (1972). A phosphate buffer solution (0.05M, KH_2PO_4 and Na_2HPO_4) and bacteria solution (*Micrococcus lysodeiticus*) (0.0075%, w/v) were prepared and kept at pH 6.2 and temperature 25°C, respectively. Using a 96-well microplate, 50 µl of the lysozyme standard (0.85% w/v) or blood serum was added to 250 µl of the bacteria solution. After shaking for 2 minutes, absorbance was measured at 490 nm and subsequently 20 minutes after shaking, it was measured again. Lysozyme concentration was determined on a standard curve of change in absorbance. Total serum protein was determined by methods of Zor and Selinger (1996) utiliZing the Bradford assay. Total serum protein was determined by diluting 20 µl of blood serum with 1500 µl dilution agent in an Eppendorf tube. Using a 96-well microplate, 50 µl of the mixture was added to 200 µl of the Bradford dye reagent in triplicates. An absorbance ratio of 630 nm/ 450 nm was to plot a standard curve and measure the total serum concentration. The methods of Ardó et al. (2008) were used to determine immunoglobulin, with 12% w/v poly-ethylene glycol (PEG) solution (Sigma-Aldrich, Average molecular weight 10000 Dalton). A 1:1 dilution of the blood serum and PEG was allowed to stand at room temperature for 2 hours and was subsequently centrifuged for 10 minutes at 14000 rpm. The resulting supernatant was used to measure immunoglobulin, by taking the difference between the total serum protein and supernatant, taking into account the dilution.

6.3.8 Tissue mineral analysis

The filet, liver, and vertebrae were analyzed for minerals after the 96-day feeding trial. Fish were sacrificed by overexposure to anaesthetic (800 mgl^{-1} of tricane methanesulfonate, MS-222, Sigma-Aldrich, Johannesburg, South Africa, using 800 mgl^{-1} of sodium bicarbonate as a buffer). To test for the minerals, the liver and filet were ground in a Hobart meat grinder (Hobart Food Equipment, Troy, OH, USA). The vertebra was treated with an enzyme (*Alcalase® 2.4L FG, Novozyme,* Denmark) at 60°C for 1 hour, rinsed with distilled water, dried in an oven at 80°C for 6 hours and finally ground. All the samples were digested in nitric acid and hydrogen peroxide using the CEM MAR microwave digester. The Thermo iCAP6000 series, ICP Emission Spectrometer was employed for determining the mineral concentrations at Stellenbosch University by the Central Analytical Facility (ASTM D 4239; ASTM D 5373).

6.3.9 Apparent digestibility coefficient

The apparent digestibility coefficient trial took place over 14 days to determine the apparent digestibility coefficient (ADC) of iron. An inert marker included at 5 gkg^{-1} was used (Chromium (III) oxide, Cr_2O_3, Sigma-Aldrich, Johannesburg, South Africa), its concentration was measured in both the feed and the feces, and the apparent digestibility coefficient of iron was calculated according to Harter et al., (2015) with the equation:

$$\mathrm{ADC} = \left(\frac{1 - \left(\frac{\mathrm{N_f}}{\mathrm{M_f}}\right)}{\left(\frac{\mathrm{N_d}}{\mathrm{M_d}}\right)} \right) \times 100$$

where:

ADC = the apparent digestibility coefficient (%)

M= marker

N= nutrient

M_d = marker concentration in the diet

M_f = marker concentration in the feces

N_d = nutrient concentration in the diet

N_f = nutrient concentration in the feces

6.3.10 Fecal collection method

The stripping method, which is a non-lethal way of obtaining fish faeces, was used to obtain the feces. The fish were anaesthetized (400 mgl^{-1} of tricane methanesulfonate, MS-222, Sigma-Aldrich, Johannesburg, South Africa, using 800 mgl^{-1} of sodium bicarbonate as a buffer) during stripping and they were gently pressed above the anus until feces was produced. The procedure was gentle to avoid contaminating the feces with urine, sperm or eggs of the fish (Ramsay et al. 2000). The procedure was done twice, first on week one and later at week two. The fish were stripped in the morning, 120 minutes after feeding. The feces were collected in sample bottles and all samples within a treatment were pooled and stored at -20°C until further analysis was done. Samples were pooled and, therefore, no statistical analysis was performed.

6.3.11 Water quality analysis

The water quality trial ran for two days using a static tank because a longer period would negatively affect fish walfare. In each of the tanks, no water was allowed to be exchanged and an aerator was fitted in each tank to provide oxygen. It was necessary that water quality was determined because the wastewater that is produced by fish affects the plants and the rate at which the plants are produced in the aquaponics system (Buzby et al., 2016).

Using a randomized experimental set up, the treatments were replicated 3 times, using 6 fish per 100-L tank with an average of 477 g. Fish were fed at 3 % their body weight, 3 times per day as per normal procedure. Equal amounts of feed were given to each tank and fish were fed until apparent satiation. Fish were visually observed during the feeding to avoid waste and over feeding.

Water samples were taken from each tank twice a day in the morning at 08:00 am and at 16:00 pm. The measured parameters are water temperature, pH, dissolved oxygen, total dissolved solids, total suspended solids, total ammonia nitrogen, phosphorus, potassium, and iron (Table 6.2).

Table 6.2 Summary of water quality parameters measured with method and instrument applied

Parameter	Units	Method/Instrument
Temperature	°C	YSI Pro Plus Multi-Parameter Water Quality Meter
Dissolved Oxygen	mgl^{-1}	
pH	NA	Hanna pH 211 microprocessor
Total Dissolved Solids	µS	AZ 8603 IP67 Water Quality Portable Meter
Total Suspended solids	mgl^{-1}	Photometric Method/ Hach DR 850 Colorimeter
Total Ammonia Nitrogen	mgl^{-1}	Hach DR 3900 Laboratory Spectrophotometer
Phosphorus	mgl^{-1}	
Potassium	mgl^{-1}	
Iron	mgl^{-1}	

6.3.12 Statistical analysis

The experimental design and data analysis of this study was achieved with assistance from Stellenbosch University's Centre for Statistical Consultation. The results were analyzed using Statistica 13 (Dell, Inc). All the continuous response variables are presented as mean ± SE. A one-way analysis of variance (ANOVA) was used to determine whether the treatment means of continuous response variables are the same. If the null hypothesis was rejected, the means were deemed statistically significant and the ANOVA p – value was less than 0.05. To determine which means were different from each other, Fisher's Least Significant Differences (LSD) post-hoc test was used. If the variance of the treatment groups were non-homogeneous according to the Levene test, a Games-Howell multiple comparisons procedure was used to compare the treatment means instead of LSD multiple comparisons.

6.4 Results

6.4.1 Production parameters

The effects of dietary iron supplementation for *C. gariepinus* are summarised in Table 6.3. No statistically significant results were obtained for any of the growth parameters measured ($p > 0.05$). Fish fed the treatment including $FeSO_4$ 30 had a weight gain of 352 ±12.50 and SGR of 1.44 ±0.09, while the control weight gain was 273 ± 33.26 and the SGR was 1.27 ± 0.11. An FCR of 0.97 ±0.03 was observed in the FeAA 10 diet, while an FCR of 1.12 ±0.12 was in the $FeSO_4$ 20 diet. Over the feeding trial, a minimum of 67% fish survived in the $FeSO_4$ 60 diet and a maximum of 90% in the control treatment. Mortalities in this study were as a result of the cannibalistic behaviour of the African catfish.

Table 6.3 Mean growth performance of the African catfish *C. gariepinus* fed different dietary fish feed additives at different levels (n=3).

Parameter	Treatment						
	$FeSO_4$ 20	$FeSO_4$ 30	$FeSO_4$ 60	FeAA 5	FeAA 10	FeAA 20	Control
Initial weight (g)	111±10.68	113±11.91	109±2.68	118±9.35	121±12.52	118±2.42	110±7.24
Final weight (g)	409±29.41	466±16.30	425±32.27	438±20.23	455±17.89	426±13.10	384±30.57
Weight gain (g)	297±36.51	352±12.50	316±29.63	320±14.31	333±29.65	308±13.17	273±33.26
FCR	1.12±0.12	1.0±0.01	1.05±0.08	1.0±0.05	0.97±0.03	1.04±0.05	1.11±0.10
SGR (%)	1.32±0.15	1.44±0.09	1.39±0.05	1.34±0.05	1.34±0.14	1.31±0.03	1.27±0.11
Survival (%)	70±10.00	73±8.81	67±8.81	70±11.54	83±6.66	80±5.77	90±5.77

Data presented as mean ± SE. All treatments p> 0.05.

6.4.2 Whole-body proximate composition

There was a significant difference in the whole-body proximate composition of the African catfish over the feeding trial, except for the ash and crude protein content ($p > 0.05$) (Table 6.4). The moisture content differed significantly between the treatments with the highest moisture contents recorded in the FeAA 20 and the control diet, 79% ±0.49 and 79% ±1.16, respectively. They differed significantly from FeAA 10 (76% ± 0.39). The crude fat content also differed significantly between treatments, ranging from 4.46% ± 0.29 for the FeAA 10 treatment and 2.88% ± 0.16 for the FeAA 20 treatment

Table 6.4 Proximate composition of whole body of experimental diets fed to *C. gariepinus* (n=3) over a 96-day trial period.

Component (%)	Treatment						
	$FeSO_4$ 20	$FeSO_4$ 30	$FeSO_4$ 60	FeAA 5	FeAA 10	FeAA 20	Control
Moisture	78±0.87^{ab}	78±0.61^{ab}	77±0.44^{bc}	78±0.21^{ab}	76±0.39^{c}	79±0.49^{a}	79±1.16^{a}
Ash	1.22±0.07	1.04±0.03	1.18±0.00	1.21±0.02	1.25±0.14	1.11±0.07	1.3±0.18
Crude Fat	3.52±0.49^{abc}	3.63±0.32^{abc}	3.94±0.40^{ab}	3.39±0.17^{bc}	4.46±0.29^{a}	2.88±0.16^{c}	3.3±0.38^{bc}
Crude Protein	16.54±0.65	15.97±0.39	17.04±0.01	16.93±0.32	17.15±0.23	16.30±0.43	15.3±1.11

Different superscripts in the same row indicate significant differences, $p< 0.05$.

Table 6.5 Haematological indices of *C. gariepinus* fed different experimental diets (n=3) over a 96-day trial period.

Indices	Treatment						
	$FeSO_4$ 20	$FeSO_4$ 30	$FeSO_4$ 60	FeAA 5	FeAA 10	FeAA 20	Control
HCT (%)	36.15 ± 1.78^{ab}	38.63 ± 1.01^{a}	36.01 ± 0.81^{ab}	36.15 ± 0.95^{ab}	35.53 ± 1.54^{abc}	31.86 ± 1.32^{c}	33.93 ± 0.78^{bc}
Hb (gdl^{-1})	13.73 ± 0.52^{ab}	13.98 ± 0.45^{ab}	14.11 ± 0.33^{a}	13.26 ± 0.28^{abc}	13.53 ± 0.36^{abc}	12.53 ± 0.32^{c}	12.86 ± 0.18^{bc}
RBC ($10^{12}l^{-1}$)	2.53 ± 0.13^{abc}	2.72 ± 0.07^{a}	2.68 ± 0.09^{a}	2.63 ± 0.04^{ab}	2.62 ± 0.08^{ab}	2.31 ± 0.08^{c}	2.37 ± 0.05^{bc}
MCHC (g/dl^{-1})	38.01 ± 0.41^{ab}	36.33 ± 0.28^{b}	39.30 ± 0.30^{a}	36.81 ± 0.33^{b}	38.06 ± 0.73^{ab}	39.35 ± 0.57^{a}	38.03 ± 1.08^{ab}
MCH (pg)	54.20 ± 0.63^{a}	51.50 ± 0.32^{bc}	52.81 ± 1.02^{ab}	50.60 ± 0.59^{c}	51.60 ± 0.56^{bc}	54.35 ± 0.65^{a}	54.23 ± 0.68^{a}
MCV (fl)	142.66±2.13	142.16±3.80	134.50±2.23	137.50±1.11	135.56±1.20	138.16±2.56	143.33±4.67
WBC ($10^{9}l^{-1}$)	3.27 ± 1.47^{b}	3.28 ± 3.42^{b}	6.80 ± 4.03^{ab}	3.67 ± 1.70^{b}	7.08 ± 1.99^{ab}	8.21 ± 2.20^{ab}	11.23 ± 4.32^{a}
HSI (%)	1.16±0.38	1.08±0.16	1.06±0.07	1.60±0.07	1.00±0.14	1.04±0.25	1.20±0.12

Different superscripts in the same row indicate significant differences, $p< 0.05$.

Table 6.6 Summary of non-specific immunity indicators of *C. gariepinus* fed experimental diets (n=3).

Parameters	Treatment						
	$FeSO_4$ 20	$FeSO_4$ 30	$FeSO_4$ 60	FeAA 5	FeAA 10	FeAA 20	Control
Lysozyme (μgml^{-1})	3.06±0.14	2.82±0.32	3.25±0.40	2.11±0.35	2.96±0.08	2.93±0.53	3.53±0.49
Immunoglobulin ($mgml^{-1}$)	21.35±0.38	22.97±0.42	22.84±0.49	20.90±1.89	22.12±0.32	22.71±0.64	23.97±0.36
Total protein ($mgml^{-1}$)	35.12±4.20	36.61±1.41	34.48±1.98	25.46±5.58	36.71±3.03	35.12±4.20	26.13±6.25

Data presented as mean ± SE. Different superscripts in the same row indicate significant differences, $p< 0.05$

6.4.3 Hematology and non-specific immunity

The results for hematology parameters are summarised in Table 6.5. The hematology parameters were significantly affected by the addition of iron from different dietary sources. The highest HCT levels were for $FeSO_4$ 30 (38.63%±1.01), significantly different from the lowest level FeAA 20 (31.86%±1.32) and the control (33.93%±0.78). The highest mean Hb value was 14.11 gdl^{-1} ±0.33 for the $FeSO_4$ 60 diet and it differed significantly from FeAA 20 (12.53 gdl^{-1} ±0.32) and the control (12.86 gdl^{-1} ±0.18). The inclusion of $FeSO_4$ 30 increased RBC mean values ($2.7210^{12}l^{-1}$ ±0.07) significantly higher than the inclusion of FeAA 20 ($2.3110^{12}l^{-1}$ ±0.08) and the control ($2.3710^{12}l^{-1}$ ±0.05). There were similarities in MCHC levels between $FeSO_4$ 20, $FeSO_4$ 60, FeAA 10, FeAA 20 and the control. MCH mean levels ranged from 54.35pg ±0.65 to 50.60pg ±0.59, with FeAA 20, $FeSO_4$ 20 and the control significantly differing from FeAA 5, FeAA 10 and $FeSO_4$ 60. The control diet had the highest WBC mean values, significantly differing from $FeSO_4$ 20, $FeSO_4$ 30 and FeAA 5. MCV and HSI were not affected by the different feed additives. The non-specific immunity parameters represented in Table 6.6 did not differ significantly between the treatments ($p > 0.05$). Mean values for lysozyme activity showed some variation and were between 3.53 μgml^{-1} ±0.49 and 2.11 μgml^{-1} ±0.35. Immunoglobulin concentrations ranged between 23.97 $mgml^{-1}$ ±0.36 and 20.90 $mgml^{-1}$ ±1.89. The total protein concentration was 36.71 $mgml^{-1}$ ±3.03 for FeAA 10 and for 25.46 $mgml^{-1}$ ±5.58 for FeAA 5.

6.4.4 Tissue mineral composition

The results of the mineral analysis of the bone, liver, and filet of fish fed different iron feed additives are presented in Table 6.7. There was no significant difference in iron levels in the bones of fish fed iron containing diets, however, there was a difference in the potassium levels ($p < 0.05$) between the treatments. The highest K level in the bone was in the diet including FeAA 20, (6.00 gkg^{-1} ±1.39), and was significantly higher than the control (2.15 gkg^{-1} ±0.14) and the rest of the treatments. No significant difference were observed in the iron and potassium levels in the filet of fish ($p > 0.05$). Potassium levels in the liver did not differ between diets ($p>0.05$), however, there was a significant difference in the iron levels in the liver, with fish fed the control (0.25 gkg^{-1} ±0.13) being significantly higher than all the treatments.

6.4.5 Apparent digestibility coefficient

Results for this trial are represented in Figure 6.1. Results for this trial could not be replicated because the feces samples were too small and had to be pooled, therefore, the data could not be statistically analyzed. The mineral digestibility coefficient observed ranged between 96 % for $FeSO_4$ 30 and 91% for FeAA 10 treatment.

Table 6.7 Summary of mineral analysis of *C. gariepinus* fed experimental diets over a 96- day trial period.

	Treatment						
Bone	$FeSO_4$ 20	$FeSO_4$ 30	$FeSO_4$ 60	FeAA 5	FeAA 10	FeAA 20	Control
K (gkg^{-1})	2.88 ± 0.23^{bc}	2.84 ± 0.25^{bc}	2.77 ± 0.52^{bc}	3.35 ± 0.38^{b}	3.21 ± 0.45^{bc}	6.00 ± 1.39^{a}	2.15 ± 0.14^{c}
Fe (gkg^{-1})	0.04±0.00	0.03±0.00	0.04±0.01	0.05±0.01	0.06±0.03	0.02±0.01	0.03±0.01
Filet							
K (gkg^{-1})	5.52±1.15	5.06±0.37	5.40±0.26	6.34±1.14	5.00±0.10	5.36±0.10	5.23±0.42
Fe (gkg^{-1})	0.01±0.00	0.03±0.02	0.01±0.00	0.01±0.00	0.00±0.00	0.02±0.01	0.01±0.00
Liver							
K (gkg^{-1})	4.36±0.32	4.72±0.54	4.22±0.25	4.20±0.09	4.29±0.11	5.04±0.21	4.27±0.23
Fe (gkg^{-1})	0.05 ± 0.01^{b}	0.04 ± 0.02^{b}	0.06 ± 0.01^{b}	0.05 ± 0.01^{b}	0.04 ± 0.00^{b}	0.04 ± 0.00^{b}	0.25 ± 0.13^{a}

Different superscripts in the same row indicate significant differences, $p< 0.05$.

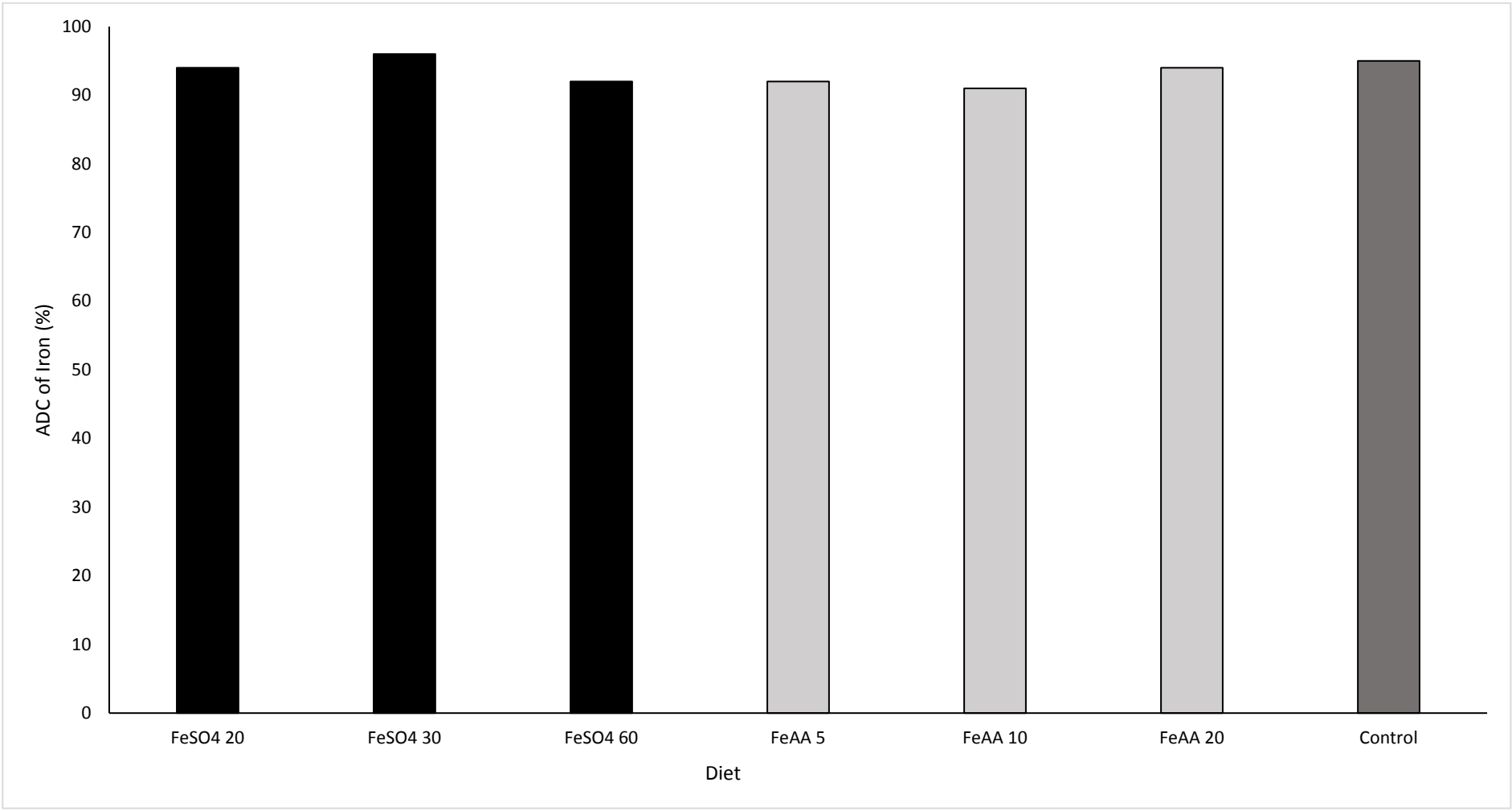

Figure 6-1 Apparent Digestibility Coefficients (ADC) of iron from feed additives; iron sulphate ($FeSO_4$) and amino acid chelated iron (FeAA) over a 14-day trial period using the African catfish.

6.4.6 Water quality analysis

The water quality analysis results are summarized in Table 6.8. The pH changed over the two days. On day 1, the pH differed significantly between the treatments, and the highest pH was 7.80 ± 0.05 for the $FeSO_4$ 60 treatment, which was significantly higher than FeAA 20 (7.69 ± 0.05 and the control (7.70 ± 0.05). On day 2, there were no significant differences between treatments for pH. DO levels were significantly different on day 1, the highest DO level was the $FeSO_4$ 60 treatment (7.91 mgl^{-1}± 0.94) and was significantly different from $FeSO_4$ 30 (6.40 mgl^{-1} ± 0.94) but neither was significantly different from the control. On day 2, no differences were observed for DO values. TDS concentrations were not affected by the different treatments on both days of the trial. No significant differences were observed for TSS on day 1, however, on day 2 $FeSO_4$ 60 (82.33 mgl^{-1} ± 7.49) had the highest TSS level and was significantly different from the control (50.16 mgl^{-1} ± 7.49), FeAA 10 (45.66 mgl^{-1} ± 7.49) and $FeSO_4$ 20 (49.83 mgl^{-1} ± 7.49). TAN was not affected by the dietary treatments on day 1, and on day 2, $FeSO_4$ 20 had the highest TAN concentration (23.37 mgl^{-1} ± 1.90) that was significantly different from FeAA 10 (19.69 mgl^{-1} ± 1.90), both treatments were not significantly different from the control. Phosphorus levels on day 1 ranged from 1.66 mgl^{-1} ± 0.17 for FeAA 10 to 1.25 mgl^{-1} ± 0.17 for $FeSO_4$ 30 and were significantly different from each other, but both were similar to the control. On day 2, the phosphorus levels were not significantly different. Potassium concentrations were similar for all the treatments on both days. There was a significant difference in iron concentrations on day 1, $FeSO_4$ 20 (0.016 mgl^{-1}± 0.00) had the highest concentration and was significantly different from the control (0.000 mgl^{-1} ± 0.00) and FeAA 20 (0.003 mgl^{-1} ± 0.00). On day 2, $FeSO_4$ 60 and FeAA 5 (0.018 mgl^{-1} ± 0.00) were significantly higher than the control (0.000 mgl^{-1} ± 0.00).

Table 6.8 Effect of potassium supplementation through feed additives on water quality in a static aerated culture system, over a two-day period (n=3).

Parameter	Treatment						
Day 1	$FeSO_4$ 20	$FeSO_4$ 30	$FeSO_4$ 60	FeAA 5	FeAA 10	FeAA 20	Control
Temperature	27.41±0.27^{b}	27.51±0.27^{b}	27.48±0.27^{b}	27.41±0.27^{b}	27.53±0.27^{ab}	27.68±0.27^{a}	27.25±0.27^{c}
pH	7.71±0.05^{ab}	7.70±0.05^{b}	7.80±0.05^{a}	7.71±0.05^{ab}	7.72±0.05^{ab}	7.69±0.05^{b}	7.70±0.05^{b}
DO(mgl^{-1})	7.72±0.94^{a}	6.40±0.94^{b}	7.91±0.94^{a}	7.49±0.94^{ab}	7.27±0.94^{ab}	7.53±0.94^{ab}	7.63±0.94^{ab}
TDS (µS)	144.03±10.5^{a}	144.18±10.5^{a}	144.50±10.5^{a}	143.31±10.5^{a}	144.91±10.5^{a}	144.21±10.5^{a}	142.80±10.5^{a}
TSS(mgl^{-1})	13.83±7.49^{a}	17.00±7.49^{a}	15.33±7.49^{a}	16.16±7.49^{a}	14.33±7.49^{a}	15.33±7.49^{a}	16.33±7.49^{a}
TAN(mgl^{-1})	5.52±1.90^{a}	5.53±1.90^{a}	5.48±1.90^{a}	5.32±1.90^{a}	5.70±1.90^{a}	5.40±1.90^{a}	6.42±1.90^{a}
P(mgl^{-1})	1.57±0.17^{ab}	1.25±0.17^{b}	1.54±0.17^{ab}	1.33±0.17^{ab}	1.66±0.17^{a}	1.46±0.17^{ab}	1.42±0.17^{ab}
K(mgl^{-1})	5.85±2.32^{a}	5.50±2.32^{a}	6.06±2.32^{a}	5.96±2.32^{a}	6.33±2.32^{a}	5.46±2.32^{a}	5.45±2.32^{a}
Fe(mgl^{-1})	0.016±0.00^{a}	0.015±0.00^{ab}	0.010±0.00^{abc}	0.010±0.00^{abc}	0.005±0.00^{abc}	0.003±0.00^{bc}	0.000±0.00^{c}
Day 2							
Temperature	22.83±0.27^{a}	23.10±0.27^{ab}	23.58±0.27^{a}	22.95±0.27^{b}	22.93±0.27^{b}	23.58±0.27^{b}	22.95±0.27^{b}
pH	7.65±0.05^{a}	7.57±0.05^{a}	7.64±0.05^{a}	7.50±0.05^{a}	7.60±0.05^{a}	7.64±0.05^{a}	7.61±0.05^{a}
DO (mgl^{-1})	7.25±0.94^{a}	6.93±0.94^{a}	6.60±0.94^{a}	3.91±0.94^{a}	7.30±0.94^{a}	7.45±0.94^{a}	6.51±0.94^{a}
TDS (µS)	218.80±10.5^{a}	228.00±10.5^{a}	221.35±10.5^{a}	224.90±10.5^{a}	218.51±10.5^{a}	223.08±10.5^{a}	211.20±10.5^{a}
TSS (mgl^{-1})	49.83±7.49^{b}	61.33±7.49^{ab}	82.33±7.49^{a}	63.33±7.49^{ab}	45.66±7.49^{b}	62.33±7.49^{ab}	50.16±7.49^{b}
TAN (mgl^{-1})	23.89±1.90^{a}	21.19±1.90^{ab}	23.05±1.90^{ab}	22.77±1.90^{ab}	19.69±1.90^{b}	23.37±1.90^{ab}	21.05±1.90^{ab}
P (mgl^{-1})	2.50±0.17^{a}	2.05±0.17^{a}	2.49±0.17^{a}	1.96±0.17^{a}	2.42±0.17^{a}	2.40±0.17^{a}	2.57±0.17^{a}
K (mgl^{-1})	13.21±2.32^{a}	13.03±2.32^{a}	15.20±2.32^{a}	13.88±2.32^{a}	15.25±2.32^{a}	14.36±2.32^{a}	18.73±2.32^{a}
Fe (mgl^{-1})	0.010±0.00^{ab}	0.016±0.00^{ab}	0.018±0.00^{a}	0.018±0.00^{a}	0.013±0.00^{ab}	0.005±0.00^{ab}	0.000±0.00^{b}

Different superscripts in the same row indicate means are significantly different, $p<0.05$

6.5 Discussion

Iron from two different dietary sources was added to fish feed at three different inclusion levels to determine their effect on production parameters, whole-body proximate composition, hematological indices, non-specific immunity, tissue mineral composition, apparent digestibility coefficient of Fe, and water quality. A control diet was used as a baseline for comparison with the different dietary treatments.

Iron levels in the water were affected on both days of the water quality trial, on day one $FeSO_4$ 30 and $FeSO_4$ 60 treatments showed increased iron excretion than the control and on day two $FeSO_4$ 60 was still significantly higher than the control treatment. The effluent excreted after fish were fed the $FeSO_4$ treatments indicated that it could be a good source of iron for aquaponics systems. Nutrients released as waste by fish in aquaponics systems can be utilized by plants, and the release of higher levels of iron into the water would be continuous as the fish are fed daily. The increased release of iron from the feed additives could be beneficial to the plants grown in aquaponics systems, because it is required as a micronutrient for plant growth and is often deficient in aquaponics systems (Rakocy et al., 2006). The inclusion levels of $FeSO_4$ 30 could be the ideal, as it showed higher concentrations than the control on day one and slightly increased on day two. $FeSO_4$ 30 had a high concentration of iron for both day one and day two. Even though the trial was in a static system with no water exchange for two days, it gives a clear indication that the $FeSO_4$ treatment had a higher excretion of iron in the wastewater. Therefore, the use of this treatment would be beneficial in an aquaponics system.

The different dietary treatments had an effect on the water quality parameters tested. The pH of the water on both days of the experiment remained above 7, which is important because in aquaponics systems bacteria convert nitrite to nitrate at a pH above 7 (Savidov et al., 2007). The dissolved oxygen levels in the water remained sufficient for fish and plant production (>6 mgl^{-1}) (Somerville et al., 2014). TDS and K concentrations in the tank were not affected on both days of the trial. TSS concentration was affected only on day two, this may be attributed to suspended metabolites and fecal matter in the tank. Total ammonia nitrogen, which is a sum of ammonium ion (NH_4^+) and un-ionized ammonia (NH_3), was only affected by the treatments on day two of the trial. The increase in ammonia on day two could be explained by accumulation of waste. Fish excrete waste in the form of ammonia, uneaten feed and other

metabolites in the tank, this could also result in increased TAN concentrations (Somerville et al., 2014). The rate at which waste decomposes is not the same (Endut et al., 2010), which could explain the difference in TAN concentration between $FeSO_4$ 20 and FeAA 5 on day 2. In a fully functioning aquaponics system, the ammonia will be used by plants for growth, therefore the increase of TAN in the static tank is expected.

Based on the results of the production parameters, there was no effect on growth, that is, none of the additives enhanced production performance, and this could indicate that the control diet had sufficient iron for the production African catfish. The results of this study are different from Gatlin and Wilson (1986) who observed a significant difference in weight gain and feed conversion when feeding channel catfish diets containing different levels of iron. Gatlin and Wilson (1986) also observed that survival was not affected by iron deficiency. Contrary to this study on the African catfish, Lim et al., (1996) did not observe a difference in feed efficiency but observed a difference in weight gain and survival. The discrepancies in the effect of dietary iron in catfish may be as a result of differences in species, size, strain, feeding management and duration of the trial period (Lim et al., 2000). Lim and Klesius (1997) suggest that for iron deficiency to have an effect on survival or mortality a trial period of more than 13 weeks is necessary and this could not be observed in this study, as the trial ran for 12 weeks.

The different feed additives impacted the whole-body proximate composition of the fish as significant differences were observed. Differences in proximate composition are commonly attributed to the quantity and quality of feed consumed by the fish (Ofudje et al., 2014). The moisture content and crude fat differed between treatments, however, because whole-body proximate composition was performed it could not be established whether differences occurred only in specific tissues or whether differences were apparent throughout all tissues. The effect was however not adverse, and would still allow for the catfish to be suitable for human consumption. Moreover, the proximate composition of the fish is in a similar range to other catfish (Fagbenro and Jauncey, 1995). There is no discernible pattern in the results obtained from the proximate composition of the fish. Further studies may be required to make a direct comparison or conclusion on the effect of different feed additives on the African catfish.

Hematological indices in fish are examined because they indicate the general health status of fish during the experimental period (Harikrishna et al., 2010). In this study, the feed additives

significantly affected the hematological indices of the fish. The diet containing $FeSO_4$ 30 had the highest HCT and RBC values compared to the control while $FeSO_4$ 60 had the highest Hb levels compared to the control. Gatlin and Wilson (1986) also observed increased Hb and HCT levels in fish fed diets including iron. Lim et al., (1996) observed a similar trend that Hb increased as the inclusion level of iron increased. Decreased HCT, Hb, MCV and MCH could be indicative of hypochromic microcytic anaemia (Shiau and Su, 2003). In this trial the lower HCT, Hb and RBC values in the fish fed the control diet may be indicative of hypochromic microcytic anaemia. However, this cannot be definitively stated as other signs of microcytic anaemia such as decreased weight gain and lowered feed intake were not observed (Lim et al., 1996). The HSI, which is a ratio of liver weight to fish weight in this study was in a similar range to other *C. gariepinus* studies (Nwanna, 2003). The non-specific immunity parameters were not different from the treatments and the control. Lim et al., (2000) observed that fish fed iron-deficient diets were more susceptible to infection than those fed iron supplemented diets. Because haematological parameters indicate the general status of fish during the experimental period, it can be concluded that the inclusion of $FeSO_4$ in the diet of the African catfish improved its haematological profile and health status. Furthermore, the susceptibility of the fish to disease could be decreased. In this study we tested the mineral levels in the tissue of catfish to ensure that the high nutritional quality of catfish is maintained as proposed by Rosa et al., 2007. Dietary iron needs to be supplemented in aquafeeds because iron found in culture water is generally not sufficient to meet the iron requirement for fish (Gatlin and Wilson, 1986, Shiau and Su, 2003). Moreover, iron toxicity is not commonly observed in fish as the culture water has negligible iron concentrations (Baker et al., 1997). However, when iron has been added in excessive levels in the diets of fish, the fish exhibit poor growth (Baker et al., 1997).

There were no significant differences observed in the iron levels of the bones, however the potassium levels in the bones of the fish differed. The iron concentration in the filet observed in this study is lower compared to findings by Fawole et al., (2007) and Toko et al., (2008) who also investigated the mineral composition of *C. gariepinus*. The differences observed may be as a result of the variations in diets fed to the catfish. There is no clear explanation for the high levels of potassium in the bones of fed FeAA treatment compared to the $FeSO_4$ treatment and the control treatment. The iron level was higher in the liver of the control than all the other

treatments, which could be a further indication that the control diet had sufficient iron for the production African catfish.

To determine if the feed additives are incorporated in the body of the African catfish, the apparent digestibility coefficient of the different feed additives were tested using a known concentration of inert marker (Chromium (III) oxide, Cr_2O_3). It is desirable that feed additives are biologically available to fish when they are added in the diet. The method used to collect feces for ADC resulted in small feces samples being obtained, therefore samples were pooled and the results could not be statistically evaluated. Even though they were not statistically analyzed, the pooled samples are seen as a reasonable representation of each treatment, and do give an indication of the apparent digestibility coefficients of these feed additives. The results indicate that all the additives provide good digestibility for both feed additives at all inclusion levels , with values for ADC ranging from 91 -96 %.

One of the aims of the research was to test the effluent produced by the different feed additives in a static tank after the feed was given to fish. It was important to test the effluent because it has the potential to be used in aquaponics systems to minimize or eliminate the use of supplemental nutrient fertilizers if the iron supplements could increase nutrient excretion. The effluent produced will affect the rate at which the plants in an aquaponics system grow (Buzby et al., 2016). The effluent in an integrated system will move from fish to plants for assimilation by plants, therefore it is important that the effluent has nutrient concentrations that can maintain optimum plant growth. The trial was ran for two days in a static tank due to lack of a system with a collection tank or sump that would separate wastewater from different treatments. Because closing the water off for more than two days would adversely affect the welfare of the fish, the experiment was ran for two days.

Based on the results from the hematological indices which indicate the health status of the fish, the additive $FeSO_4$ 30 showed the highest HCT and RBC levels. Moreover, Hb levels of $FeSO_4$ 30 were similar to $FeSO_4$ 60 which had the highest Hb levels. MCH levels for $FeSO_4$ 30 were also significantly different from the control. Moreover, the fish fed the additive $FeSO_4$ 30 had the highest digestibility coefficient, indicating its high bioavailability to fish. When observing $FeSO_4$ 30 concentration in the effluent, it can be established that it has the best potential for use in integrated aquaponics systems. Based on all the parameters tested during the study, the best mineral source is $FeSO_4$ and the best inclusion level is 30 $mgkg^{-1}$ to enhance

the hematological profile of the African catfish and excrete iron for optimum plant growth in aquaponics systems.

6.6 Conclusion

Production parameters and non-specific immunity parameters were not significantly affected, which could indicate that there is limited or no need to supplement iron in the diet of the African catfish. The high iron levels in the liver of the African catfish may further indicate that there is no need to supplement iron in the diet. The lack of discernible patterns in the results obtained from proximate composition warrant further investigations into the effects of feed additives. The ADC of iron when $FeSO_4$ was included at 30 $mgkg^{-1}$ was high. The increased iron levels in the water of the fish fed $FeSO_4$ treatment could be as a result of the feces produced by fish. The iron rich effluent produced by the fish could be beneficial to plants in an aquaponics system, reducing or even eliminating the need to add nutrient fertilisers. The inclusion of iron in the diet of the African catfish as $FeSO_4$ 30 could have implications for both fish and plants by improving hematological profile of the fish and providing iron rich effluent for improved plant production.

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Author contributions

Declaration by candidate

With regards to chapter 7, Evaluation of potassium diformate as a potassium supplement in the production of the African catfish, *Clarias gariepinus* in combination with lettuce in an integrated aquaponics the nature and scope of my contribution were as follows:

Chapter	Pages	Nature of contribution	Extent of contribution (%)
7	119 - 169	Planned and designed the experiments. Formulated the fish feed, procured the ingredients and prepared the feed. Carried out the daily feeding, the sampling of fish for production performance. Weighed the lettuce before and after the experiment. Planted the lettuce, performed the proximate analysis on the lettuce and fish feed. Collected the water samples and performed the analysis on the water quality trial. Prepared and analysed data. Wrote the manuscript and collated all the comments from the co-authors.	80

The following co-authors have contributed to chapter 7:

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Neill Goosen	njgoosen@sun.ac.za	Contributed in data interpretation, proof reading and substantially improving the manuscript.	15
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Signature of candidate……..

Date……

Declaration by co-authors

The undersigned hereby confirm that

1. The declaration above accurately reflects the nature and extent of the contribution of the candidate and the co-authors to chapter 7 in the dissertation,
2. No other author contributed to chapter 7 and

3. Potential conflict of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in chapter 7 of this dissertation.

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Chapter 7 Evaluation of potassium diformate as a potassium supplement in the production of the African catfish, *Clarias gariepinus* in combination with lettuce in an integrated aquaponics system

7.1 Abstract

When fish feed is the only nutrient input into aquaponics systems, it can be limiting to plant growth as this approach is tailored toward optimal nutrition of the fish, and not necessarily that of the plant. For aquaponics systems to operate optimally, the plants sometimes require nutrient supplementation in the form of nutrient solutions. This research aims at producing fish feed to optimise both the production of fish and plants in an aquaponics system to minimise or eliminate the need for additional nutrient solutions, through potassium supplementation by direct inclusion in the fish feed. The aims of the research were achieved by using the African catfish (*Clarias gariepinus*) as experimental species at culture densities of 40 and 47 kgm^{-3} in combination with lettuce in an integrated aquaponics system. Three consecutive production cycles were carried out for 31 days each to eliminate bias due to the environmental variation in position of the plant growing beds. The fish were fed a diet supplemented with potassium from potassium diformate (KDF, $C_2H_3KO_4$) at 9 gkg^{-1} and a control diet with no supplementation. The lettuce growth, yield, proximate, and mineral composition along with fish growth and water quality parameters were monitored throughout. The fish grew at an acceptable rate, with the specific growth rate ranging from 0.49 – 0.78 %/day and the feed conversion ratio ranged from 1.3 – 2. During the first two production cycles, the average biomass and shoot weight was higher in the lettuce grown using the potassium supplemented feed (57.9 g ± 20.4 and 51.2 g ± 14.4 for cycles 1 and 2, respectively) compared to the control treatment (17.2 g ± 5.30 and 14.5 g ± 5.48 for cycles 1 and 2, respectively). During the third production cycle, the KDF performed poorer than the control (7.97 g ± 3.28 of average plant weight vs 13.64 g ± 4.65 for the control), but plant growth for both treatments was noticeably poorer, and was attributed to the high temperatures experienced during this time. The KDF treatment consistently showed higher levels of potassium and total dissolved solids in the wastewater of the growing beds compared to the control treatment, which also translated to higher levels of potassium in the lettuce

grown in the KDF treatment. It is therefore concluded that the supplementation of KDF in the diet of the African catfish can lead to increased potassium levels in the circulating water in aquaponics systems, leading to overall improved lettuce production and higher potassium levels in the produced lettuce.

7.2 Introduction

Aquaponics integrates aquaculture and hydroponics and has been proven as a sustainable food producing method (Goddek et al., 2015). This production method provides better water management solutions than conventional aquaculture and agricultural methods by using significantly less water (Somerville et al., 2014; FAO, 2016). Moreover, it does not require the use of soil as plant roots are immersed directly in water, thereby improving their capacity and rate for nutrient absorption from water (Saufie et al., 2015).

The main nutrient input in aquaponics system is the fish feed fed to the cultured animals, and it is designed to satisfy the nutritional needs of the fish. However, the nutrient levels in the wastewater excreted by fish (that acts as the nutrient source of the cultured plants in the system) do not fully meet the nutritional requirements of plants. This has resulted in the use of nutrient fertilizer solutions to supplement plant needs in aquaponics systems to achieve optimal plant production (Seawright et al., 1998). Nutrients such as potassium and iron are supplemented to aquaponics systems to obtain a balanced nutrient profile for optimum plant growth (Seawright et al., 1998; Pantanella et al., 2012).

Potassium, the most abundant cation in plants is often limiting in aquaponics systems, and because of its importance to optimal plant production, it is supplemented (Wang et al., 2013). This is done because sub-optimal K levels will result in poor plant performance which will negatively impact economics of aquaponics systems.

In aquaponics systems, it is important to use dietary sources of minerals that will be available to both fish and plants. Organic sources of potassium include potassium diformate (KDF), a salt of formic acid that has been used as a feed additive to improve growth in fish (De Wet, 2005; Lückstädt and Mellor, 2011). KDF has an active formate ion that is utilised by fish to increase the absorption of minerals in the gut and improve protein and amino acid digestibility (De Wet 2005; Lückstädt, 2006), but the potassium that forms part of the KDF may be excreted by the cultured animals, which means that it may increase the potassium levels in the water circulating through grow beds in aquaponics systems. These increased potassium levels resulting from feed additives can potentially provide additional nutrients to the plants in aquaponics systems.

When using the feed additive KDF, there is the potential to achieve a dual benefit in aquaponics systems: improved fish production and simultaneously increase potassium

excretion in the wastewater. There is prior evidence that dietary KDF supplementation can have this desired dual effect (Siqwepu et al., 2020a). In the previous study, we characterised the wastewater produced when feeding fish and found that KDF at inclusion of 9 g/kg provided a good potassium concentration and improved the haematological profile of the African catfish. The current study was to validate the findings from the previous study that the concentration of potassium produced by KDF can optimally grow lettuce in an aquaponics production system. Aquaponics production combining leafy green plants and the African catfish, *C. gariepinus* has been evaluated before (Endut el at., 2010; 2011; 2012; Palm et al., 2014). In this study, lettuce was selected because it is an economically important plant with shorter culture periods compared to fruiting plants (Diver, 2000).

The aim of this study was to evaluate potassium from KDF as a mineral supplement and feed additive on lettuce growth, yield, proximate, and mineral composition along with fish growth and water quality parameters in an integrated aquaponics system.

7.3 Materials and methods

7.3.1 System design

The aquaponics research unit at the Welgevallen Experimental Farm of Stellenbosch University was based on the designs from University of Virgin Islands (Rakocy et al., 2006). The system is illustrated in Figure 7.1 and consisted of three main components: The fish culture component, solid removal component, and hydroponic plant growing component. The fish tanks, filter tanks, and sump constructed from polyethylene (LLDPE) roto-moulded tanks. Two of the aquaponics plant-growing beds were constructed from bricks, which were rendered and then lined with high density 4 mm thick construction grade polyethylene sheeting and one was constructed with wood and sealed with fibreglass. The hydroponic component consisted of three plant-growing beds that used floating Styrofoam rafts (18 m long x 1.3 m broad, 52.5 cm deep with water depth of 41.5 cm) with propagation net pots for plant growth. The fish culture component consisted of circular fish rearing tanks (1000 l volume) covered with polyethylene sheeting. The solid removal component consisted of three cone-bottomed cylindrical silo tanks used as a clarifier (1000 l volume, 1100mm diameter, 1900 mm height) and three rectangular filter tanks (1000 l animal drinking troughs) containing Polyvinyl Chloride cross corrugated structures packed with shading cloth as bio-filter media. Pipes distributed the effluent from fish tanks to the plant-growing beds. Each of the plant growing

beds were aerated by 10 aquarium air-stone diffusers. The water flowed from the fish tanks to the solid removal component of the system consisting of cone-bottomed clarifiers. The water then flowed to the filter tanks which acted as a bio-filter and pipes circulated the water to the plant growing beds. Individual sumps collected the water from the plant growing beds and water was circulated from the sumps to the fish tanks by a pump (0.75 kW). The aquaponics system outflow was circulated to the fish tanks and water levels were maintained by flow level valves in the sump tanks. The water from the different growing beds and fish tanks never mixed. Because the system consists of three plant growing beds at different positions, the experiment was repeated three times to eliminate any bias and the effect of environmental variation due to the position of the plant growing bed.

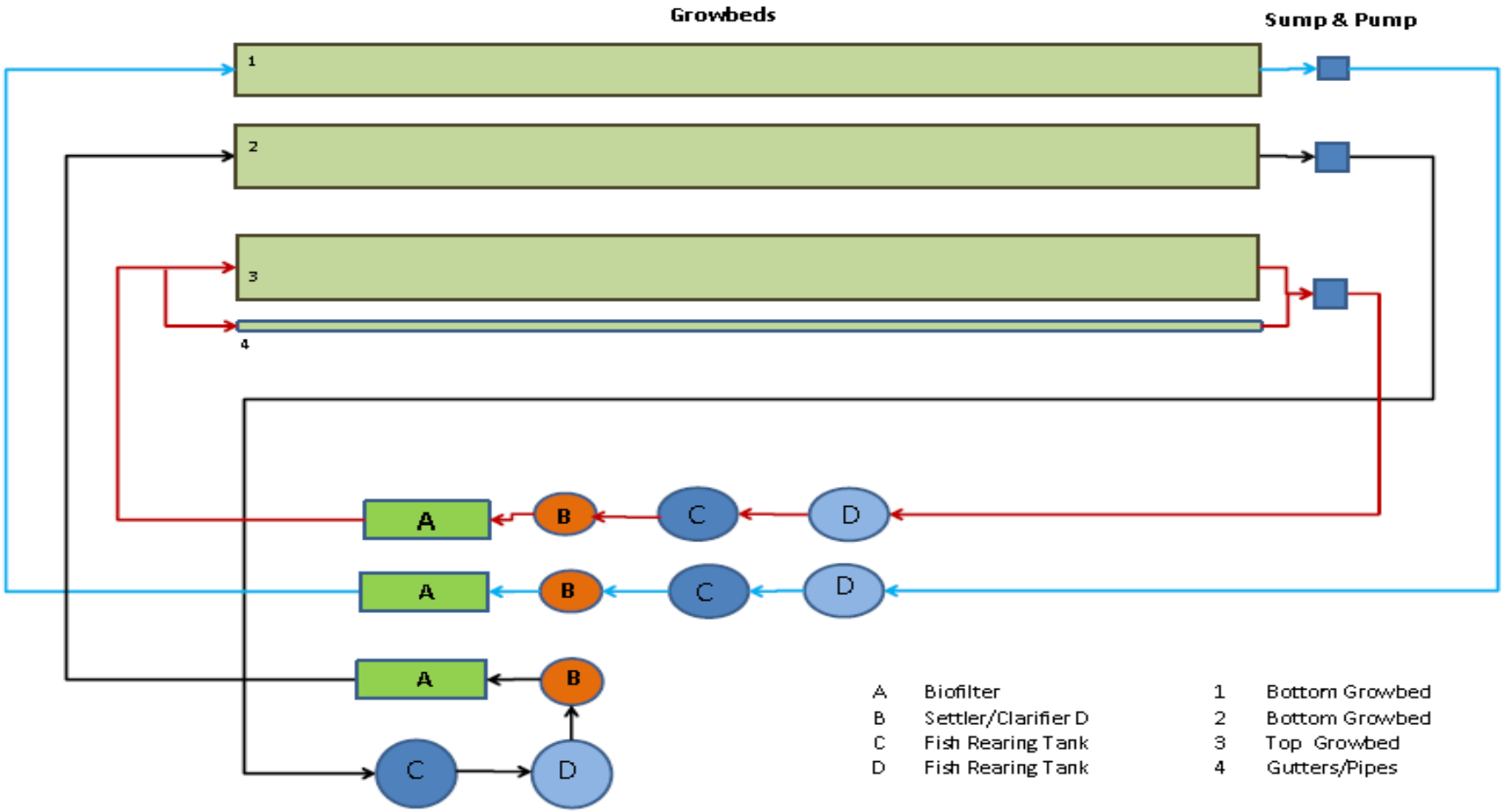

Figure 7-1 Schematic overview of the aquaponics research unit at the Welgevallen Experimental Farm of Stellenbosch University used during the trial period.

7.3.2 Experimental design

The three-month trial consisted of two dietary treatments, a control diet used as a reference and one diet supplemented with potassium diformate (KDF) at 9 gkg^{-1} (Table 7.1). The isonitrogenous and isocaloric diets were formulated to meet the needs of the catfish (NRC, 1993), the inclusion level of 9 gkg^{-1} has been used in the previous chapter (Siqwepu et al., 2020a) for catfish production. The experiment was performed three consecutive times using each of the dietary treatments while alternating the plant growing beds.

At the beginning of each production cycle the fish were randomly selected, weighed and divided between the treatments to ensure that each dietary treatment had the same initial stocking density. The first, second and third production cycle had initial stocking densities of 40 kgm^{-3} and 47 kgm^{-3}, respectively. The system was reset each time a new production cycle began. At the end of each production cycle, the fish from each dietary treatment were weighed and pooled to prepare for the next production cycle. The fish were pooled from the tanks each time and randomly divided between the treatments at the beginning of each cycle.

7.3.3 Diet preparation and analysis

The experimental diets were prepared by mixing dry ingredients with warm water and oils in a commercial dough mixer (MacAdams SM 401) (McAdams International, Cape Town, South Africa). Pellets were extruded from a single-screw extruder (custom model, Reomach Engineering, South Africa) and dried overnight at 55° C in a convection oven (Envirowatch, Cape Town, South Africa). The feed was analysed for moisture, ash, crude fat, crude protein, and crude fibre after size reduction by grinding in a hammer mill (Centrotec, Cape Town, South Africa) with a 1.5 mm sieve. The moisture content of the feed was determined by drying samples in an oven at 100° C for 24 hours (AOAC, 2002a). Feed samples were then incinerated overnight in a muffle furnace at 600 ° C for measurement of ash content (AOAC, 2002b). Crude protein was measured by the combustion Dumas method with a LECO FP 528 (AOAC, 2002c), and the fat in feed samples was determined using the ether extraction method (AOAC, 2002d). Table 7.1 represents the feed formulation and proximate composition of the experimental diet and control.

Table 7.1 Feed formulation and proximate composition of feed of experimental diets fed to *C. gariepinus* (gkg^{-1}).

Ingredients (gkg^{-1})	Treatment	
	Control	KDF
Fish meal	120	120
Soya	570	570
Maize	200	200
Cellulose	15	6
Vit/Min premix[a]	15	15
MDCP[b]	20	20
Fish oil	30	30
Sunflower oil	30	30
KDF[c]		9
Proximate composition (gkg^{-1})		
Moisture	77	71
Ash	121	116
Crude Lipids	86	84
Crude Protein	370	375
Crude Fibre	44	45
Carbohydrates[d]	346	354

[a]Vit/Min premix -Vitamins: Vitamin A, 12 500 000 IU; Vitamin D3,2 500 000 IU; Vitamin E, 150 000; Vitamin K3, 8g; Vitamin B1, 15g; Vitamin B2, 20g; Vitamin B6,15g; Vitamin B12,0.035g; Niacin, 80g Cal Pnth, 50g; Folic Acid, 2.50g; Biotin, 0.350g; Iodine, 2.50g; Cobalt, 0.55g; Selenium, 0.25g; Vitamin C (Stay 35), 300g. Minerals: Manganese, 60g; Zinc, 60g; Copper, 6g; Choline, 1000g.
[b]MDCP: Monodicalcium phosphate. [c]KDF: Potassium diformate.
[d]Determined by difference as: 1000 – Moisture - Crude Protein - Crude Lipids – Ash.

7.3.4 Experimental conditions

The experiment was conducted between November 2018 and March 2019. The system was operated for 3 weeks with only fish. The fish were fed the control diet at a reduced rate (1 % body weight) to establish biofilm and acclimate the bio-filters. During the trial, environmental conditions such as temperature were observed and recorded but were not controlled, as both the fish tanks and the aquaponics systems are located outdoors. The photoperiod was not manipulated and the system was exposed to natural light during the study. The experiment was repeated three times to ensure that each of the treatments was evaluated on all three plant growing beds. The three production cycles were to eliminate any bias in growing bed position and any possible environmental variation that each growing bed could experience. Water temperature was measured between 9:00 and 14:00. It was in the range of 16.0- 25.8° C, DO was 6.6- 7.2 mgl^{-1} and pH 5.3 – 7.8 throughout the trial, within the acceptable ranges for African catfish culture. At the start of the production cycle, the stocking density in the fish rearing tanks was 40 kgm^{-3} except for the last production cycle where the stocking density was 47 kgm^{-3} (Akinwole and Faturoti, 2007). The quantity of water lost through evaporation and spillage was not measured during the study.

7.3.5 Fish production

The experimental animals chosen for this study were relatively large (510 g ± 15.2 initial weight), to have sufficient biomass in the aquaponics system. Before the experiment, the fish were acclimated in rearing tanks for three weeks. Each of the fish rearing tanks had air stones connected to an air blower placed inside the tank to maintain sufficient oxygen supply to the fish. During the acclimation period, the fish were hand fed the control diet twice a day (08:00 am and 16:00 pm) (1 % body weight). The fish rearing tanks were covered with nets to prevent the fish from jumping out of the tanks. Temperature, dissolved oxygen, and pH were measured daily in the fish rearing tanks using OxyGaurd Handy Polaris (OxyGuard International A/S, Farum, Denmark) and pH with a Hanna pH 211 microprocessor (Hanna Instruments, Sarmeola di Rubano, Italy). To assess fish growth, the fish were netted and weighed at the start and end of each production cycle. Handling was done under anaesthesia (400 mgl^{-1} of tricane methanesulfonate, MS-222, Sigma-Aldrich, Johannesburg, South Africa), using 800 mgl^{-1} of sodium bicarbonate as a buffer. Initial weight, final weight, weight gain,

feed conversion ratio (FCR), specific growth rate (SGR %), and survival rate (%) were measured. The parameters were calculated as follows: Weight gain = ($W_2 - W_1$)

$$\text{Specific growth rate (SGR)} = \frac{\ln W_2 - \ln W_1}{T_1 \times 100}$$

$$\text{Survival rate (\%)} = \frac{\text{No. of fish at end of experiment}}{\text{No. of fish at start of experiment} \times 100}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Dry weight of feed (g)}}{\text{Weight gain (g)}}$$

Where:

W_1 = Initial mean weight (g)

W_2 = Final mean weight (g)

T_1 = Duration of the experiment

7.3.6 Lettuce production

Lettuce (*Lactuca sativa*) seedlings were obtained from Radical Seedlings and Landorf nursery in Cape Town, South Africa. The lettuce seedlings were two weeks old and grown in a peat moss medium and were subjected to a one-week withdrawal period before being transplanted into the aquaponics system. During the withdrawal period, the seedlings were watered manually once daily. The seedlings were then transplanted directly to floating polystyrene rafts in the aquaponics growing beds after one week. The plants were transplanted by removing them from the growth media, rinsing them with water, and placing them in propagation pots, while ensuring that the roots were immersed in the aquaponics system water. Three hundred and thirty plants per treatment were used. The propagation

pots were then filled with Leca growing medium (8 – 16 mm) to stabilise and support the newly transplanted plants. No fertilizers or pesticides were used on the plants.

For the water quality parameters in the plant growing beds, removal efficiency for the measured nutrients was calculated according to Endut el at. (2011), using the equation:

$$\mathrm{RE}\ (\%) = 100\ \times \frac{(\mathrm{C_i} - \mathrm{C_0})}{\mathrm{C_i}}$$

Where:

RE = Removal efficiency (%)

Ci = Influent

C_0 = Effluent

In the systems that was used, a high nutrient load was removed as solid particles by the solid removal component of the system. Therefore, some nutrients are removed before they reach the plant growing beds. Therefore, it can be said that the efficiency that is measured is one resulting from only lettuce after the solids have been removed.

For each nutrient, the samples were taken once a week and analysed immediately. After each production cycle, all the plants were removed from the plant growing beds and this procedure was repeated for each production cycle.

7.3.7 Monitoring and data collection

For plant growth and yield, the total number of transplanted plants, live plants, and harvested plants were recorded. Furthermore, the average biomass, shoot weight, root weight and root: shoot ratio were recorded. Plants were measured at the beginning and end of each growing cycle. At the end of each production cycle, in each of the growing beds, plants (10 %) were randomly selected and taken to measure their wet weight, shoot and root weight. The plants selected were assumed to be representative of the plants in the growing bed. Plant growth was monitored throughout the 31-day period and data on parasitic snails observed on the plants. A lettuce was considered live and healthy when it reached a minimum of 35 g at the time of harvest.

7.3.8 Proximate and mineral analysis

At the end of each production cycle, 1 kg of plants from each plant growing bed and both treatments were weighed and analysed for proximate and mineral composition. The wet weight of the plants was recorded, then the plants were oven dried at 60° C for 48 hours. The dry biomass was pooled and prepared as a single composite sample and subsequently ground. The moisture content of the plants was determined by drying samples in an oven at 100°C for 24 hours (AOAC, 2002a). The samples were then incinerated overnight in a muffle furnace at 600° C for measurement of ash content (AOAC, 2002b). Crude protein was measured by the combustion Dumas method with a LECO FP 528 (AOAC, 2002c). The total fat in feed samples was determined using the ether extraction method (AOAC, 2002d). The plants were sent for mineral analysis to Elsenburg Institute for Plant Science at the Department of Agriculture, Western Cape. The minerals in the plant leaves were measured on an iCAP 6000 Series Inductive Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan, Italy). The plant leaves were dried for 48 hours at 60°C. A finely ground sample of 0.5 g was then dried and defatted. The sample was then ashed for six hours at a temperature of 460 - 480° C, then the sample was allowed to cool at room temperature, and subsequently 5 ml of 6 M HCl was added. The sample was placed in an oven for 30 minutes at 50° C, then 35 ml of distilled water was added to the sample. The sample was filtered into a brown container and distilled water was added to obtain 50 ml of the sample. The elemental concentrations in the sample were then calculated with iTEVA Analyst software.

7.3.9 Water quality analysis

Weekly water quality analysis was performed on the influent and effluent water of the plant growing beds from the experimental treatment and the control. The water was collected as it flowed into and exited the growing bed and analysed immediately in the laboratory for total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), reactive phosphorus (orthophosphate, PO_4^{3-}-P), iron (Fe), potassium (K), total suspended solids (TSS), and total dissolved solids (TDS). The samples were analysed using a Hach DR 900 Laboratory Spectrophotometer (Hach Company, Loveland, Colorado, USA). Daily water quality measurements of temperature, dissolved oxygen (DO), and pH were taken. The pH was measured with a Hanna pH 211 microprocessor (Hanna Instruments, Sarmeola di Rubano,

Italy) and temperature and DO with OxyGuard Handy Polaris (OxyGuard International A/S, Farum, Denmark).

7.3.10 Statistical analysis

The fish and plant production, along with water quality data, were not statistically analysed because there were no independent replicates, only descriptive statics were used. However, the proximate and mineral composition of the plants were statistically analysed using Statistica 13 (Dell, Inc), using one-way analysis of variance (ANOVA) to determine whether there were any differences between treatments. Differences were deemed statistically significant between means when $p < 0.05$.

7.4 Results

7.4.1 Fish production

The results of fish production parameters are presented in Table 7.2.There was an increase in the initial total weight of fish at the end of each production cycle. There was an average increase of 6.61 kg (control) and 7.6 kg (KDF treatment) in the first production cycle. In the second and third production cycle, the average weight increase was 7.6 and 7.7 kg for the control and 6.6 and 12.5 kg for the KDF treatment. The FCR ranged from 1.3 – 2 over the three planting cycles. The FCR of the control was 1.9 and 2, while that of the KDF treatment in the first and the third cycle of planting was 1.7 and 1.3, respectively. The temperature in the fish tanks during the trial ranged between 16.7- 25.8° C, while the DO and pH was 6.6- 7.2 mgl^{-1} and 5.3 – 7.8 respectively. The experimental conditions were acceptable to the African catfish (Ali and Jauncey, 2004).

Table 7.2 Fish production parameters of the African catfish, *C. gariepinus* fed the control diet and KDF treatment.

Production cycle	Parameters	Control	KDF
First (14 Nov - 15 Dec)	Initial Weight (kg)	40.0	40.0
	Final Weight (kg)	46.7	47.6
	Weight Gain (kg)	6.61	7.6
	Survival (%)	90	96
	FCR	1.9	1.7
	SGR (%)	0.49	0.56
	Number of fish	80	80
Second (29 Dec - 29 Jan)	Initial Weight (kg)	40	40
	Final Weight (kg)	46.7	45.6
	Weight Gain (kg)	7.6	6.6
	Survival %	93	90
	FCR	1.6	1.8
	SGR (%)	0.57	0.5
	Number of fish	60	60
Third (04 Feb - 06 March)	Initial Weight (kg)	46.7	44.5
	Final Weight (kg)	54.4	56
	Weight Gain (kg)	7.7	12.5
	Survival %	95	100
	FCR	2	1.3
	SGR (%)	0.49	0.78
	Number of fish	56	53

No replicates were available, thus no SE is shown.

7.4.2 Plant production parameters

Results for the total survival of plants, their initial weight, average final wet weight, and shoot and root weight are presented in Table 7.3. Plants grown in combination with fish fed the control treatment had a lower average biomass compared to plants grown with fish fed the KDF treatment at the end of each production cycle, except for the last production cycle. The control had a total average biomass of 17.2 g ± 5.30, 14.5 g ± 5.48, and 13.7 g ± 4.65, for the first, second and third cycle respectively, while the average biomass in the KDF treatment had values of 57.9 g ± 20.4, 51.2 g ±14.4, and 7.97 g ± 3.28, respectively. Upon visual observation, the plants did not exhibit any visible nutritional deficiencies at the time of harvest. During cycle one and two, the total number of harvested plants were lower in the control treatment than the KDF treatment. The number of plants harvested was high during the last production cycle but the plants for both treatments were wilted and had very small roots.

Table 7.3 Plant production and yield from November 2018 to March 2019.

Production cycle	Production parameters	Control	KDF
First (14 Nov – 15 Dec)	Total No. of plants	340	340
	No of live plants	320	333
	No. of plants harvested	320	333
	Average biomass (fresh) g	17.2±5.30	57.9±20.4
	Average shoot weight(g)	14.3±4.75	50.9±19.0
	Root (fresh) g	2.85±0.94	6.94±2.4
	Root:Shoot	0.20	0.14
	Initial plant weight (g)	5.25±0.90	5.33±0.91
Second (29 Dec – 29 Jan)	Total No. of plants	340	340
	No of live plants	290	335
	No. of plants harvested	290	335
	Average biomass (fresh) g	14.5±5.48	51.2±14.4
	Average shoot weight(g)	10.7±4.86	44.0±13.0
	Root (fresh) g	3.80±1.11	7.20±2.38
	Root:Shoot	0.36	0.16
	Initial plant weight (g)	5.2±0.89	5.22±0.90
Third (04 Feb Dec – 06 March)	Total No. of plants	340	340
	No of live plants	340	329
	No. of plants harvested	340	329
	Average biomass (fresh) g	13.7±4.65	7.97±3.28
	Average shoot weight(g)	12.4±4.57	6.62±2.45
	Root (fresh) g	1.28±1.21	1.35±1.01
	Root:Shoot	0.10	0.2
	Initial plant weight (g)	5.15±0.88	5.18±0.89

Data presented as average ± SD

Table 7.4 Proximate composition of the lettuce from November 2018 to March 2019.

Production cycle	Proximate composition (%)	Control	KDF
First (14 Nov - 15 Dec)	Moisture	9.5±0.16[a]	10.1±0.06[b]
	Ash	13.2±0.04[a]	14.3±0.04[b]
	Crude Fat	4.8±0.08[a]	4.5±0.01[a]
	Crude Protein	21.2±0.22[a]	24.2±0.07[a]
	Crude Fibre	12.8±0.09[a]	12.4±0.12[a]
Second (29 Dec - 29 Jan)	Moisture	9.7±0.09[a]	7.4±0.04[b]
	Ash	11.4±0.00[a]	23.0 ±0.09[b]
	Crude Fat	5.5±0.19[a]	4.2±0.05[b]
	Crude Protein	18.1±0.13[a]	18.6±0.21[a]
	Crude Fibre	12.6±0.22[a]	11.4±0.06[b]
Third (04 Feb - 06 March)	Moisture	6.6±0.03[a]	8.1±0.05[b]
	Ash	23.7±0.04[a]	16.1±0.06[b]
	Crude Fat	4.4±0.07[a]	5.2±0.07[b]
	Crude Protein	19.6±0.17[a]	18.4±0.20[b]
	Crude Fibre	11.1±0.10[a]	12.3±0.08[b]

Data is presented as mean ±SE. Different superscripts in the same row indicate significant differences, $p > 0.05$.

7.4.3 Proximate and mineral composition of lettuce leaves

The proximate composition of the lettuce leaves was analysed and is presented in Table 7.4. The moisture and ash differed between the leaves of lettuce grown with wastewater of the control treatment and those of wastewater from the KDF dietary treatment ($p<0.05$) for all the production cycles. Crude fat and crude fibre followed a similar trend to each other and were only different on the second and third cycle ($p<0.05$). Crude protein was similar for the leaves of the control and leaves of the KDF feed on the first and second cycle but differed on the third cycle ($p<0.05$). The mineral analysis of the plants is presented in Table 7. 5. The second and third cycle followed a similar trend. The plants grown with KDF supplemented fish feed had significantly higher K concentration in the leaves compared to the control feed ($p<0.05$). The NH_4-N concentration did not differ on the first and second cycle, however, the control had a significantly higher NH_4-N concentration in the leaves at the end of the third production cycle. In all three production cycles, PO_4^{3-}-P and Mn differed significantly between the treatments. Fe was significantly different in cycle one and two, however, on the third cycle both treatments were similar. In the aquaponics plant growing beds, the water temperature ranged between 16.0 – 25° C, while the DO and pH was 6.6- 7.2 mgl^{-1} and 5.8 – 7.10, respectively (Appendix B).

Table 7.5 Lettuce mineral composition of lettuce from November 2018 to March 2019

Production cycle	Nutrient (gkg^{-1})	Control	KDF
First (14 Nov - 15 Dec)	NH_4-N	37.85±1.95[a]	38.85±1.95[a]
	PO_4^{3-}-P	5.15±0.05 [a]	5.95±0.05 [b]
	K	46.55±0.81[a]	48.55±0.81[a]
	Fe	0.18±0.00[a]	0.12±0.00[b]
	Mn	0.28±0.01 [a]	0.98±0.01[b]
Second (29 Dec - 29 Jan)	NH_4-N	31.95±0.65[a]	32.7±0.65[a]
	PO_4^{3-}-P	5±0.03[a]	4.45±0.03[b]
	K	37.1±0.32[a]	52.3±0.32[b]
	Fe	0.22±0.01[a]	0.25±0.01[b]
	Mn	0.40±0.00[a]	0.24±0.00[b]
Third (04 Feb - 06 March)	NH_4-N	32.8±2.22[a]	28.9±2.22[b]
	PO_4^{3-}-P	5.45±0.03[a]	3.8±0.03[b]
	K	45±0.73[a]	48.6±0.7[b]
	Fe	0.16±0.01[a]	0.18±0.01[a]
	Mn	0.33±0.00[a]	0.19±0.00[b]

Data is presented as mean ±SE. Differen t superscripts in the same row indicate significant differences, $p > 0.05$.

7.4.4 Water quality

The water quality parameters measured during the study were total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), reactive phosphorus (PO_4-P), iron (Fe), potassium (K), total suspended solids (TSS), and total dissolved solids (TDS). The results of the water quality analysis along with the percentage removal efficiency in the plant growing beds are presented in Table 7.6 – 7.11. The removal efficiency of lettuce for the control and potassium treatment during the three production cycles ranged from 0 – 100 %. Potassium from the diet supplemented with KDF was efficiently removed at a rate of 20 - 49 %.

The TAN concentrations during the study in both fish tanks and plant growing beds ranged from 0.18 – 2.12 mgl^{-1}, while NO_3-N concentration was 0.5 – 7.2 mgl^{-1}. NO_2-N concentrations did not exceed 4 mgl^{-1}. Graphs representing the influent and effluent dynamics over the trial period in the plant growing beds are presented as Figures 7.2 – 7.4. The concentration of potassium in the water was increased in the KDF treatment compared to the control treatment in all three production cycles. The concentration of potassium was higher in the influent than in the effluent, which indicates the absorption of nutrients by plants in the system. The TDS concentration was also higher in all the production cycles in the KDF treatment compared to the control treatment. The water temperature over the three production cycles was measured and is presented in Appendix B, Table B1 - B6. During the first production cycle, atmospheric temperatures ranged from 9 - 29° C while water temperature ranged from 16.0 – 20.1° C. During the second cycle, the atmospheric temperature ranges were 10 - 36° C and 17.0 – 22.5° C respectively. During the last production cycle, the temperatures were high and ranged from 12 - 39° C and 17.2 – 24.5° C for the atmosphere and water temperature respectively.

Table 7.6 Water quality parameters of the control diet for the first production cycle (mgl^{-1}) (14 November – 15 December)

Week		Water quality parameters							
		TAN	NO_2-N	NO_3-N	PO_4^{3-} P	Fe	K	TSS	TDS
Week 1	Influent	0.33	3	2.60	1.62	0.01	6.40	6	120.5
	Effluent	0.31	2	2.	1.40	0.01	4.20	6	118.2
	Removal efficiency (%)	6	33	23	14	0	34	0	2
Week 2	Influent	0.88	2	4.40	1.55	0.01	3.20	4	117.1
	Effluent	0.72	1.50	4.20	1.32	0.00	2.90	3	115.1
	Removal efficiency (%)	18	25	5	15	100	9	25	2
Week 3	Influent	1.28	2	4.20	6.52	0.01	4.30	6	189.8
	Effluent	0.60	1	1.50	5.40	0.00	4	3	109.4
	Removal efficiency (%)	53	50	64	17	100	7	50	42
Week 4	Influent	0.51	4	5.40	7.68	0.02	3.70	5	100.2
	Effluent	0.32	3	4	6.60	0.01	3.50	2	94.1
	Removal efficiency (%)	37	25	26	14	50	5	60	6

Table 7.7 Water quality parameters of the KDF diet for the first production cycle (mgl^{-1}) (14 November – 15 December)

Week		Water quality parameters							
		TAN	NO_2-N	NO_3-N	PO_4^{3-} P	Fe	K	TSS	TDS
Week 1	Influent	2.12	2	7	1.56	0.03	5.50	9	139.9
	Effluent	0.56	0	4.60	1.52	0.01	4.20	6	137.9
	Removal efficiency (%)	74	100	34	3	67	24	33	1.4
Week 2	Influent	1.52	2	3.30	1.77	0.07	8	11	157.6
	Effluent	1.4	1	2.80	1.68	0.02	6.10	9	157.2
	Removal efficiency (%)	8	50	15	5	71	24	18	0.3
Week 3	Influent	1.68	4	7.70	7.08	0.05	14.60	4	178.5
	Effluent	1.3	2	6.60	6.96	0.03	7.5	2	106.3
	Removal efficiency (%)	23	50	14	2	40	49	50	40
Week 4	Influent	0.98	2	2.70	7.24	0.06	7.50	8	198.7
	Effluent	0.9	1.50	2.30	6.88	0.04	5.50	5	197.9
	Removal efficiency (%)	8	25	15	5	33	27	38	0.4

Table 7.8 Water quality parameters of the control diet for the second production cycle (mgl^{-1}) (29 December – 29 January)

Week		Water quality parameters							
		TAN	NO_2-N	NO_3-N	PO_4^{3-} P	Fe	K	TSS	TDS
Week 1	Influent	4.6	3	5	1.61	0.03	2.80	3	55.6
	Effluent	2.8	0	3.20	1.04	0.02	4.60	2	54.9
	Removal efficiency (%)	39	100	36	35	33	39	33	1
Week 2	Influent	0.28	0	1.80	7.44	0.06	1.50	3	58.4
	Effluent	0.27	0	1.30	4.88	0.04	1	0	57.6
	Removal efficiency (%)	4	0	28	34	33	33	100	1
Week 3	Influent	0.93	2	4.20	1.60	0.00	0.60	7	57.7
	Effluent	0.82	0	3.90	1.45	0.00	0.40	6	56.4
	Removal efficiency (%)	12	100	7	9	0	33	14	2.
Week 4	Influent	1.40	2	5.80	5.60	0.03	1.50	9	56.6
	Effluent	1.30	1	5.40	5.50	0.01	1.10	4	56.1
	Removal efficiency (%)	7	50	7	2	67	27	56	1

Table 7.9 Water quality parameters of the KDF diet for the second production cycle (mgl^{-1}) (29 December – 29 January)

Week		Water quality parameters							
		TAN	NO_2-N	NO_3-N	PO_4^{3-} P	Fe	K	TSS	TDS
Week 1	Influent	0.36	2	1.3	3.28	0.04	6.8	2	111.95
	Effluent	0.14	0.5	0.9	1.79	0.02	4.5	1	110.3
	Removal efficiency (%)	61	75	31	45	50	34	50	1.5
Week 2	Influent	0.43	3	1.1	1.15	0.05	7.5	5	60.3
	Effluent	0.39	1	0.8	1.03	0.04	5.5	2	59.1
	Removal efficiency (%)	9	67	27	10	20	27	60	2
Week 3	Influent	0.34	4	1.1	1.37	0.03	2.6	4	95.1
	Effluent	0.31	2	0.9	1.28	0.03	1.5	4	93.3
	Removal efficiency (%)	9	50	18	7	0	42	0	2.2
Week 4	Influent	0.31	0.05	1.1	2.7	0.05	3.6	6	112.3
	Effluent	0.29	0.04	0.9	1.4	0.01	2.4	3	110.3
	Removal efficiency (%)	6	20	18	48	80	33	50	2

Table 7.10 Water quality parameters of the control diet for the third production cycle (mgl^{-1}) (04 February – 06 March)

Week		Water quality parameters							
		TAN	NO_2-N	NO_3-N	PO_4^{3-} P	Fe	K	TSS	TDS
Week 1	Influent	0.39	0	0.7	1.12	0.01	0.7	13	121.5
	Effluent	0.18	0	0.5	0.91	0.01	0.6	7	59
	Removal efficiency (%)	54	0	29	19	0	14	46	51
Week 2	Influent	0.27	0	1.0	0.71	0.01	0.7	3	60.6
	Effluent	0.20	0	0.5	0.56	0.01	0.5	0	58.6
	Removal efficiency (%)	26	0	50	21	0	29	100	3.3
Week 3	Influent	0.35	0	0.8	0.99	0.01	0.7	1	64.2
	Effluent	0..27	0	007	0.89	0.00	0.6	1	63.8
	Removal efficiency (%)	23	0	13	10	100	14	0	1
Week 4	Influent	0.46	2	2.7	4.4	0.02	1.6	4	75.8
	Effluent	0.4	1	2.1	0.36	0.01	0.8	3	74.8
	Removal efficiency (%)	13	50	22	92	50	50	25	1.3

Table 7.11 Water quality parameters of the KDF diet for the third production cycle (mgl^{-1}) (04 February – 06 March)

Week		Water quality parameters							
		TAN	NO_2-N	NO_3-N	PO_4^{3-} P	Fe	K	TSS	TDS
Week 1	Influent	0.94	2	1.7	4.38	0.02	6.4	12	77
	Effluent	0.6	1	1.5	3.46	0.01	4.4	4	75.3
	Removal efficiency (%)	36	50	12	21	50	31	67	2.2
Week 2	Influent	1.06	3	1.7	4.58	0.03	2.3	6	73.4
	Effluent	0.82	2	1.4	3.66	0.01	1.4	0	70.8
	Removal efficiency (%)	23	33	18	20	67	39	100	4
Week 3	Influent	0.92	0.5	1.0	1.67	0.03	4.4	4	72.4
	Effluent	0.63	0.3	0.8	1.31	0.02	2.4	3	68.5
	Removal efficiency (%)	32	40	20	22	33	45	25	5.4
Week 4	Influent	1.09	1	1.3	2.62	0.03	6.1	7	70.4
	Effluent	0.61	0	1.2	1.79	0.01	4.9	4	64
	Removal efficiency (%)	44	100	8	32	67	20	43	9.1

7.5 Discussion

Lettuce growth, yield, proximate, and mineral composition were evaluated along with water quality and fish production in an integrated production system using the African catfish. A potassium feed additive, potassium diformate was used as a dietary treatment along with a control treatment that was used as a reference point in the diet of the African catfish.

The production parameters changed over the three-month study period, as the weight of the experimental fish increased. The FCR in this study was higher than the ideal FCR of 1.0 for the African catfish in recirculating aquaculture systems (Eding and Kamstra, 2001), which could be attributed to overall poor conversion of feed leading to a high FCR in the aquaponics system. The FCR was also high compared to other studies of African catfish in aquaponics systems using water spinach (1.23 – 1.39) (Endut et al., 2010; 2012), mustard green (1.13 – 1.32) (Endut et al., 2011) and herbs (0.61) (Knaus and Palm, 2017). The growth rate was in a comparable range to other African catfish grown in aquaponics systems in combination with basil, cucumber, lettuce, and tomato (Palm et al., 2014). Despite the high FCR values obtained and the variable SGR in this study the African catfish gained weight over the trial period for both dietary treatments.

The inclusion of KDF to the diet of the African catfish led to good lettuce growth when the environmental and water temperature was favourable (growth cycles one and two). The increase in average biomass and shoot weight of lettuce from the KDF dietary treatment compared to the control treatment during cycle one and two proved the importance of supplementing K to lettuce in aquaponics systems. Increased production has been reported in aquaponics systems when fish wastewater from Tilapia was supplemented with micronutrients and K compared to lettuce grown with non-supplemented wastewater (Nozzi et al., 2018). It has been suggested that supplementing nutrients such as potassium and phosphorus should be considered essential for optimum plant production (Nicoletto et al., 2018). This is because when lettuce is grown in systems with insufficient nutrients, including potassium, bolting (the rapid elongation of the stem) is observed (Al-Hafedh et al., 2008; Fukuda et al., 2009), but this phenomenon was not observed in this study for any of the treatments or during any of the production cycles.

During the last cycle of production, the average biomass and shoot weight of plants significantly decreased for both dietary treatments. Seasonally high temperature had a direct effect on lettuce growth; the atmospheric temperatures reached a high of 39° C while water temperatures reached 24.5° C maximum. Weather conditions have been reported to affect production in hydroponic systems when cucurbits, lettuce, and pepper were grown in hydroponics systems (Urrestarazu et al., 2008; Fallovo et al., 2009; Amalfitano et al., 2017). In this study, low average biomass and shoot weight could be attributed to longer photoperiods and increased temperature during the summer month of February. Lettuce grows well in temperatures ranging from 7 – 24° C, with an average temperature of 18° C (Maboko and Du Plooy, 2007), but environmental temperatures during this production cycle were well above the optimum for lettuce. The higher light incidence and high air and water temperatures (Supplementary Table B1 –B3) during this production cycle reduced the growth of the plants significantly by lowering the nutrient uptake by plants (Nicoletto et al., 2018).

Lettuce was able to remove nutrients efficiently from wastewater during this study. Lettuce has been used in aquaponics systems before and has shown the ability to remove nutrients from wastewater (Endut et al., 2010; 2011). There were fluctuations and variations in the uptake of nutrients throughout the trial period. The efficiency or ability to remove nutrients by plants is dependent on the nutritional needs of the plant (Graber and Junge, 2009) and climatic conditions (Fallovo et al., 2009), explaining the periodic fluctuations observed in removal efficiency of nutrients by lettuce during the trial period.

The water quality parameters between the two treatments did not show any consistent trends, with the exception of K and TDS concentrations. Although not statistically analysed, the wastewater from the control treatment had lower K and TDS concentrations than the wastewater from the KDF dietary treatment (Figures 7.2 – 7.4). The differences observed in K and TDS concentration between the treatments can be attributed to the addition of KDF to the fish diet. The supplementation of K through KDF may not have only increased K concentration in the water, but also TDS as a result of the K^+ cation. The increased K concentration in wastewater of KDF treatment during the trial is proof that including KDF in the diet of the African catfish can lead to high K concentrations which may in turn lead to good lettuce production under good climatic conditions.

The lettuce produced during the trial had variable levels of Fe and Mn in the leaves. It cannot be concluded whether the levels of minerals in lettuce leaves were influenced purely by the dietary treatments, especially in the first and second production cycles, as there is no obvious pattern to mineral levels. The Fe and Mn levels in lettuce leaves could be as a result of the variability in nutrient uptake and utilization by plants (Fallovo et al., 2009). The mineral levels in lettuce leaves during this trial were not adversely affected by the dietary feed additive, as higher Fe and comparable Mn concentrations in lettuce leaves have been reported in aquaponics systems before (Delaide et al., 2016).

Significant differences were observed when evaluating the proximate composition of lettuce. Although the differences did not follow a trend, they could be attributed to the effects of the dietary treatments and to seasonality as they can affect the nutritional quality of plants (Wheeler et al., 1995, Fallovo et al., 2009). Reports of lettuce grown in aquaponics and hydroponics systems showed comparable results to this study (Wheeler et al., 1995; Fallovo et al., 2009), indicating that the production of lettuce was normal during the first and second production cycle of this study.

The requirement of some nutrients like K, decreases as the plant grows (Voogt, 2002), this may affect their uptake by plants, depending on the needs of the plants. This explains the variation and fluctuations in the uptake of K from wastewater during the trial. The plants were absorbing K depending on their need for it and on the growth stage. The increased K concentration in the wastewater resulted in higher K levels in the leaves of lettuce grown in the KDF dietary treatment compared to the control treatment. There were significantly higher K levels in the leaves of lettuce from the KDF dietary treatment in the second and third cycle of production, although not significantly different, the first production cycle also showed higher K levels, which may be attributed to the KDF feed additive in the fish feed.

The nitrate and TAN concentrations in this study, ranging from 0.5 – 7.2 and 0.18 – 2.12 mgl^{-1} respectively, were lower than values reported in literature (Al-Hafedh et al., 2008; Endut et al., 2010; 2011; Knaus and Palm 2017). The NO_3–N concentration did not exceed the threshold concentration of 140 mgl^{-1} for the production of the African catfish (Schram et al., 2014). Similarly, TAN did not exceed 4.96 mgl^{-1}, a TAN concentration that African catfish withstood in aquaponics system with no adverse effect (Baßmann et al., 2017). The nitrate and TAN concentration in this study were regulated through production by fish waste, conversion by

bacteria and uptake by plants. The uptake of TAN and NO_3-N by plants resulted in the lower concentration of these nutrients compared to other studies in aquaponics systems (Al-Hafedh et al., 2008; Endut et al., 2010; 2012; Knaus and Palm, 2017). Results in this study indicate that the NO_3 – N and TAN concentrations did not adversely affect the production of the African catfish and produced sufficient concentrations for optimum plant growth for both treatments. TAN removal was accompanied by an increase in NO_3-N, the observed increase in TAN removal could be attributed of nitrification occurring in the system (Endut et al., 2010).

Phosphorus had a removal efficiency of 2 – 92 % for both dietary treatments and the concentration of phosphorus in the influent was higher compared to the concentration in the effluent in the plant growing beds. This is an indication that phosphorus produced from fish waste was absorbed by plants. The phosphorus concentrations were sufficient for optimum plant production and did not result in poor lettuce growth and the plants did not exhibit any deficiencies (Al-Hafed et al., 2008). Although our phosphorus concentrations varied from other catfish producers in aquaponics systems (Endut et al., 2010; 2012; Palm et al., 2014; Knaus and Palm, 2017), the influent and effluent concentrations along with high removal efficiency of phosphorus shows it was absorbed by plants.

The pH was not manipulated in any way during the study. The pH range (5.50 – 7.10) in the aquaponics growing bed in this study allowed for nitrification to occur in the bio-filter and at the root zone, while plants were able to absorb nutrients from the water. The pH was an ideal compromise for all organisms during this study because nitrification occurs efficiently at a pH of 7.5 -8.0 (Savidov et al., 2007) while plants absorb nutrients efficiently at a pH of 6.0 – 6.5 (Goddek et al., 2015). A suboptimal pH in the aquaponics systems may have led to an accumulation of nitrite which is detrimental to plant and fish production (Palm et al., 2014). This was not seen during this study, as nitrite concentrations ranged from 0 – 4 mgl^{-1} which is below the nitrite concentrations of 5 – 10 mgl^{-1} that can be harmful to both fish and plants (Tyson et al., 2007). A pH allowing for a symbiotic co-existence of fish, plants and bacteria was maintained (Goddek et al., 2015). Furthermore, NO_2-N accumulation in the system may further hinder the plants ability to absorb certain nutrients (Tyson et al., 2004) resulting in stunted growth. The concentration of NO_2-N during the trail is in the reported ranges for other studies using the African catfish in aquaponics systems (Endut et al., 2010).

7.6 Conclusion

The study showed that increased potassium levels resulting from the feed additive, potassium diformate, can provide additional nutrients to the plants in aquaponics systems. The increased potassium concentration in the wastewater increased the average biomass and shoot weight of lettuce, simultaneously yielding acceptable fish production. Therefore, supplementation of potassium from KDF at 9 gkg^{-1} can enhance lettuce growth in aquaponics growth systems.

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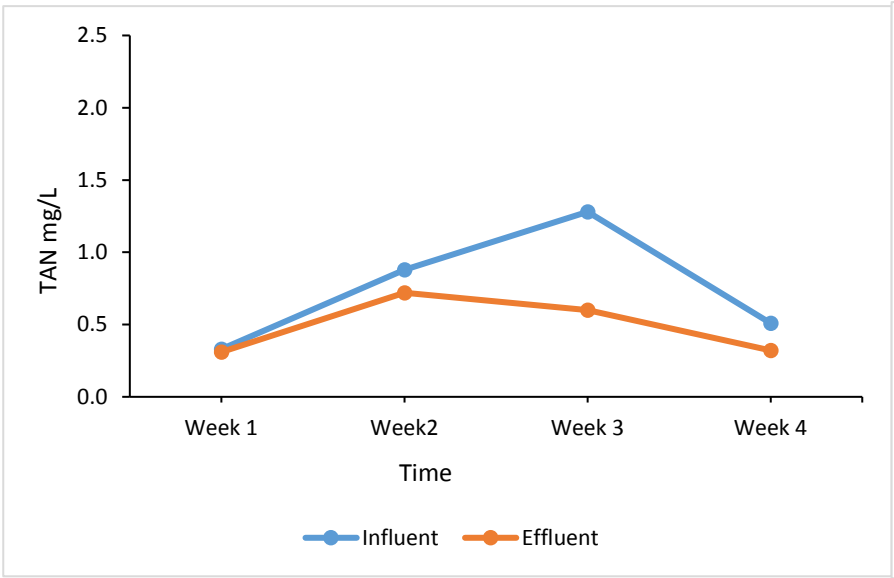
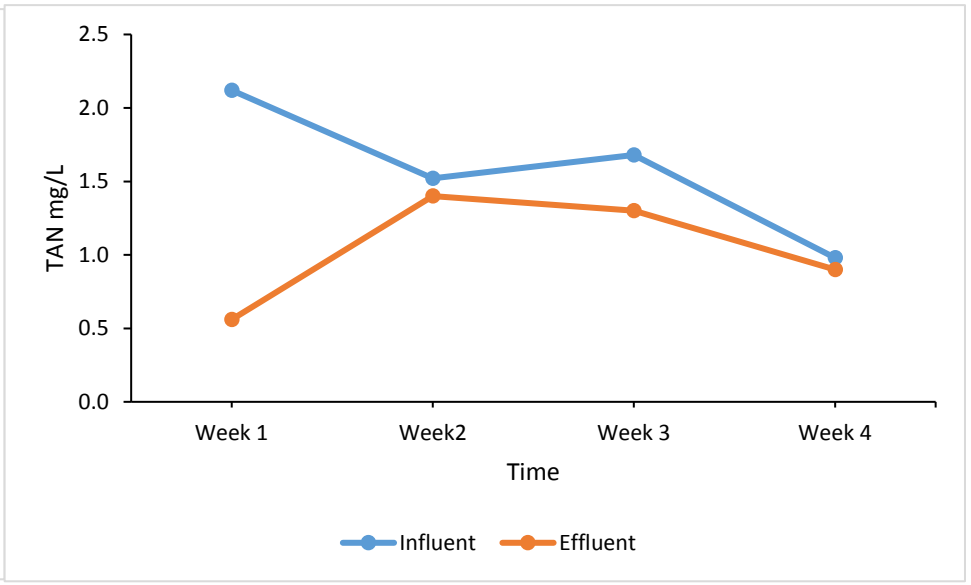
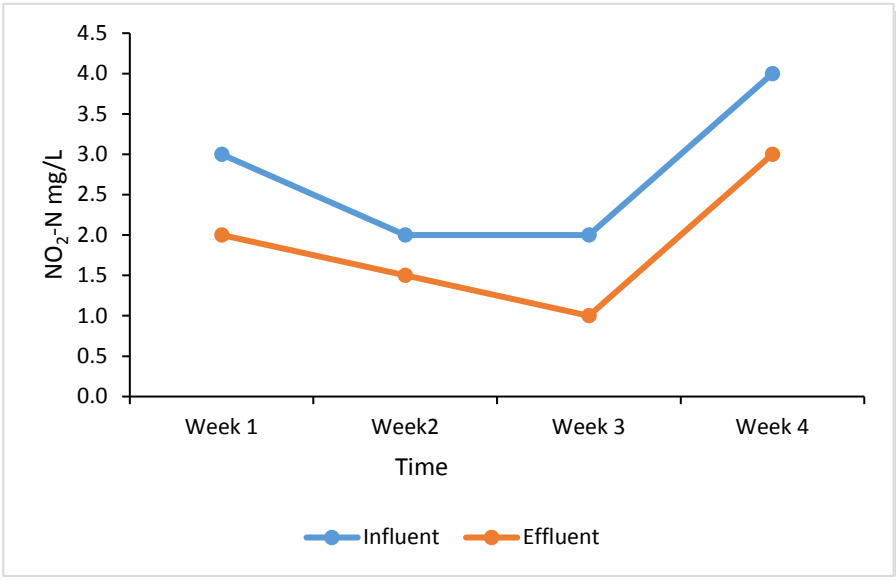
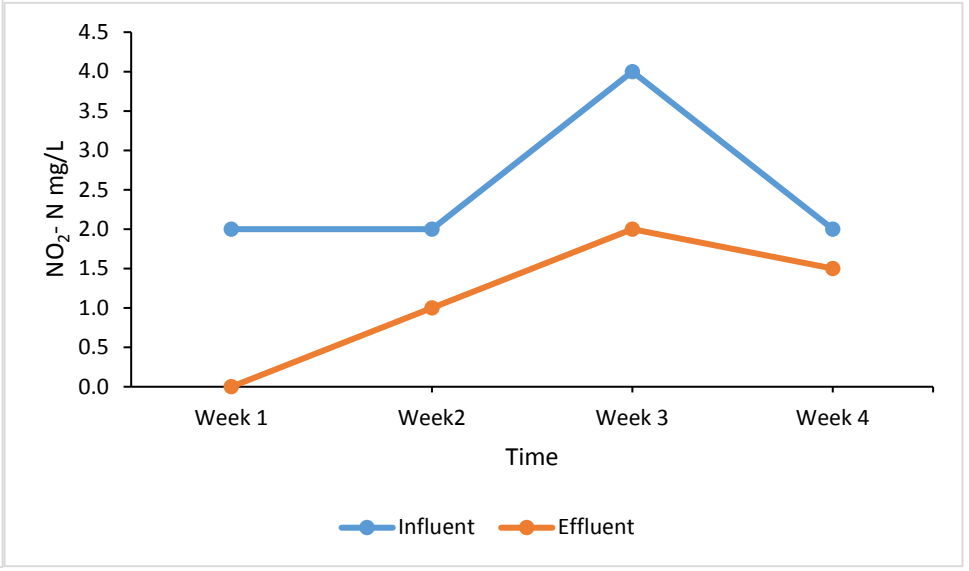
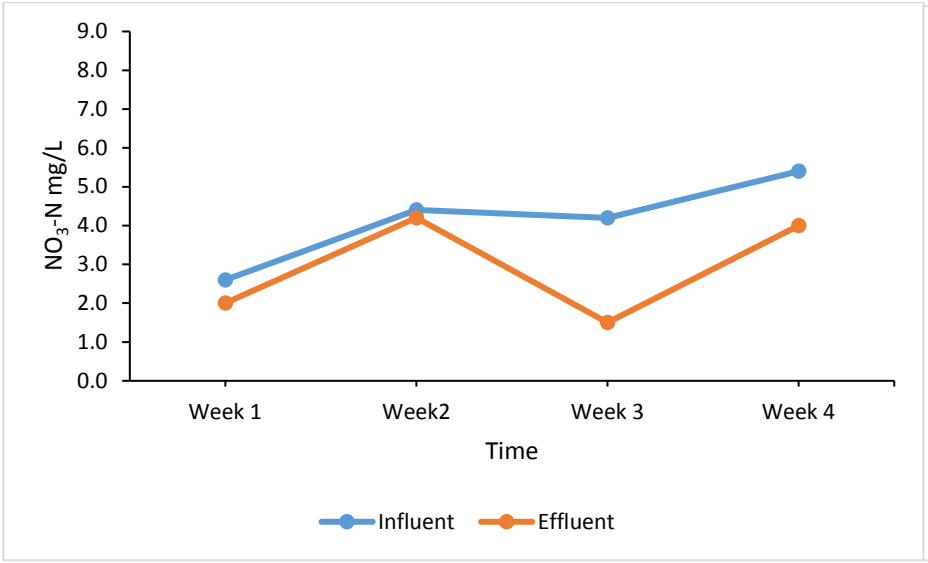
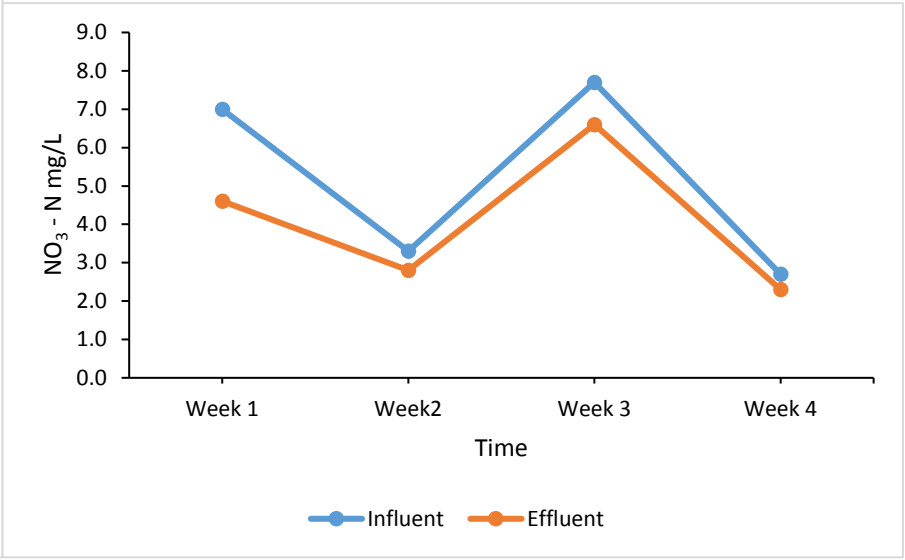


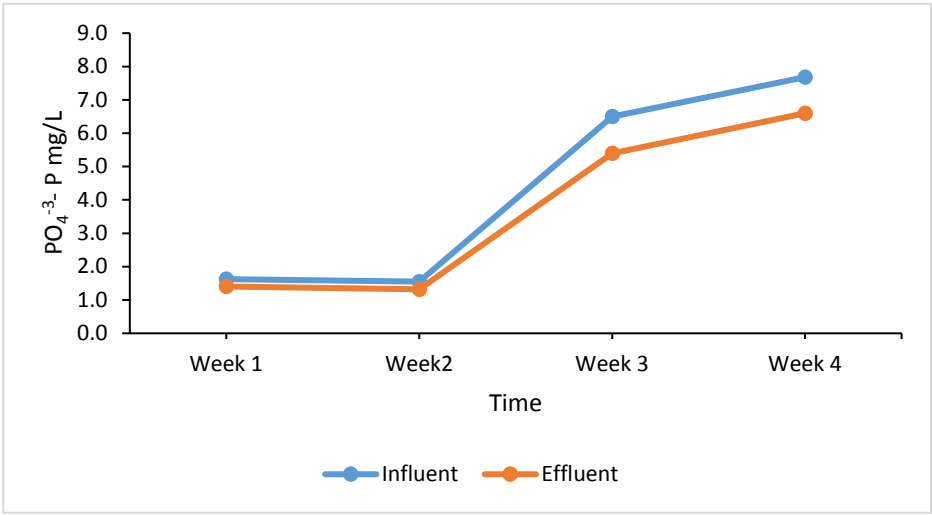


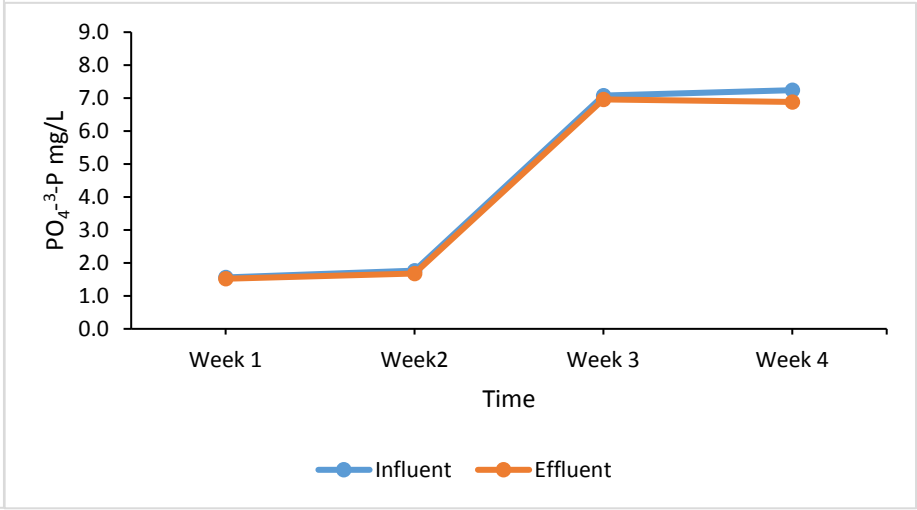


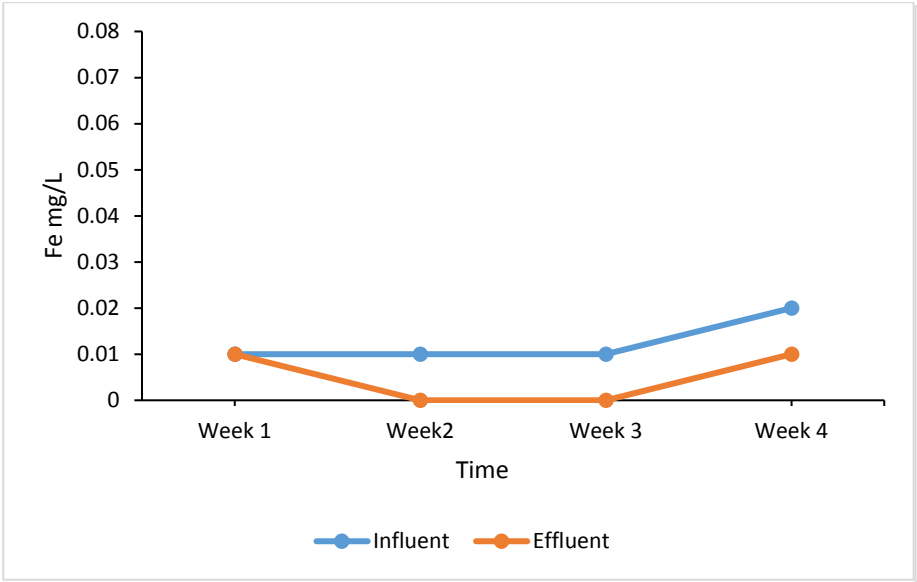


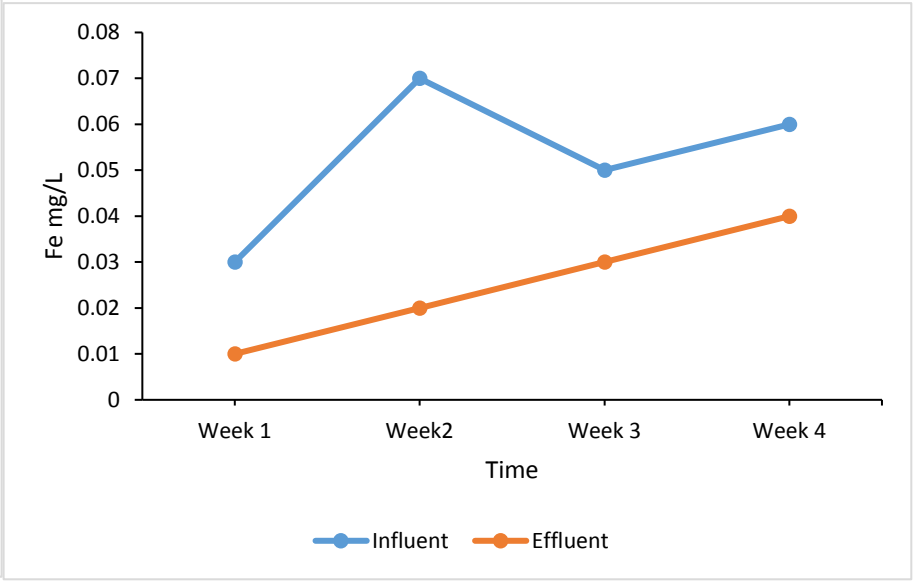


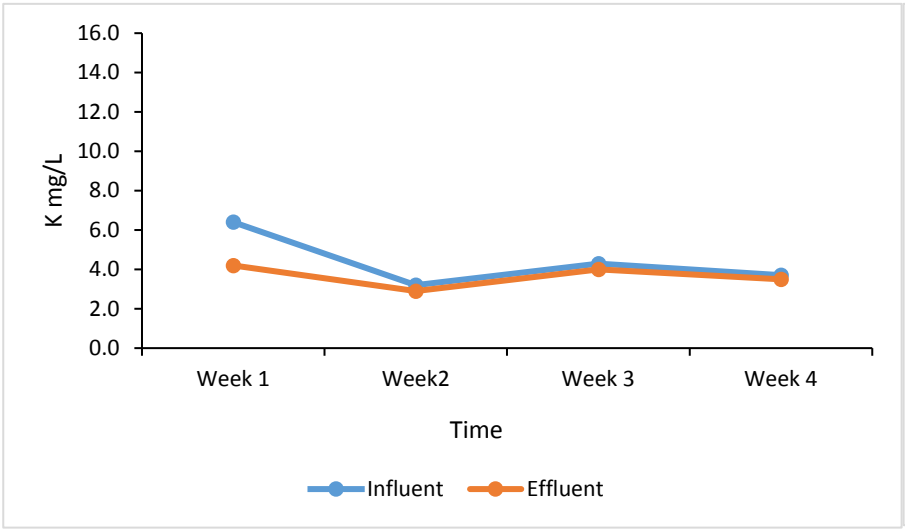


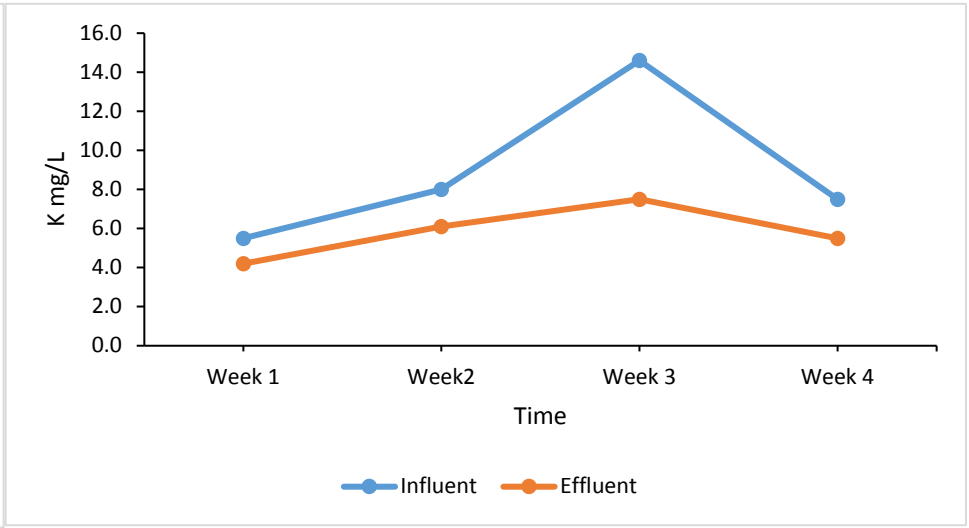












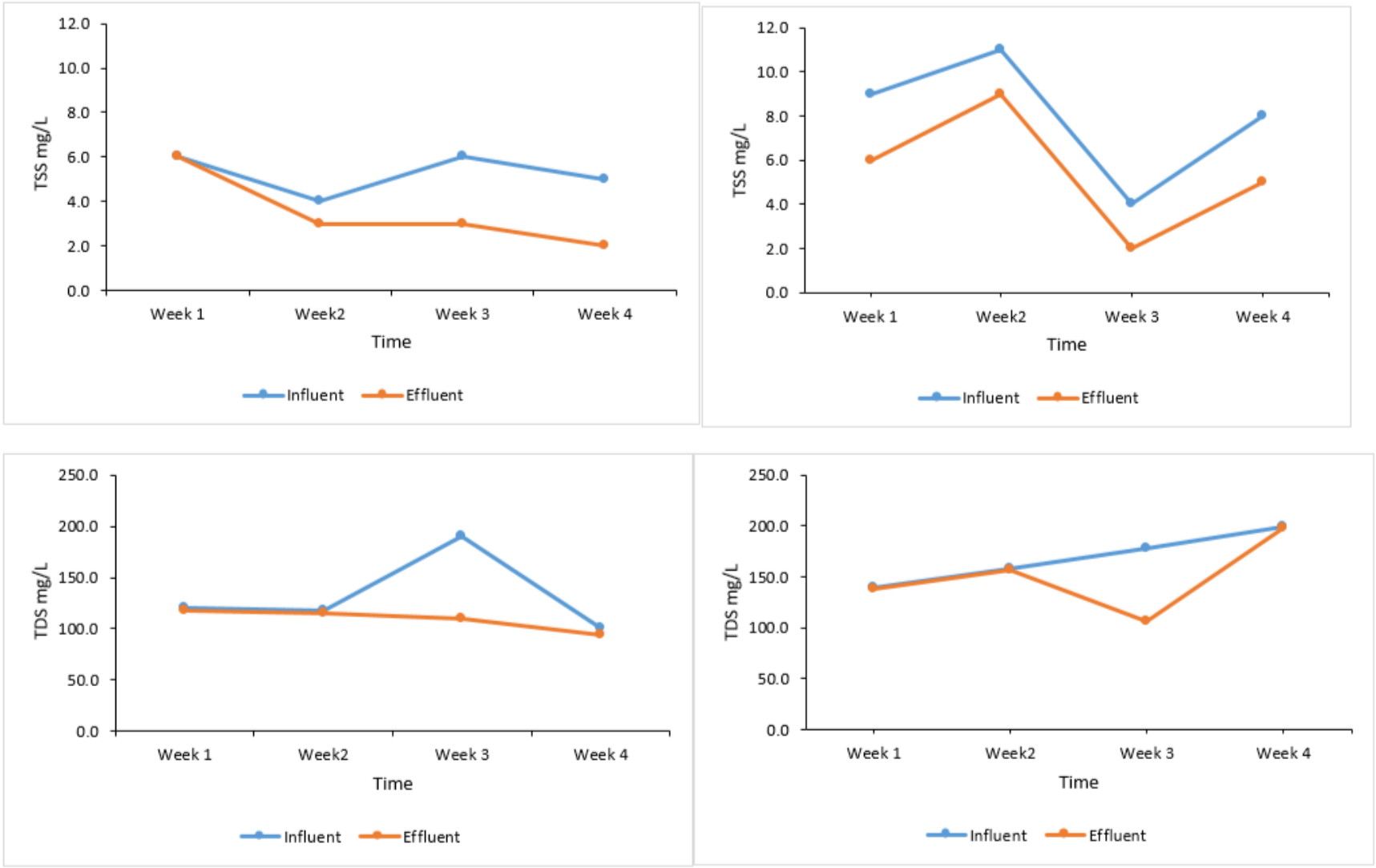
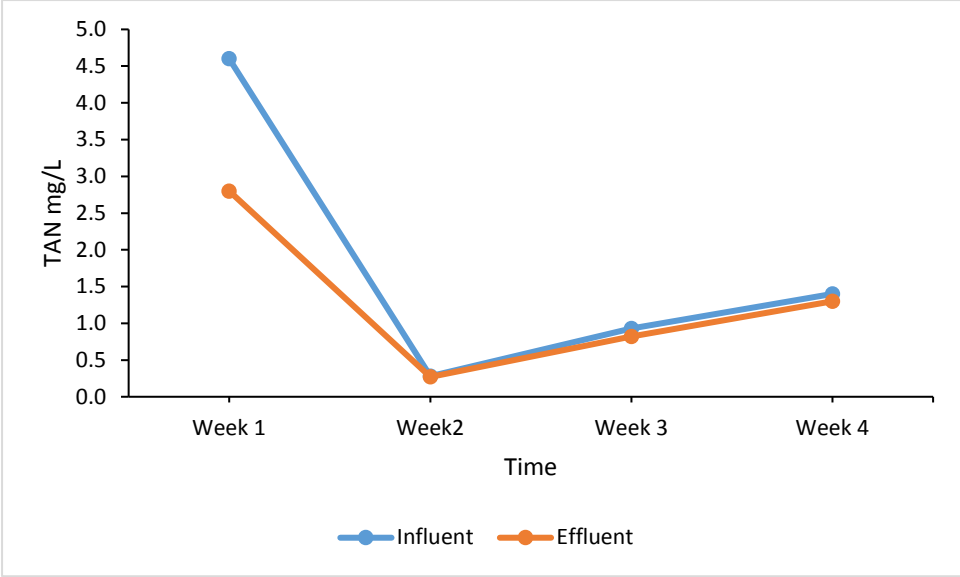
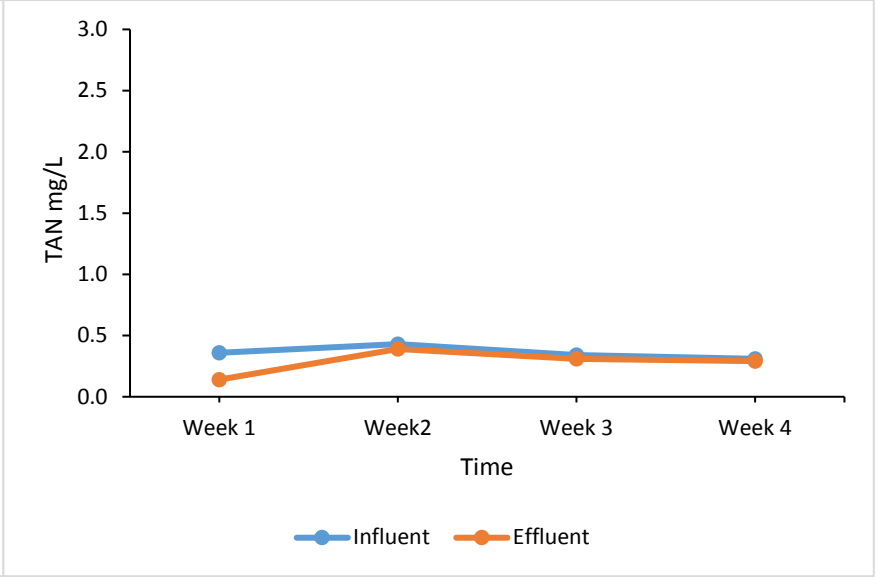
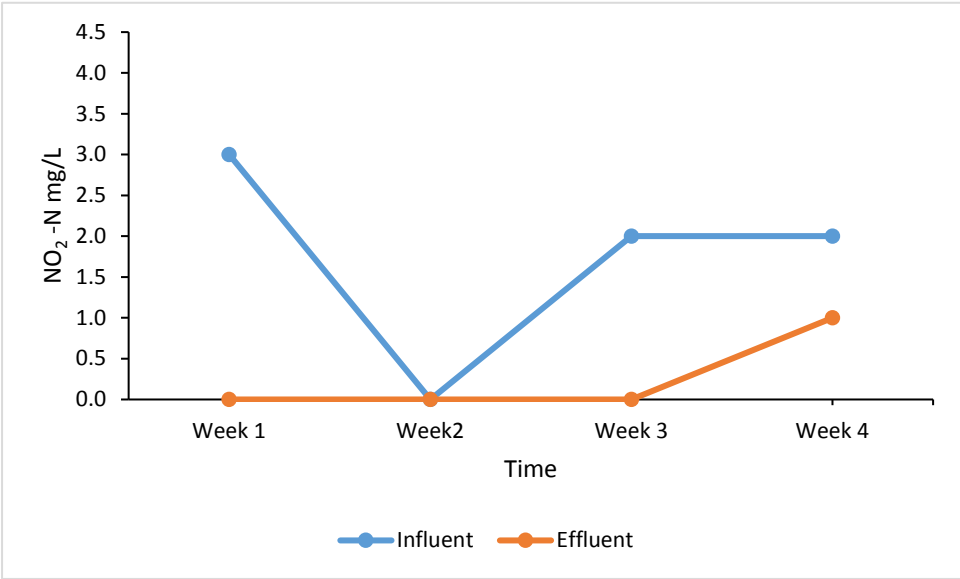
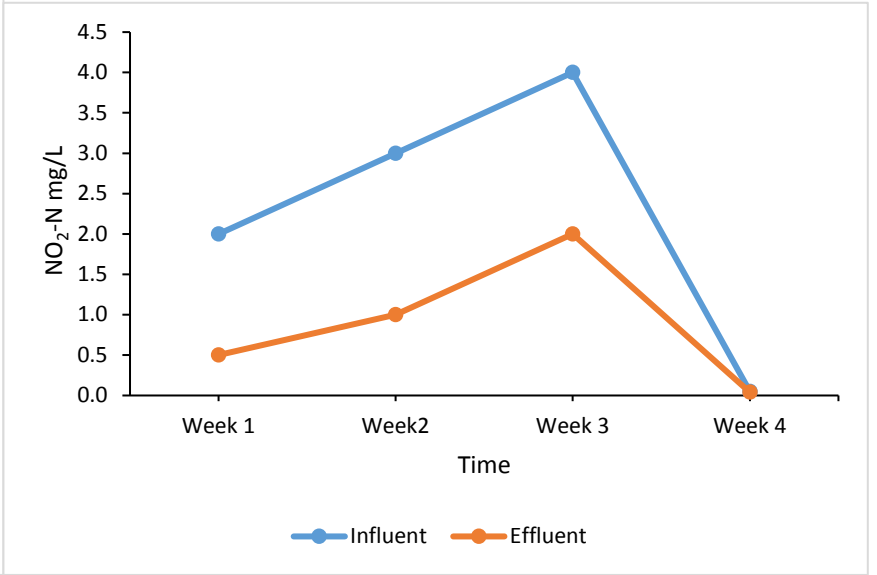


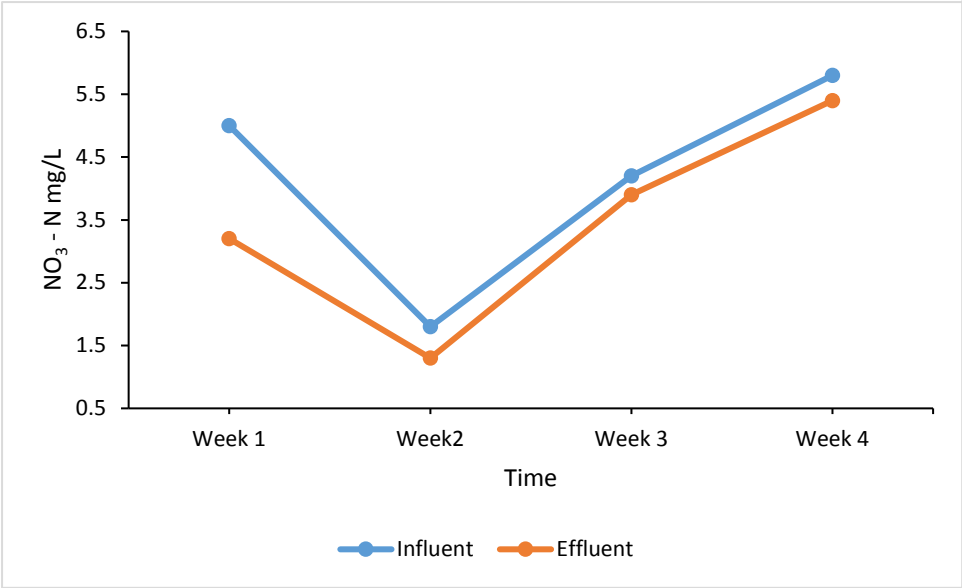
Figure 7-2 Influent and effluent in the plant growing beds in the integrated aquaponics system during the first cycle of production. Graphs on the left represent the control treatment, the right-hand side is the KDF treatment.

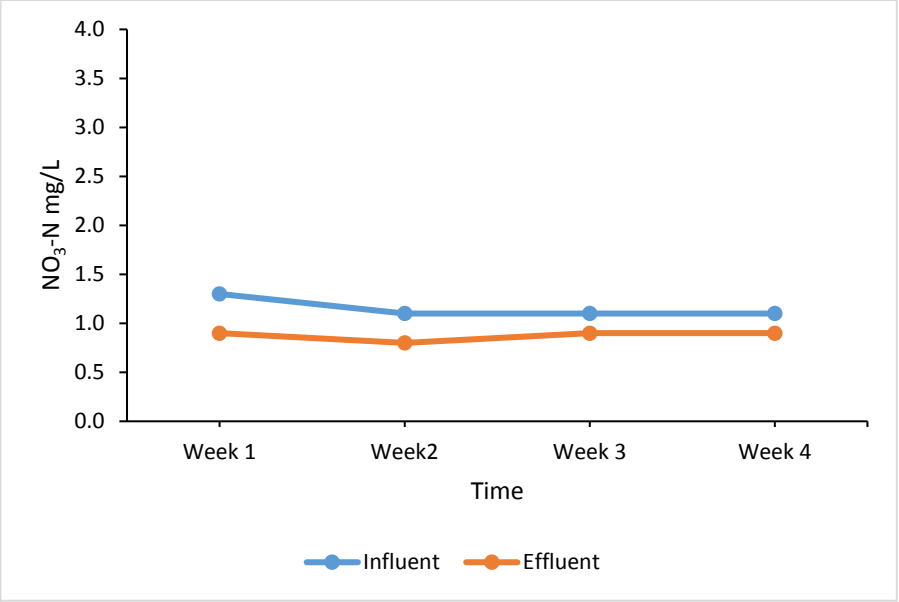


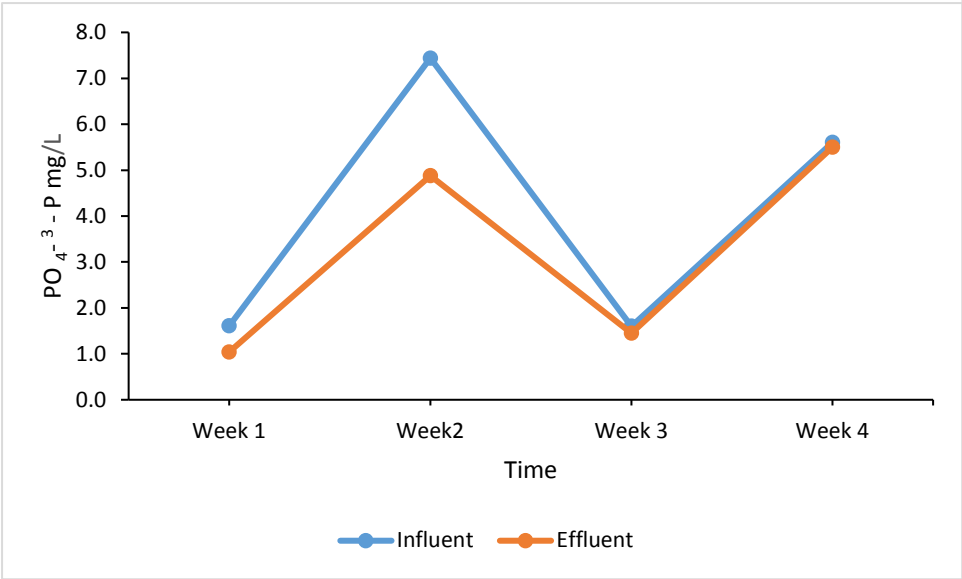


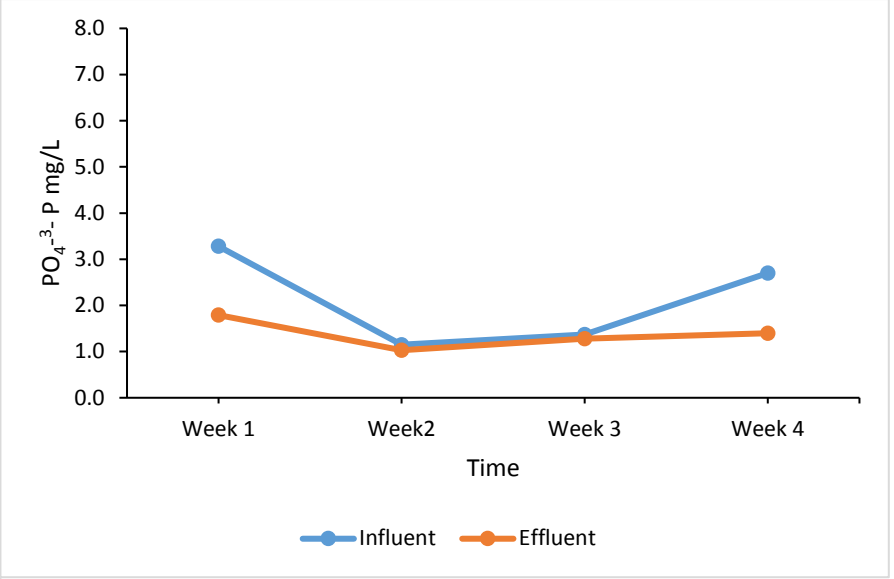


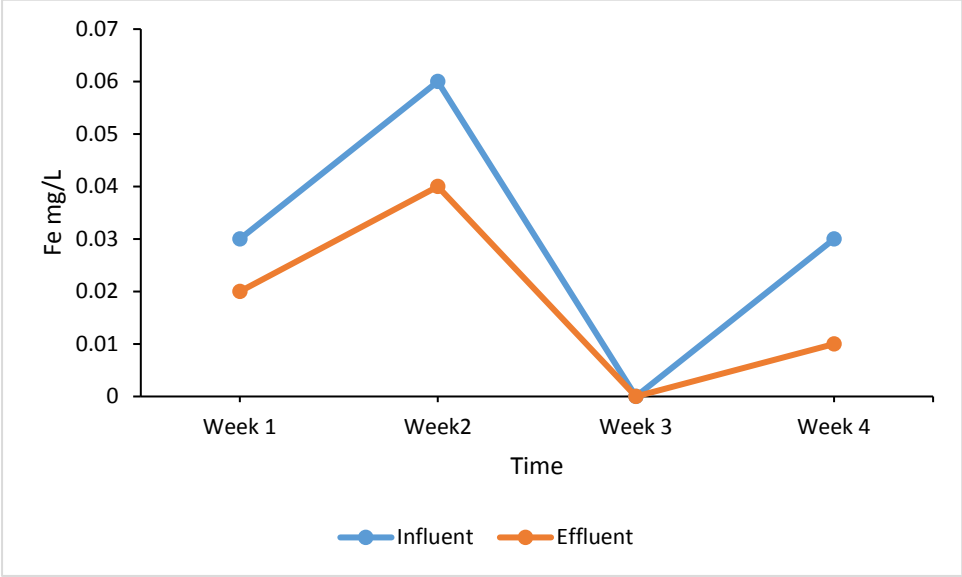


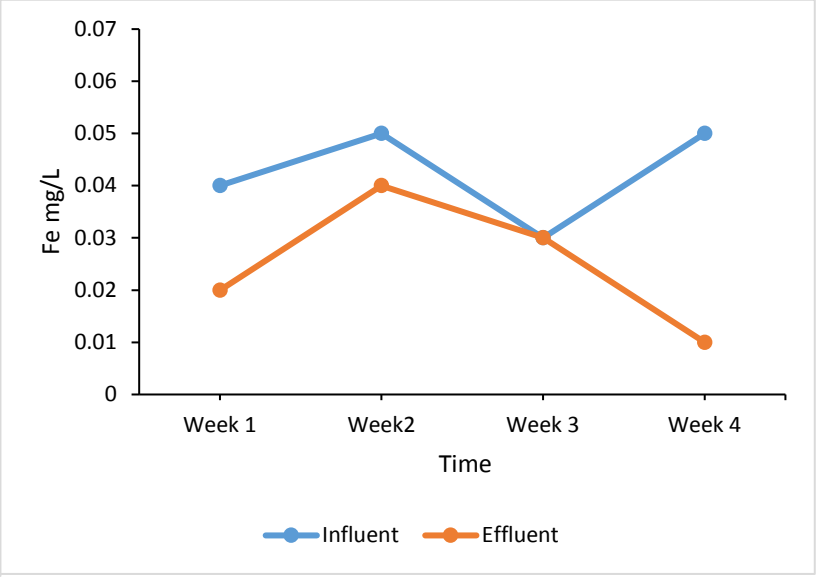


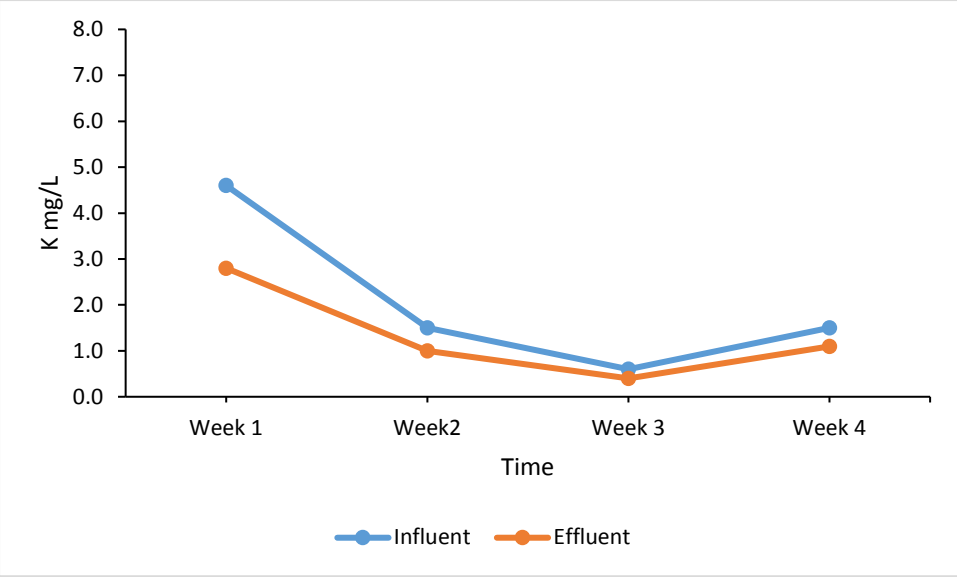


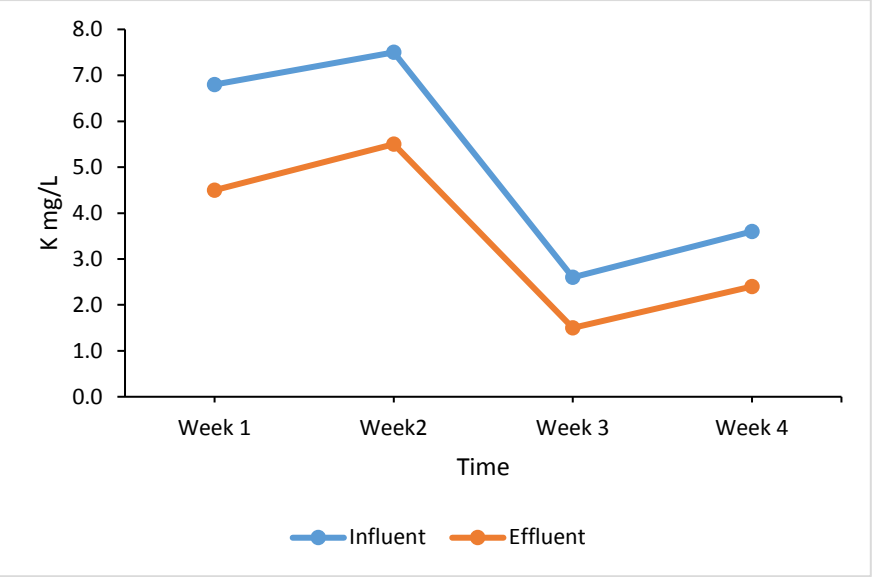












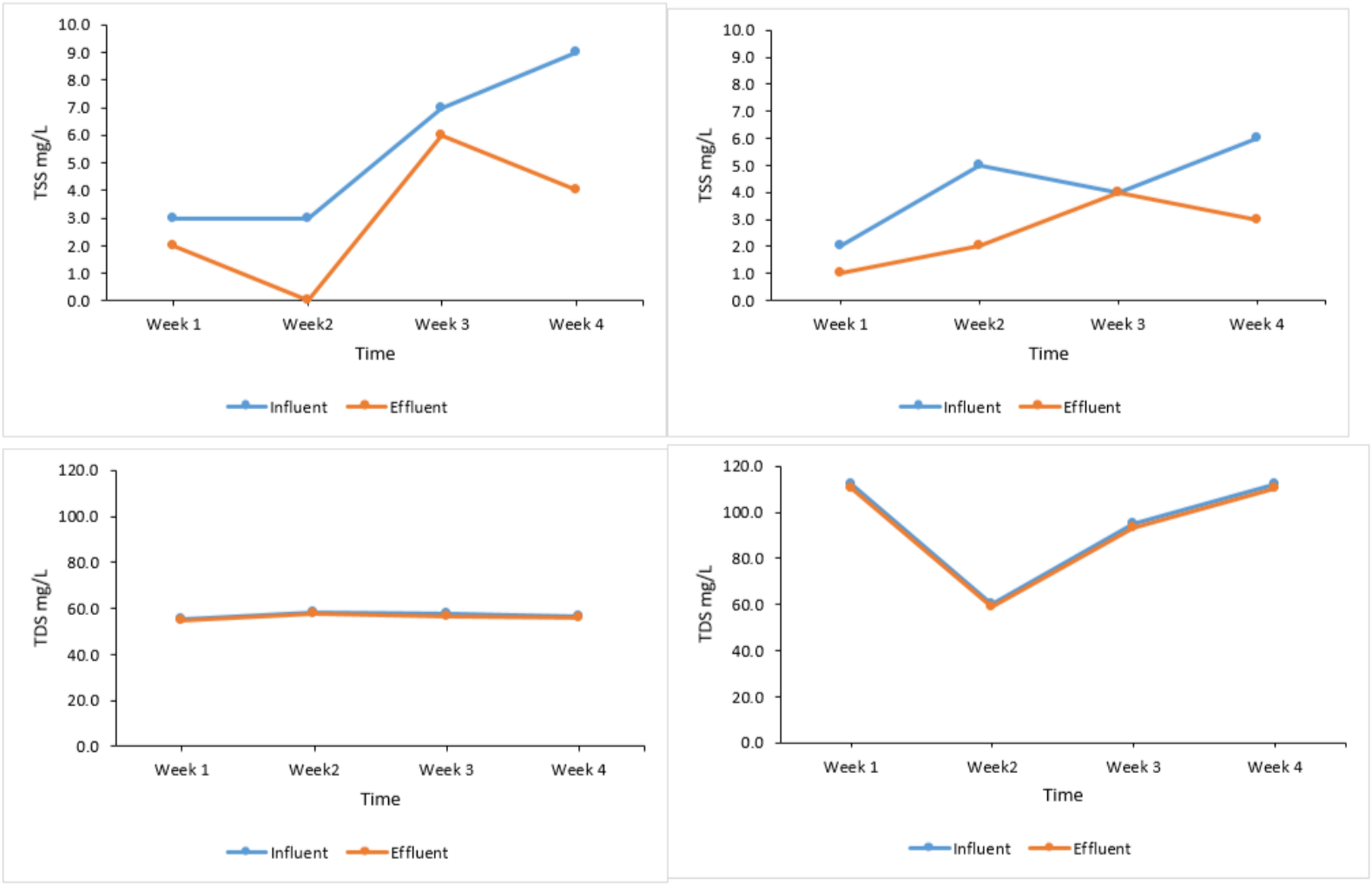
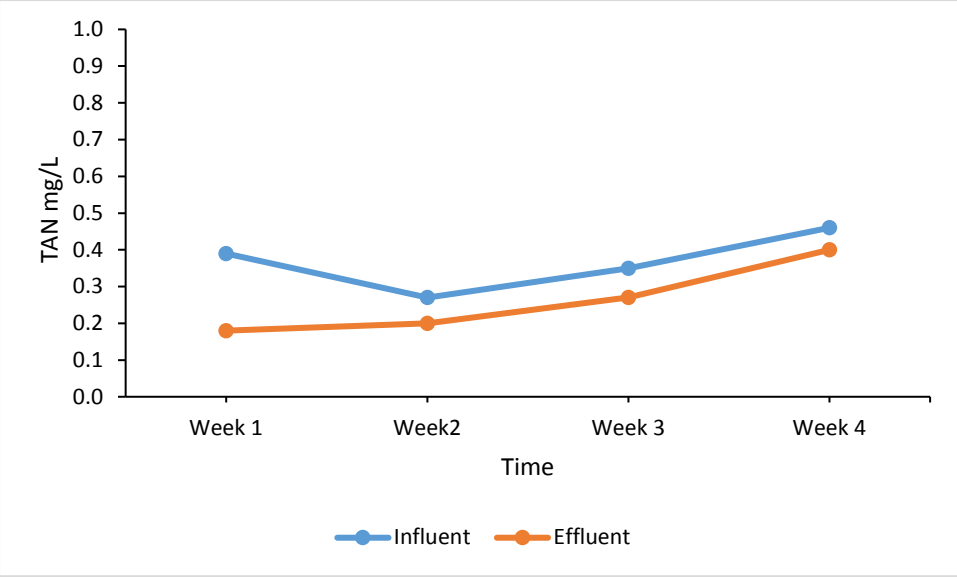
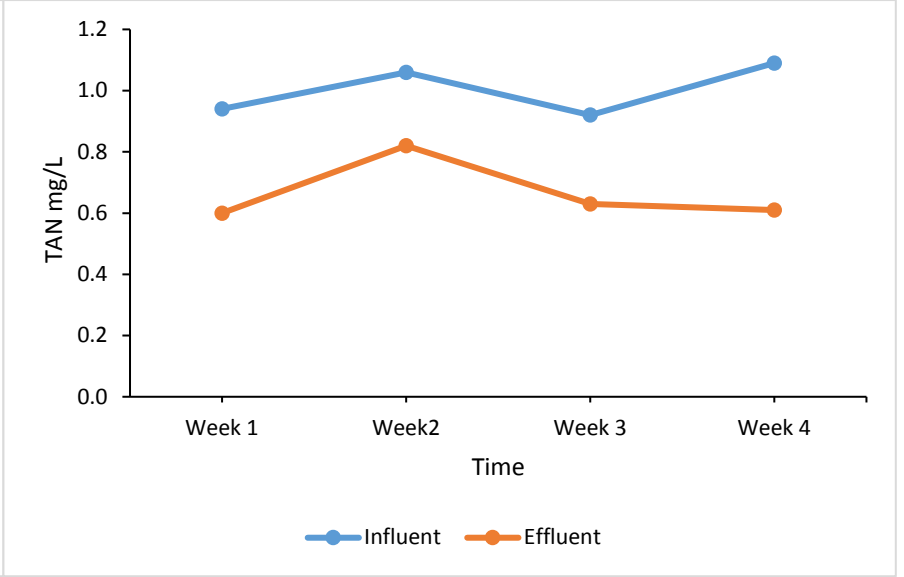
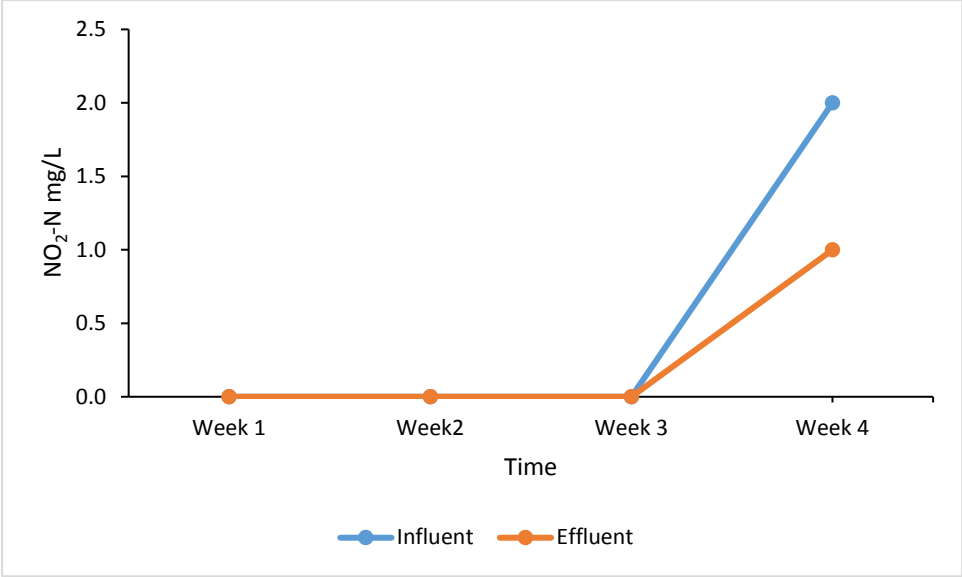
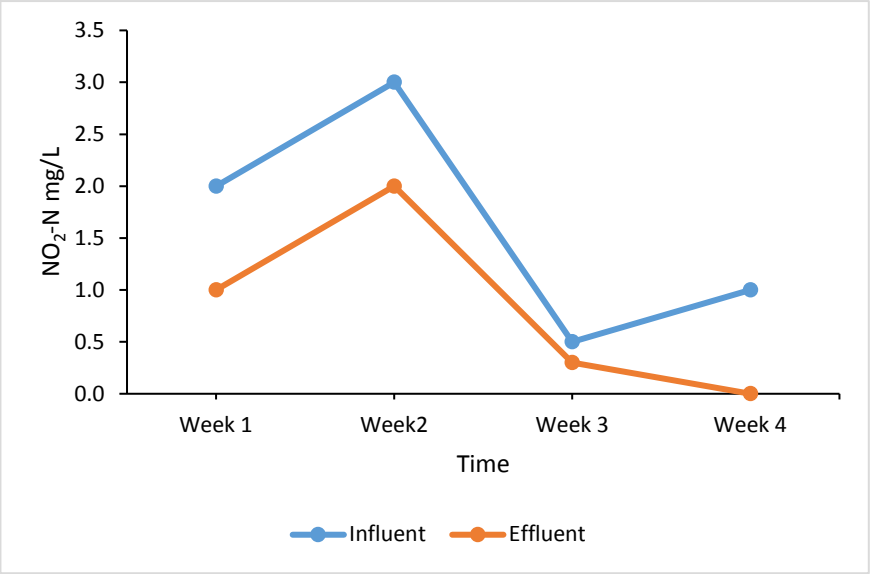


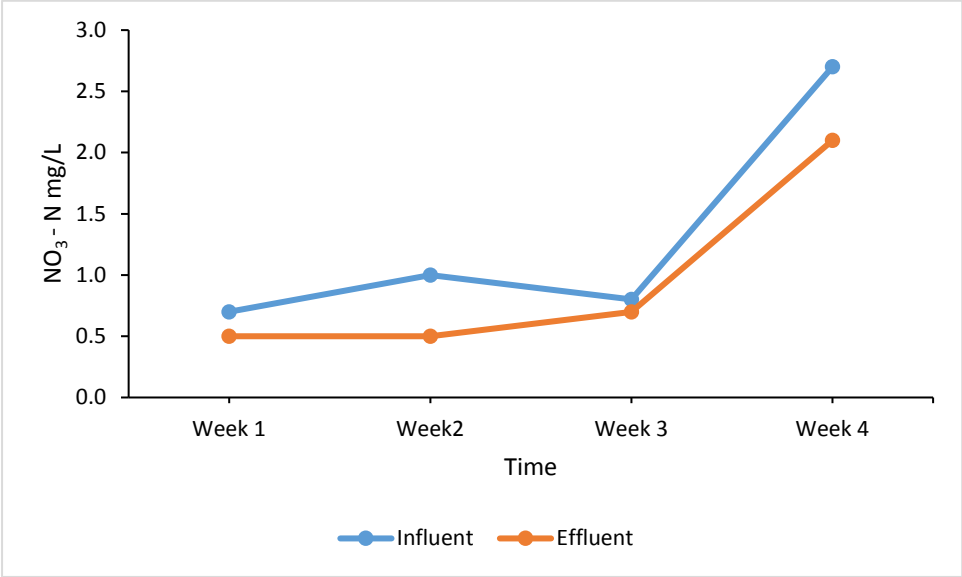
Figure 7-3 Influent and effluent in the plant growing beds in the integrated aquaponics system during the second cycle of production. Graphs on the left represent the control treatment, the right-hand side is the KDF treatment.

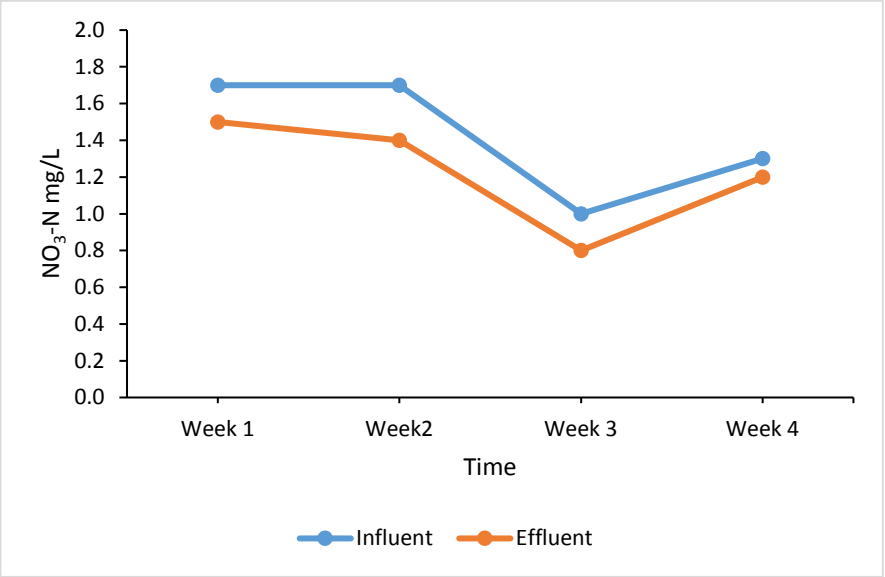


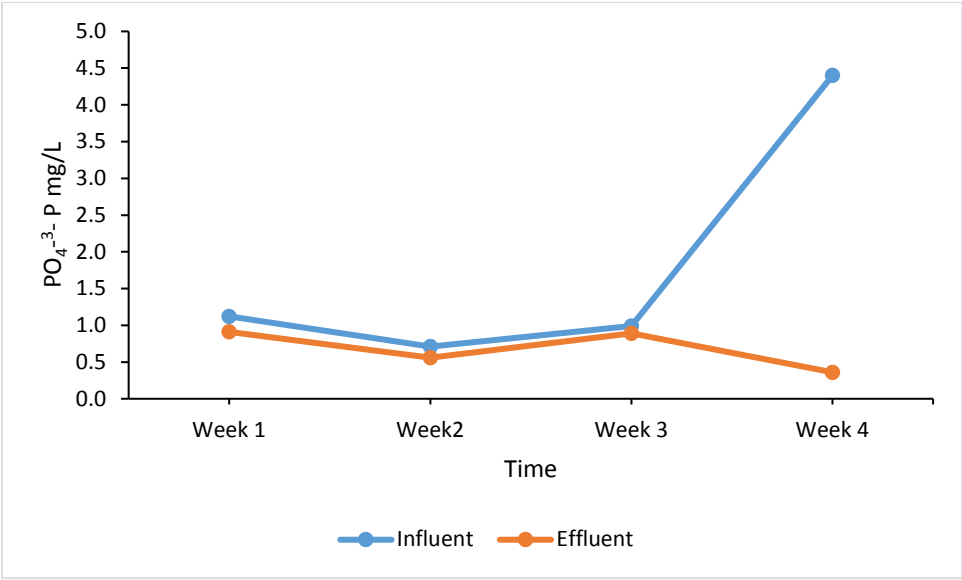


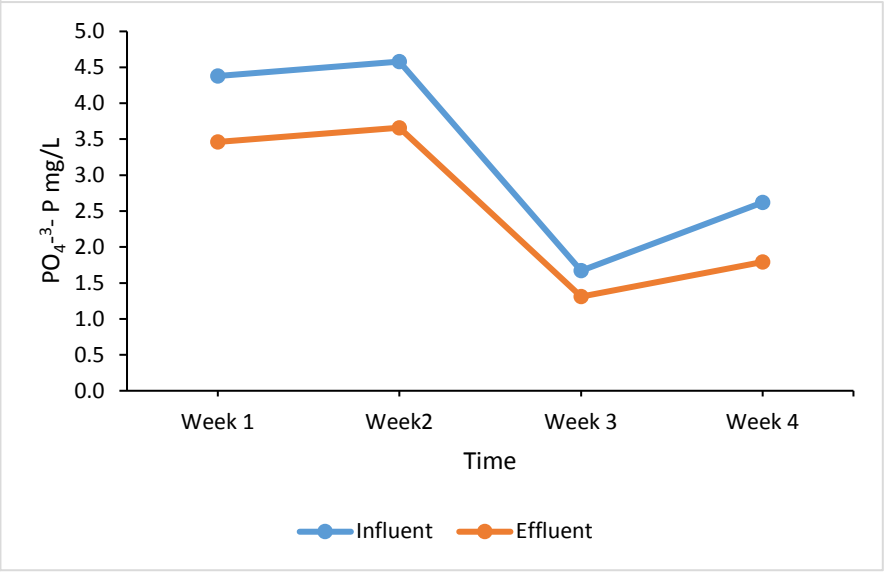


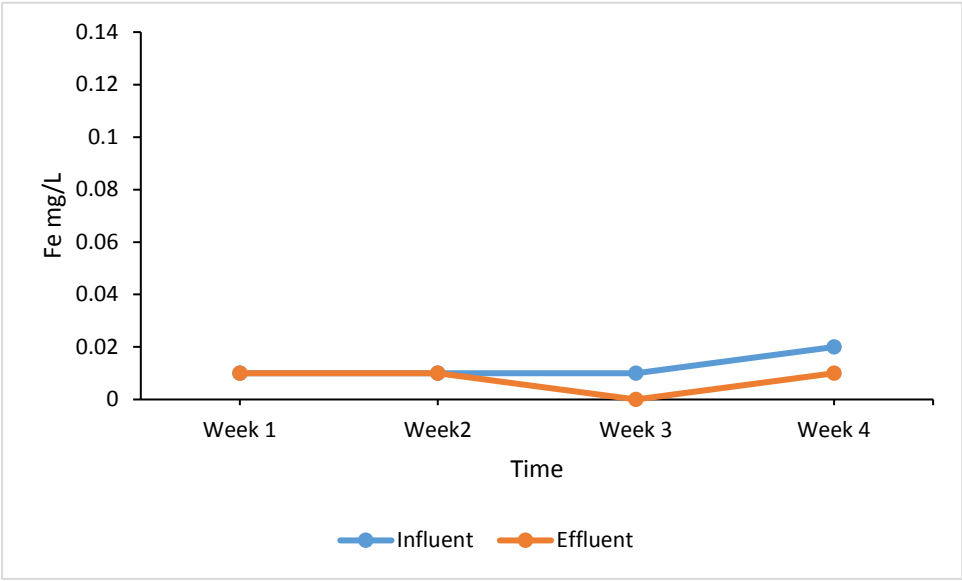


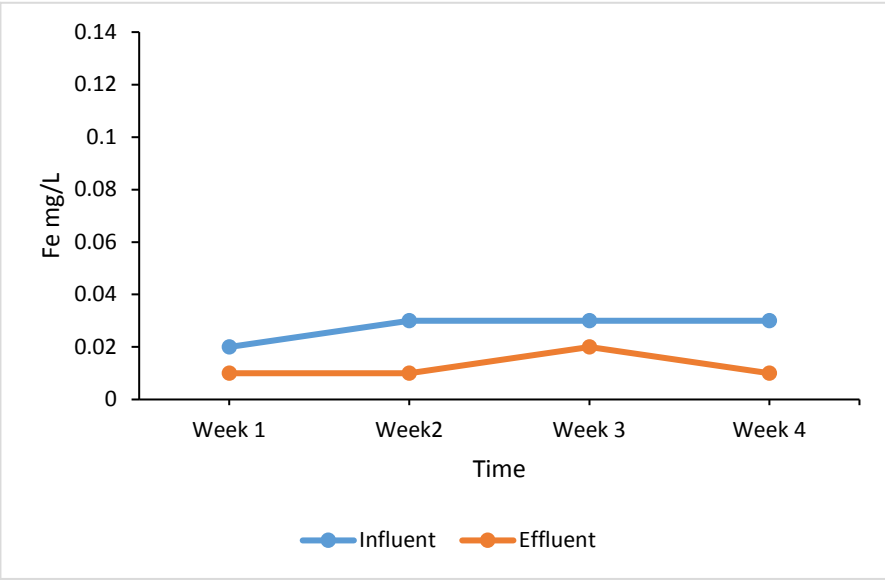


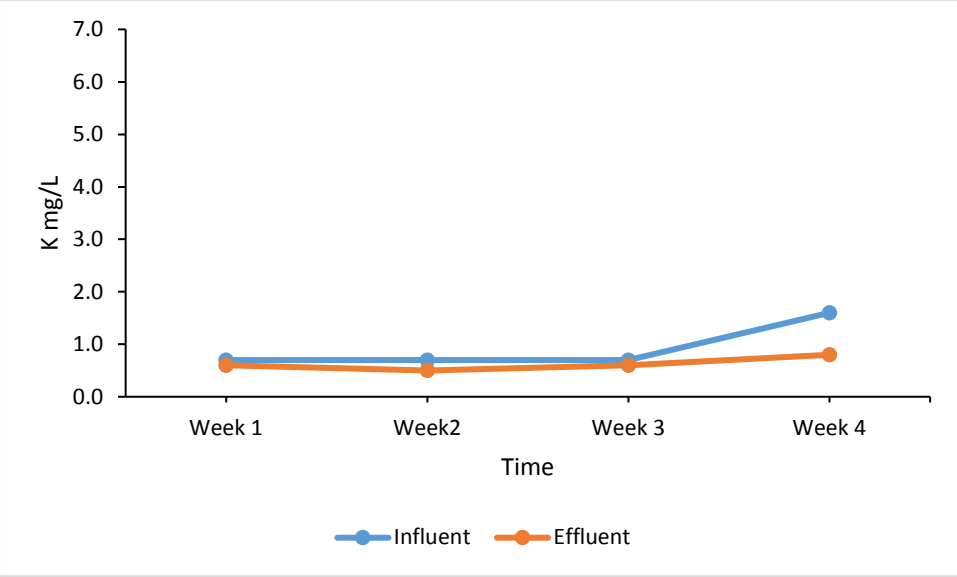


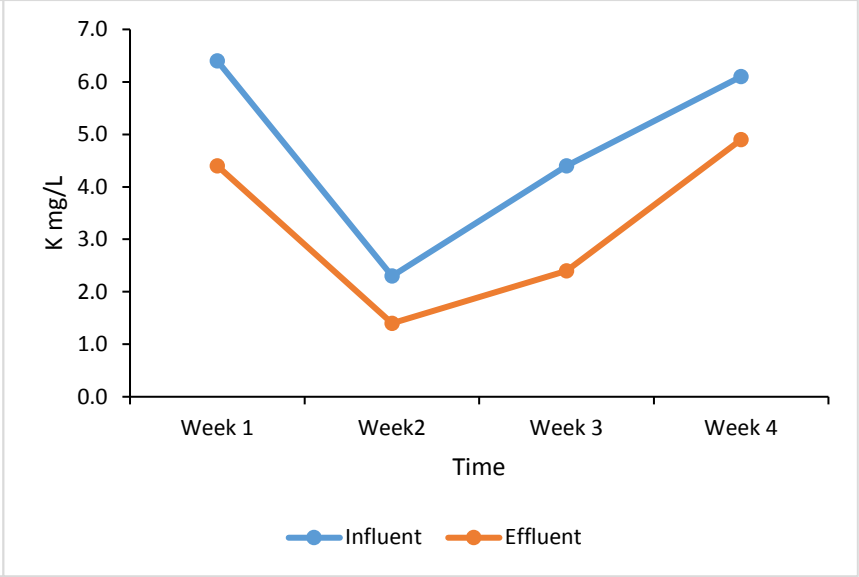












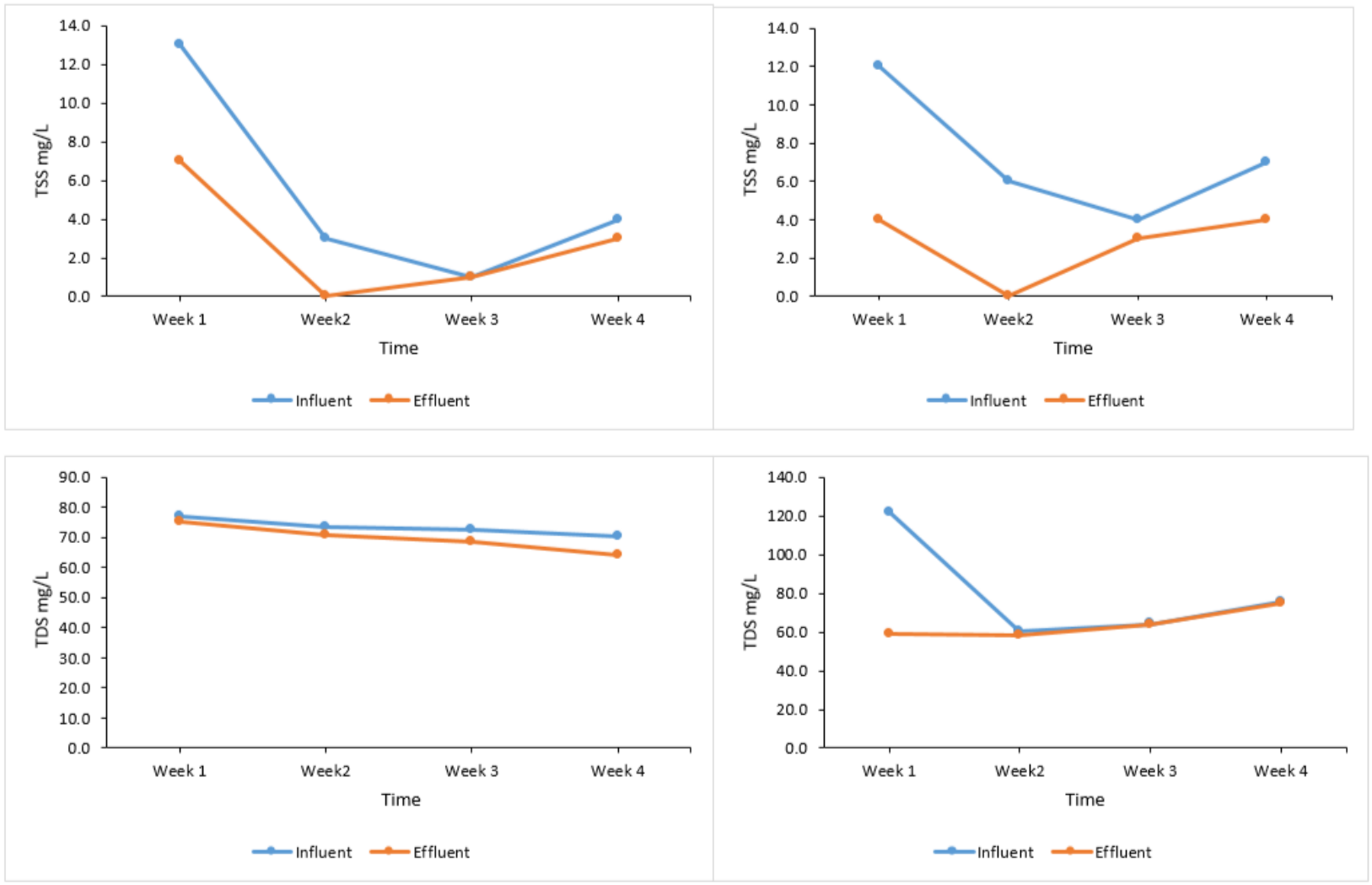


Figure 7-4 Influent and effluent in the plant growing beds in the integrated aquaponics system during the second cycle of production. Graphs on the left represent the control treatment, the right-hand side is the KDF treatment.

Author contribtions

Declaration by candidate

With regards to chapter 8: The supplementation of iron from iron sulphate (FeSO4) to the diet of the African catfish, *Clarias gariepinus* for lettuce (*Lactuca sativa*) production in an integrated aquaponics system, the nature and scope of my contribution were as follows:

Chapter	Pages	Nature of contribution	Extent of contribution (%)
8	171 - 212	Planned and designed the experiments. Formulated the fish feed, procured the ingredients and prepared the feed. Carried out the daily feeding, the sampling of fish for production performance. Weighed the lettuce before and after the experiment. Planted the lettuce, performed the proximate analysis on the lettuce and fish feed. Collected water samples and performed the analysis on the water quality trial. Prepared and analysed data. Wrote the manuscript and collated all the comments from the co-authors.	80

The following co-authors have contributed to chapter 8:

Name	Email address	Nature of contribution	Extent of contribution (%)
Neill Goosen	njgoosen@sun.ac.za	Contributed in data interpretation, proof reading and substantially improving the manuscript.	15
Khalid Salie	Ks1@sun.ac.za	Assisted with proof reading the manuscript and general discussions around the manuscript.	5

Signature of candidate……..

Date……

Declaration by co-authors

The undersigned hereby confirm that

1. The declaration above accurately reflects the nature and extent of the contribution of the candidate and the co-authors to chapter 8 in the dissertation,
2. No other author contributed to chapter 8 and

3. Potential conflict of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in chapter 8 of this dissertation.

Signature	Institutional affiliation	Date
	Department of Process Engineering, Stellenbosch University	
	Department of Animal Sciences, Stellenbosch University	

Chapter 8 The supplementation of iron from iron sulphate ($FeSO_4$) to the diet of the African catfish, *Clarias gariepinus* for lettuce (*Lactuca sativa*) production in an integrated aquaponics system

8.1 Abstract

The supplementation of plant nutrients in aquaponics systems through nutrient fertilizers is standard practice. This is made necessary by the lack of certain nutrients in the wastewater produced by normal fish feed used in aquaponics systems. The aim of this study was to design fish feed aimed at meeting both the needs of fish and plants, minimising or possibly eliminating the need to use supplemental nutrient fertilizers. This was done by evaluating the impact of supplementing ferrous sulphate to a formulated aquafeed. To meet the aims of the research, the African catfish, *Clarias gariepinus* was cultured at a density of 40 and 47 kgm^{-3} in combination with lettuce (*Lactuca sativa*) in an integrated aquaponics system, in three consecutive production cycles lasting 31 days. Fish feed supplemented with $FeSO_4$ at a level of 30 $mgkg^{-1}$ and a control treatment was used. Fish production, lettuce production, and proximate and mineral analysis of lettuce was evaluated along with water quality parameters. Fish survival was high for both treatments (90 – 100 %), while the feed conversion ratio ranged from 1.2 – 2. In the first two production cycles when the environmental conditions were favourable, the average biomass and average shoot weight were higher in the $FeSO_4$ treatment (27.1 g ± 10.1 and 22.9 g ±9.1, respectively) compared to the control treatment (17.2 g ± 5.30 and 14.3 g ± 4.75, respectively). The third production cycle displayed overall poor lettuce growth for the control (13.7 g ±4.65) and $FeSO_4$ treatment (9.84 g ± 8.48), which was due to seasonably high temperatures. The water quality measures showed that the inclusion of $FeSO_4$ in the diet of the African catfish resulted in higher concentrations of iron in the wastewater compared to the control treatment, and this translated to higher levels of iron in the lettuce grown in $FeSO_4$ treatment, and better lettuce growth compared to the control. The study showed that the inclusion of $FeSO_4$ at 30 $mgkg^{-1}$ in the diet of the African catfish improved the overall production of lettuce in the integrated aquaponics system.

8.2 Introduction

Aquaponics systems integrate aquaculture and hydroponics for an environmentally sustainable way of producing food (Goddek et al., 2015). Waste produced by fish in the form of NH_3, NO_2^-, NO_3^-, and PO_4^{3-} is absorbed by plants which act as a biofilter in the aquaponics system (Saufie et al., 2015). Aquaponics systems also offer the benefit of producing food in arid areas where soil and water are scarce (FAO, 2016). Although aquaponics is an innovative way of producing food, it faces many challenges (Goddek et al., 2015).

The imbalance of nutrients for optimum plant development is one such challenge. These nutrient imbalances are caused by the lack of important plant nutrients in the feed to the fish in the system, with the consequence that sub-optimal amounts of these essential nutrients are excreted, leading to suboptimal plant production (Somerville et al., 2014; Goddek et al., 2015). To mitigate the problem of fish feed lacking plant nutrients, fertilizers or nutrient solutions are used in aquaponics production systems to supplement plants with the deficient nutrients (Seawright et al., 1998). The nutrients that are largely supplemented in aquaponics systems include iron, calcium, and potassium (Rakocy et al., 2006).

Iron is an essential micronutrient for both plants and fish (Nenova, 2006; Lim et al., 1996) and is therefore an important nutrient in aquaponics systems. Fish require iron for cellular respiration and oxygen transport (Lim et al., 1996), and different fish species have different nutritional requirements for this nutrient. Fish mostly obtain iron from their diet as the iron in the water may not be sufficient to meet their requirement (Lim et al., 1996; Shiau and Su, 2002). Plants require iron for enzyme and chlorophyll synthesis (Hochmuth, 2011) and a deficiency of iron in plants generally results in poor growth and production (Nenova, 2006; Hochmuch, 2011).

For fish and plants to grow optimally in an aquaponics system, they need a good source of iron in sufficient amounts to prevent deficiencies while also ensuring that it is not supplied in excess. Because both plants and fish require iron, the supplementation of iron in fish feed that is used in aquaponics systems may be beneficial to both fish and plants, provided that dietary iron supplementation also leads to increased iron excretion in the water circulating in the system. In aquaponics, if additional iron supplemented to fish feed is excreted through faecal matter or metabolite waste and then transported via the water to the plant production

component in the system, it can be utilised by plants as a source of iron. This iron enrichment may decrease or eliminate the need to supplement iron through nutrient solutions.

Apart from potentially increasing the overall iron available to the plants, there is evidence that dietary iron supplementation can also have benefits to the cultured fish (Siqwepu et al., 2020b). In Siqwepu et al. (2020b), when the African catfish, *Clarias gariepinus*, was fed a diet supplemented with $FeSO_4$ at an inclusion level of 30 $mgkg^{-1}$ the animal's haematological profile was improved. The supplementation of iron in the diet of catfish further increased the iron concentration in the wastewater, showing its potential efficiency as an iron supplement for plant production in aquaponics systems. In this study, we therefore investigate the effect of $FeSO_4$ as a fish feed additive on the production of lettuce, *Lactuca sativa*, and the African catfish in an integrated aquaponics system. The combination of lettuce and the African catfish in an integrated system was evaluated by Palm et al. (2014b), whilst the African catfish has also been cultivated in combination with several other plant species successfully (Endut el at., 2010; 2011; 2012; Palm et al., 2014b). Furthermore, lettuce is a good candidate plant species for use in aquaponics, as it has been proven to remove nutrients from wastewater and has a short culture period, making it a good model species for experimental purposes (Diver, 2000; Akinbile and Yusoff, 2012; Dediu et al., 2012).

The study aims to investigate the effect of supplementing fish feed with iron from iron sulphate, $FeSO_4$, on the production, mineral, and proximate composition of lettuce. The production of the African catfish was also investigated along with the water quality parameters during three consecutive production cycles.

8.3 Material and methods

The material and methods for this chapter are identical to chapter 7 with the exception of diet preparation, where $FeSO_4$ was included. Table 8.1 represents the feed formulation and proximate composition of the experimental diet and control.

Table 8.1 Feed formulation and proximate composition of feed of experimental diets fed to *C. gariepinus* (gkg^{-1}).

Ingredients (gkg^{-1})	Treatment	
	Control	$FeSO_4$
Fish meal	120	120
Soya	570	570
Maize	200	200
Cellulose	15	15
Vit/Min premix[a]	15	15
MDCP[b]	20	20
Fish oil	30	30
Sunflower oil	30	30
$FeSO_4$		0.03
Proximate composition (gkg^{-1})		
Moisture	77	71
Ash	121	112
Crude Lipids	86	86
Crude Protein	370	360
Crude Fibre	44	45
Carbohydrates[d]	346	371

[a]Vit/Min premix -Vitamins: Vitamin A, 12 500 000 IU; Vitamin D3,2 500 000 IU; Vitamin E, 150 000; Vitamin K3, 8g; Vitamin B1, 15g; Vitamin B2, 20g; Vitamin B6,15g; Vitamin B12,0.035g; Niacin, 80g Cal Pnth, 50g; Folic Acid, 2.50g; Biotin, 0.350g; Iodine, 2.50g; Cobalt, 0.55g; Selenium, 0.25g; Vitamin C (Stay 35), 300g.
Minerals: Manganese, 60g; Zinc, 60g; Copper, 6g; Choline, 1000g.
[b]MDCP: Monodicalcium phosphate. [c]KDF: Potassium diformate.
[d]Determined by difference as: 1000 – Moisture - Crude Protein - Crude Lipids – Ash.

8.4 Results

8.4.1 Fish production

The fish production performance during the three production cycles is presented in Table 8.2. The FCR on the first production cycle was 1.9 and 1.2 for the control and $FeSO_4$ treatment, respectively. On the second and third cycle, the FCR for the control was 1.6 and 2 and for the $FeSO_4$ treatment was 1.6 and 1.4, respectively. The specific growth rate (SGR) ranged from 0.49 – 0.78 % during the three production cycles. Survival was high ranging from 90 – 100 %.

8.4.2 Plant production parameters

The production of lettuce over the three production cycles is presented in Table 8.3. During the first production cycle the average biomass and average shoot weight was higher for the $FeSO_4$ treatment (27.1 g ± 10.1 and 22.9 g ± 9.1) compared to the control (17.2 g ± 5.30 and 14.3 g ± 4.75). During the second production cycle, the number of plants surviving until harvest was higher for the $FeSO_4$ treatment, along with the average biomass (28.7 g ± 10.9) and average shoot weight (24.9 g ± 10.2). During the last production cycle, the average biomass and average shoot weight were low for both the control and the $FeSO_4$ treatment, which was attributed to seasonally high environmental temperatures. The average daily maximum temperature was 29° C during the last production cycle, whereas optimum growth conditions for lettuce requires that maximum environmental temperature remain below 24° C (Maboko and Du Plooy, 2007).

Table 8.2 Fish production parameters of the African catfish, *C. gariepinus* fed the control diet and $FeSO_4$ treatment.

Production cycle	Parameters	Control	$FeSO_4$
First (14 Nov - 15 Dec)	Initial Weight (kg)	40.09	40
	Final Weight (kg)	46.7	51
	Weight Gain (kg)	6.61	11
	Survival (%)	90	100
	FCR	1.9	1.2
	SGR (%)	0.49	0.78
	Number of fish	80	80
Second (29 Dec - 29 Jan)	Initial Weight (kg)	40	40
	Final Weight (kg)	46.7	46.9
	Weight Gain (kg)	7.6	7.9
	Survival %	93	97
	FCR	1.6	1.6
	SGR (%)	0.57	0.59
	Number of fish	60	60
Third (04 Feb - 06 March)	Initial Weight (kg)	47	47
	Final Weight (kg)	54.4	58
	Weight Gain (kg)	7.7	11.1
	Survival %	95	91
	FCR	2	1.4
	SGR (%)	0.49	0.69
	Number of fish	56	58

No replicates were available, thus no SE is shown.

Table 8.3 Plant production and yield from November 2018 to March 2019.

Production cycle	Production parameters	Control	$FeSO_4$
First (14 Nov – 15 Dec)	Total No. of plants	340	340
	No of live plants	320	257
	No. of plants harvested	320	257
	Average biomass (fresh) g	17.2±5.30	27.1±10.1
	Average shoot weight(g)	14.3±4.75	22.9±9.1
	Root (fresh) g	2.85±0.94	4.21±1.45
	Root:Shoot	0.20	0.18
	Initial plant weight (g)	5.25±0.90	5.12±0.89
Second (29 Dec – 29 Jan)	Total No. of plants	340	340
	No of live plants	290	300
	No. of plants harvested	290	300
	Average biomass (fresh) g	14.5±5.48	28.7±10.9
	Average shoot weight(g)	10.7±4.86	24.9±10.2
	Root (fresh) g	3.80±1.11	3.60±1.08
	Root:Shoot	0.36	0.14
	Initial plant weight (g)	5.2±0.89	5.3±0.91
Third (04 Feb – 06 March)	Total No. of plants	340	340
	No of live plants	340	335
	No. of plants harvested	340	335
	Average biomass (fresh) g	13.7±4.65	9.84±8.48
	Average shoot weight(g)	12.4±4.57	8.41±3.81
	Root (fresh) g	1.28±1.21	1.43±1.51
	Root:Shoot	0.10	0.17
	Initial plant weight (g)	5.15±0.88	5.18±0.89

Data presented as average ± SD.

8.4.3 Proximate and mineral composition of lettuce leaves

On the first production cycle, the proximate composition of harvested lettuce differed significantly between the control treatment and $FeSO_4$ treatment ($p< 0.05$), except for the moisture content. The ash and crude protein were significantly higher on the $FeSO_4$ treatment compared to the control. On the second production cycle, the proximate composition differed significantly between the two treatments ($p < 0.05$), the control treatment had a higher moisture (9.7 % ±0.09) and crude fibre content (12.6 % ±0.22). During the last production cycle, the proximate composition differed between the two treatments except for crude protein (Table 8.4).

The mineral composition of the lettuce leaves is presented in Table 8.5. The mineral content in the lettuce leaves differed significantly between the treatments on the first production cycle; the NH_4-N, PO_4^{3-} -P, Fe, and Mn were significantly higher in the $FeSO_4$ treatment compared to the control treatment. On the second production cycle, the NH_4-N, PO_4^{3-} -P, K were significantly higher than the control treatment. During the last production cycle, NH_4-N, Fe and Mn were similar ($p > 0.05$).

Table 8.4 Proximate composition of the plants from November 2018 to March 2019.

Production cycle	Proximate composition (%)	Control	$FeSO_4$
First (14 Nov - 15 Dec)	Moisture	9.5±0.16[a]	9.1±0.01[a]
	Ash	13.2±0.04[a]	14.4±0.03[b]
	Crude Fat	4.8±0.08[a]	4.0±0.00[b]
	Crude Protein	21.2±0.22[a]	25.4±0.09[b]
	Crude Fibre	12.8±0.09[a]	11.5±0.11[b]
Second (29 Dec - 29 Jan)	Moisture	9.7±0.09[a]	7.8±0.04[b]
	Ash	11.4±0.00[a]	23.1±0.02[b]
	Crude Fat	5.5±0.19[a]	4.8±0.00[b]
	Crude Protein	18.1±0.13[a]	19.8±0.15[b]
	Crude Fibre	12.6±0.22[a]	11.6±0.15[b]
Third (04 Feb - 06 March)	Moisture	6.6±0.03[a]	9.9±0.08[b]
	Ash	23.7±0.04[a]	13.1±0.06[b]
	Crude Fat	4.4±0.07[a]	5.0±0.07[b]
	Crude Protein	19.6±0.17[a]	19.9±0.39[a]
	Crude Fibre	11.1±0.10[a]	13.9±0.29[b]

Data is presented as mean ±SE. Differen t superscripts in the same row indicate significant differences, $p > 0.05$.

Table 8.5 Plant mineral composition of lettuce from November 2018 to March 2019

Production cycle	Nutrient (gkg^{-1})	Control	$FeSO_4$
First (14 Nov - 15 Dec)	NH_4-N	37.85±1.95[a]	46.95±1.95[b]
	PO_4^{3-}-P	5.15±0.05 [a]	6.0±0.05[b]
	K	46.55±0.81[a]	45.7±0.81[a]
	Fe	0.18±0.00[a]	0.23±0.00[b]
	Mn	0.28±0.01 [a]	1.20±0.01[b]
Second (29 Dec - 29 Jan)	NH_4-N	31.95±0.65[a]	35.2±0.65[b]
	PO_4^{3-}-P	5.0±0.03[a]	5.4±0.03[b]
	K	37.1±0.32[a]	47.1±0.32[b]
	Fe	0.22±0.01[a]	0.29±0.01[b]
	Mn	0.40±0.00[a]	0.39±0.00[b]
Third (04 Feb - 06 March)	NH_4-N	32.8±2.22[a]	33.4±2.22[a]
	PO_4^{3-}-P	5.45±0.03[a]	5.2±0.03[b]
	K	45±0.73[a]	37.9±0.73[b]
	Fe	0.16±0.01[a]	0.14±0.01[a]
	Mn	0.33±0.00[a]	0.33±0.00[a]

Data is presented as mean ±SE. Differen t superscripts in the same row indicate significant differences, $p > 0.05$.

8.4.4 Water quality

The measured water quality parameters and the percentage removal efficiency during the trial period are presented in Tables 8.6 – 8.11. During the first production cycle, the TAN in the control treatment ranged from 0.31 – 0.88 mgl^{-1} while in the $FeSO_4$ treatment it ranged from 0.43 – 3.28 mgl^{-1}. The $FeSO_4$ treatment had an iron concentration of 0.03 – 0.11 mgl^{-1} while the control had 0.00 – 0.02 mgl^{-1}. During the second production cycle, the control treatment had a TAN concentration ranging from 0.27 – 4.6 mgl^{-1} compared to the $FeSO_4$ treatment which had a range of 0.36 – 1.76 mgl^{-1}. The iron concentration was higher in the $FeSO_4$ treatment (0.00 – 0.15 mgl^{-1}) than the control treatment (0.00 – 0.06 mgl^{-1}). In the first and second production cycle, TDS was higher in the $FeSO_4$ treatment (156.9 – 223 and 51.20 – 85.35 mgl^{-1}) than the control (94.1 – 189.8 and 54.9 – 58.4 mgl^{-1}). During the last production cycle, the iron levels were higher in the control treatment (0.07 – 0.12 mgl^{-1}) than the $FeSO_4$ treatment (0.00 – 0.02 mgl^{-1}).

Table 8.6 Water quality parameters of the control diet for the first production cycle (mgl^{-1}) (14 November – 15 December)

Week		Water quality parameters							
		TAN	NO_2-N	NO_3-N	PO_4^{3-} P	Fe	K	TSS	TDS
Week 1	Influent	0.33	3	2.60	1.62	0.01	6.40	6	120.5
	Effluent	0.31	2	2	1.40	0.01	4.20	6	118.2
	Removal efficiency (%)	6	33	23	14	0	34	0	2
Week 2	Influent	0.88	2	4.40	1.55	0.01	3.20	4	117.1
	Effluent	0.72	1.50	4.20	1.32	0.00	2.90	3	115.1
	Removal efficiency (%)	18	25	5	15	100	9	25	2
Week 3	Influent	1.28	2	4.20	6.52	0.01	4.30	6	189.8
	Effluent	0.60	1	1.50	5.40	0.00	4	3	109.4
	Removal efficiency (%)	53	50	64	17	100	7	50	42
Week 4	Influent	0.51	4	5.40	7.68	0.02	3.70	5	100.2
	Effluent	0.32	3	4	6.60	0.01	3.50	2	94.1
	Removal efficiency (%)	37	25	26	14	50	5	60	6

Table 8.7 Water quality parameters of the $FeSO_4$ diet for the first production cycle (mgl^{-1}) (14 November – 15 December)

Week		Water quality parameters							
		TAN	NO_2-N	NO_3-N	PO_4^{-3-} P	Fe	K	TSS	TDS
Week 1	Influent	0.63	5	4	1.48	0.09	2	4	158.2
	Effluent	0.43	4	3	1.38	0.03	1.8	2	156.9
	Removal efficiency (%)	32	20	25	7	67	10	50	1
Week 2	Influent	3.28	3	3.1	1.55	0.11	4.2	8	172.8
	Effluent	2.24	0	2.4	1.39	0.05	4	6	171.3
	Removal efficiency (%)	32	100	23	10	55	5	25	1
Week 3	Influent	1.82	4	7	7.64	0.09	7.5	6	192.2
	Effluent	1.68	3	6	6.32	0.05	7.1	4	177.9
	Removal efficiency (%)	8	25	14	17	44	5	33	6
Week 4	Influent	1.14	3	3.1	6.76	0.07	5.9	10	223
	Effluent	1.10	2	2.8	6.08	0.03	5.5	4	220.5
	Removal efficiency (%)	4	33	10	10	57	7	60	1

Table 8.8 Water quality parameters of the control diet for the second production cycle (mgl^{-1}) (29 December – 29 January)

Week		Water quality parameters							
		TAN	NO_2-N	NO_3-N	PO_4^{3-} P	Fe	K	TSS	TDS
Week 1	Influent	4.6	3	5	1.61	0.03	2.80	3	55.6
	Effluent	2.8	0	3.20	1.04	0.02	4.60	2	54.9
	Removal efficiency (%)	39	100	36	35	33	39	33	1
Week 2	Influent	0.28	0	1.80	7.44	0.06	1.50	3	58.4
	Effluent	0.27	0	1.30	4.88	0.04	1	0	57.6
	Removal efficiency (%)	4	0	28	34	33	33	100	1
Week 3	Influent	0.93	2	4.20	1.60	0.00	0.60	7	57.7
	Effluent	0.82	0	3.90	1.45	0.00	0.40	6	56.4
	Removal efficiency (%)	12	100	7	9	0	33	14	2.
Week 4	Influent	1.40	2	5.80	5.60	0.03	1.50	9	56.6
	Effluent	1.30	1	5.40	5.50	0.01	1.10	4	56.1
	Removal efficiency (%)	7	50	7	2	67	27	56	1

Table 8.9 Water quality parameters of the $FeSO_4$ diet for the second production cycle (mgl^{-1}) (29 December – 29 January)

Week		Water quality parameters							
		TAN	NO_2-N	NO_3-N	PO_4^{-3-} P	Fe	K	TSS	TDS
Week 1	Influent	0.62	2	1.1	1.71	0.15	1.2	4	59.1
	Effluent	0.36	0	1	1.24	0.06	0.9	3	56.1
	Removal efficiency (%)	42	100	9	27	60	25	25	5
Week 2	Influent	0.80	2	1.7	1.71	0.13	1.5	3	65.65
	Effluent	0.37	0	1.5	1.45	0.06	0.7	1	60.2
	Removal efficiency (%)	54	100	12	15	54	53	67	8
Week 3	Influent	1.76	4	1.4	5.12	0.10	0.9	9	85.35
	Effluent	0.53	3	0.5	1.48	0.06	0.7	6	51.20
	Removal efficiency (%)	70	25	64	71	40	22	33	40
Week 4	Influent	1.04	3	4.7	1.81	0.07	1.1	5	63.75
	Effluent	0.63	0	1.1	1.34	0.03	1	3	59.75
	Removal efficiency (%)	39	100	77	26	57	9	40	6

Table 8.10 Water quality parameters of the control diet for the third production cycle (mgl^{-1}) (04 February – 06 March)

Week		Water quality parameters							
		TAN	NO_2-N	NO_3-N	PO_4^{3-} P	Fe	K	TSS	TDS
Week 1	Influent	0.39	0	0.7	1.12	0.01	0.7	13	121.5
	Effluent	0.18	0	0.5	0.91	0.01	0.6	7	59
	Removal efficiency (%)	54	0	29	19	0	14	46	51
Week 2	Influent	0.27	0	1.0	0.71	0.01	0.7	3	60.6
	Effluent	0.20	0	0.5	0.56	0.01	0.5	0	58.6
	Removal efficiency (%)	26	0	50	21	0	29	100	3.3
Week 3	Influent	0.35	0	0.8	0.99	0.01	0.7	1	64.2
	Effluent	0..27	0	007	0.89	0.00	0.6	1	63.8
	Removal efficiency (%)	23	0	13	10	100	14	0	1
Week 4	Influent	0.46	2	2.7	4.4	0.02	1.6	4	75.8
	Effluent	0.4	1	2.1	0.36	0.01	0.8	3	74.8
	Removal efficiency (%)	13	50	22	92	50	50	25	1.3

Table 8.11 Water quality parameters of the $FeSO_4$ diet for the third production cycle (mgl^{-1}) (04 February – 06 March)

Week		Water quality parameters							
		TAN	NO_2-N	NO_3-N	PO_4^{-3-} P	Fe	K	TSS	TDS
Week 1	Influent	1.04	0	3.6	5.26	0.12	4.5	13	89.9
	Effluent	0.89	0	2.8	2.96	0.07	3	9	88.5
	Removal efficiency (%)	14	0	22	44	14	33	31	1
Week2	Influent	0.77	0	2.5	5.5	0.09	1.9	7	77.05
	Effluent	0.50	0	1.9	5.18	0.07	1.6	2	73.7
	Removal efficiency (%)	35	0	24	6	22	16	71	4
Week 3	Influent	0.98	1.0	2.5	5.8	0.07	1.8	4	89.95
	Effluent	0.78	0.9	2	5.72	0.06	0.8	1	86.3
	Removal efficiency (%)	20	10	20	1	14	56	75	4
Week 4	Influent	0.85	2	5.3	8	0.12	1.7	6	130.3
	Effluent	0.79	1	5.1	5.8	0.07	1.6	4	122.3
	Removal efficiency (%)	7	50	4	28	42	6	33	6

8.5 Discussion

The study evaluated the supplementation of iron from $FeSO_4$ to the diet of the African catfish to produce lettuce in an integrated aquaponics system. The trial was run over a three-month period in three consecutives production cycles.

The African catfish showed good fish growth and high survival (90-100 %); any mortalities experienced could be attributed to natural death. The FCR was high (1.2 - 2) compared to other studies growing the African catfish in aquaponics systems (Endut et al., 2010; 2012; Knaus and Palm, 2016) and in RAS (Baßmann et al., 2017), the reason for this is not clear. The production of fish in this study was acceptable and supported the growth and welfare of the African catfish (Baßmann et al., 2017). This was important because fish welfare in aquaponics systems is as important as plant production (Yildiz et al., 2018) and fish should grow at acceptable rates.

Under good climatic conditions, when the temperature ranged between 17 - 27° C (first and second production cycles), improved production of lettuce was observed in the $FeSO_4$ dietary treatment compared to the control treatment. During the first two production cycles, the $FeSO_4$ dietary treatment had a higher average biomass and shoot weight compared to the control at point of harvest. The better growth of lettuce in the $FeSO_4$ dietary treatment can be attributed to Fe which resulted from the supplementation of $FeSO_4$ in the fish feed. The need for Fe in plants has long been established (Christ, 1974; Nenova, 2006; Hochmucth, 2011). Generally, aquaponics system have suboptimal plant production due to iron deficiency (Seawright et al., 1998; Rakocy et al., 2006; Goddek et al., 2015), which was observed in the control treatment where, lower production of lettuce was found compared to the $FeSO_4$ dietary treatment. The inclusion of $FeSO_4$ in the fish feed was therefore beneficial, and improved the overall production in the system as it produced acceptable fish production, along with improved plant production.

In aquaponics and hydroponics systems, iron is usually supplemented in a chelated form as Fe-EDTA or Fe-DTPA. However, the chelated iron needs to be added continuously and is unstable at pH >7 (Rakocy et al, 2006). The addition of Fe through the feed additive $FeSO_4$ in the fish feed in this study resulted in consistent improved growth of fish and plants under

favourable conditions without requiring continuous addition of Fe in any part of the system. This is an important aspect in aquaponics, as the need to regularly supplement iron in aquaponics system increases management efforts. Furthermore, iron needs to be consistently monitored to ensure that it is does not adversely affect bacteria, fish and plants in the system (Kasozi et al., 2019).

On the third production cycle, poor plant growth was observed as a result of high water and air temperatures (Supplementary Table B1 – B3). According to Maboko and Du Plooy (2007), lettuce grows optimally at temperature ranges of 7 - 24° C, but during this production cycle atmospheric temperatures ranged between 12 - 39° C while water temperatures ranged between 17.2 – 24.5° C. Both the $FeSO_4$ dietary treatment and the control had poor average biomass and average shoot weight because both the treatments were exposed to the same environmental elements. Several authors have cited the different effects of temperature and/or season on plant growth and yield while working on different plant species in hydroponic systems (Fallovo et al., 2009; Amafitano et al., 2017; Nicoletto et al., 2018). Results obtained during this production cycle further highlight the importance of climatic conditions in the production of lettuce.

The wastewater excreted from the $FeSO_4$ treatment had higher levels of iron compared to the control treatment throughout the trial. The iron concentration in the wastewater from the $FeSO_4$ dietary treatment ranged from 0.03 – 0.15 mgl^{-1}. The higher iron concentration in the wastewater from the $FeSO_4$ dietary treatment shows that the inclusion of $FeSO_4$ increased the Fe concentration in wastewater. The removal efficiency of Fe by lettuce ranged between 14 – 67 % and resulted in better growth than the control treatment, which can be attributed to the Fe in the treatment. The higher Fe concentration in the wastewater provided iron required by lettuce for optimum growth, resulting in its higher average biomass and shoot weight. The nutrients in the wastewater excreted by fish were removed efficiently by lettuce in the plant growing beds.

The removal of nutrients by lettuce during this study varied (Table 8.6 – 8.11) (Figure 8.1 – 8.3) because the nutrient uptake by plants varies throughout the growth stages of the plant (Endut et al., 2011). There was a fluctuation in nutrient removal by lettuce, which can be attributed to the fact that nutrient accumulation in aquaponics systems does not occur at equal rates between the nutrients (Endut et al., 2011). The uptake of nutrients is also

dependent on factors such as pH, temperature (Tyson et al., 2004; Fallovo et al., 2009), and the requirement of the nutrients by plants in their particular growth stage (Graber and Junge, 2009). The absorption and removal of wastewater nutrients by lettuce in this study can be regarded as suitable as the nutrients did not accumulate, an indication that the plants were absorbing the nutrients produced by fish. The water quality conditions were acceptable for optimum plant growth as evidenced by the plant biomass. The efficiency of the removal was also evidenced by the influent concentration in plant growing beds which was consistently found to be higher than the effluent levels. The results indicate that the feed provided did not deteriorate the quality of the water and allowed nutrients to be taken up by plants.

The pH was good for all the organisms cultured in the system as it was within a range that allowed, fish, plants and bacteria to coexist (Goddek et al., 2015). The pH in the plant growing beds was monitored but not manipulated in any way, and it ranged between 5.50 - 7.22. The pH also allowed for absorption of Fe by lettuce as the uptake of minerals such as Fe is reduced at pH >7 levels (Bugbee 2004) therefore requiring the addition of buffers (Rakocy et al., 2006; Goddek et al., 2015). The feed provided during the trial did not adversely affect the pH, this is an important consequence in aquaponics systems, as pH is one of the significant factors affecting fish, plants and bacteria (Goddek et al., 2019).

The nitrite concentration did not exceed 5 mgl^{-1}, which is a range acceptable for both fish and plant production in the system (0 – 5 mgl^{-1}) (Tyson et al., 2007). It was also within a similar range to studies culturing the African catfish successfully in aquaponics systems (Endut et al., 2010; 2012). It was important to monitor nitrite throughout the study because at high concentrations it is harmful to organisms in the aquaponics system (Palm et al., 2014). Nitrite accumulation is linked to high pH in the system, which may be detrimental to organisms in the system. In this study, nitritre concentration was within an acceptable range and plant growth was observed without any compromise to fish production.

The NO_3-N and TAN levels in the plant growing beds were sufficient for plant production and were also within acceptable range for the production of the African catfish (0.5 – 9 mgl^{-1}, NO_3-N, 0.27 – 4.6 mgl^{-1}, TAN)(Schram et al., 2014; Baßmann et al., 2017). Water quality conditions in aquaponics systems need to accommodate all organisms, including fish (Baßmann et al., 2017), as was the case in this study. The levels of these nutrients in our system were lower compared to other aquaponics production systems using the Africa catfish (Endut et al., 2010;

2012). The lower levels of NO_3 and TAN in the system along with the acceptable water quality conditions can be attributed to lettuce in the systems which acted as a good biological filter and absorbed the nitrates in the water. These results translate to feed's ability to produce not only increased Fe concentration but also sufficient NO_3-N and TAN concentration for plant production in the system.

The phosphorus levels were sufficient for plant growth and no deficiencies as a result of phosphorus were observed during the trial. When plants are deficient in phosphorus, they show stunted growth and older leaves die (Fitzsimmons and Posadas, 1997). Phosphorus that was produced in wastewater was absorbed by plants, which is proven by the differences in the influent and effluent concentrations in the growing beds. Phosphorus levels in this study, ranging from 0.36 – 7.68 mgl^{-1}, were lower compared to studies by other authors working on African catfish in aquaponics systems (Endut et al., 2010; 2012; Knaus and Palm 2017) with the exception of Palm et al (2014b). The excreted phosphorus concentrations from the treatment met the nutritional requirements of the plants in the growing beds during the trial.

Differences in the proximate composition of lettuce as a result of the treatments were observed. These results were comparable to proximate composition of lettuce grown in hydroponics systems (Wheeler et al., 1995; Fallovo et al., 2009). In this study, on the first and second production cycle, when conditions were optimal, $FeSO_4$ supplemented feed not only resulted in increased plant production, but also more nutritious plants. The plants from the $FeSO_4$ treatment produced more biomass that had significantly higher protein content compared to the control. These results show that the $FeSO_4$ treatment is able to produced healthier plants, with a higher nutritional value, an indication that this feed may improve the overall products harvested from the aquaponics systems.

The mineral analysis conducted during this study showed differences in the Fe content in the leaves of the control and $FeSO_4$ dietary treatment. During the first and second production cycle, the Fe content in the leaves of the control was significantly lower compared to the $FeSO_4$ dietary treatment. This can be attributed to the supplementation of iron in the fish feed through $FeSO_4$ as a dietary treatment. The Fe content in lettuce leaves was lower in this study compared to Delaide et al. (2016) who found that supplemented aquaponics water had higher leaf mineral content compared to the non-complimented aquaponics water. The third production cycle had similar levels of Fe in the leaves between the control and $FeSO_4$ dietary

treatment. The results of the last production cycle are difficult to interpret due to the effects of temperature during the summer month. Results from the first and second production cycle are an indication that Fe supplemented through $FeSO_4$ resulted in overall improved lettuce production. These findings are similar to improvement observed by Rono et al., (2018) working the growth performance of spinach supplemented with iron from an amino acid chelate in an integrated aquaponics system.

The mineral content in the lettuce leaves differed significantly between the treatments on the first and second production cycle. This was as a result of the $FeSO_4$ treatment and the control. The NH_4-N, PO_4^{3-}-P were significantly higher in the $FeSO_4$ treatment compared to the control treatment while K and Mn varied over the two production cycles. The K, Mn and P content in lettuce was comparable to Fallovo et al. (2009) and Delaide et al. (2016). The results of the mineral analysis obtained in this study indicate that the lettuce leaves though variable from some studies had mineral levels within the normal range compared to other studies. The inclusion of Fe in fish feed from $FeSO_4$ improved the overall mineral content of the plants, an important and significant implication for plants grown in aquaponics systems.

8.6 Conclusion

The inclusion of FeSO4 as a dietary feed additive in the fish diet resulted in increased Fe concentration in the wastewater without deteriorating the quality of the water. The higher Fe concentration in the wastewater resulted in an increaseof the average biomass and shoot weight of lettuce without adversely affecting fish production. . Plants from the $FeSO_4$ treatment had significantly higher protein content compared to the control. The inclusion of this feed additive produced healthier plants, with a higher nutritional value. It can therefore be concluded that iron from $FeSO_4$ at an inclusion level of 30 $mgkg^{-1}$ can improve lettuce growth and the overall efficiency of the aquaponics system. These findings show that the production of fish and plants in aquaponics system can be improved through the design of fish feed. Furthermore, system management can be optimised by including iron as a fish feed additive in the form of $FeSO_4$, eliminating the need to continuously supplement iron to the system.

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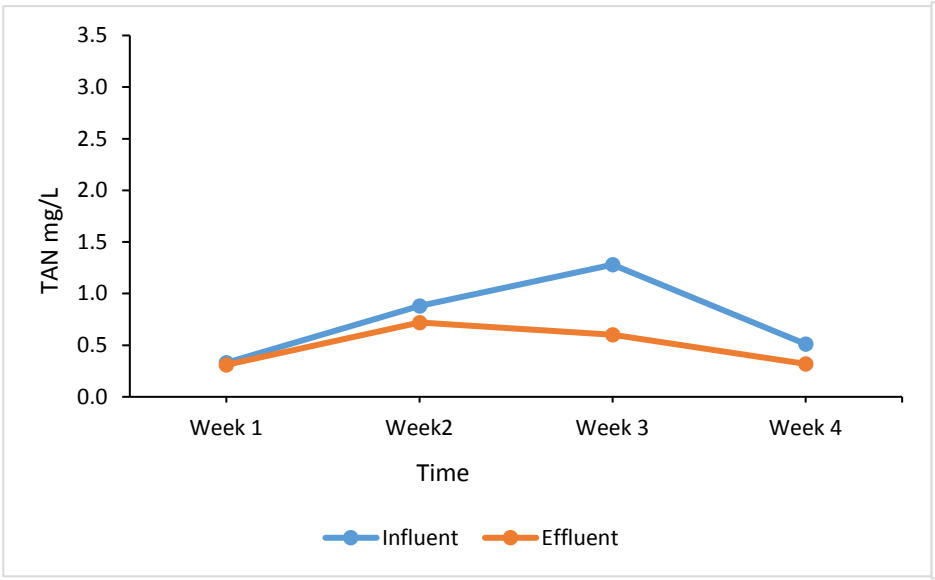
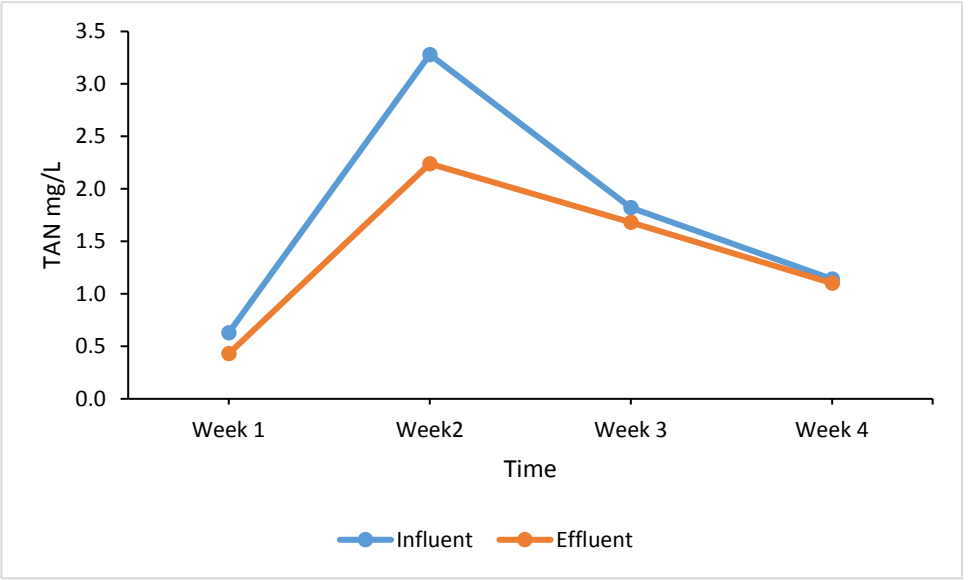
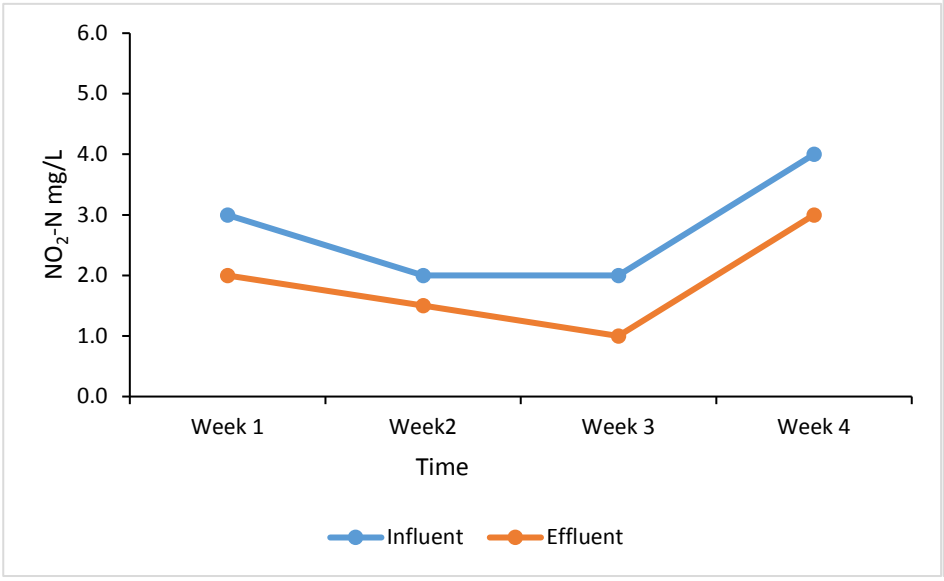
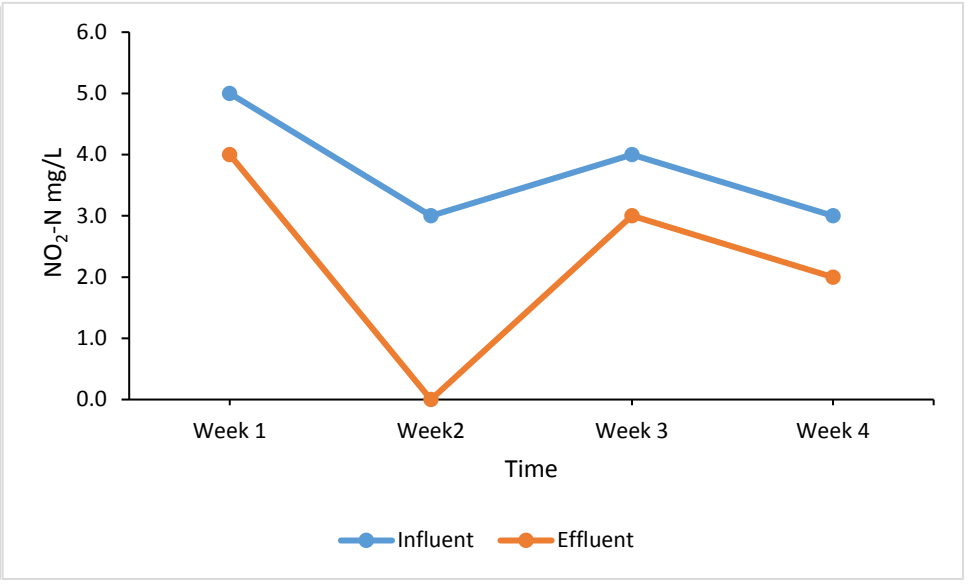
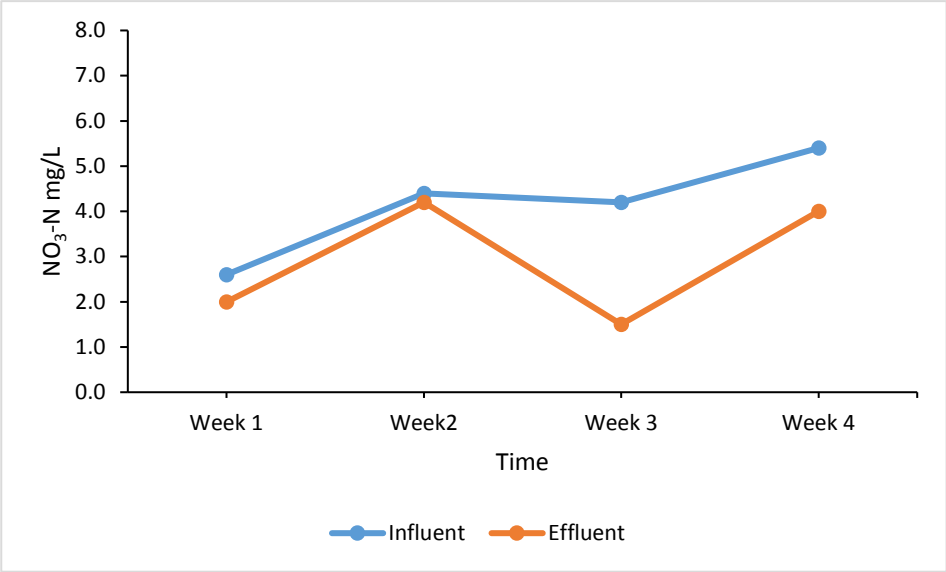
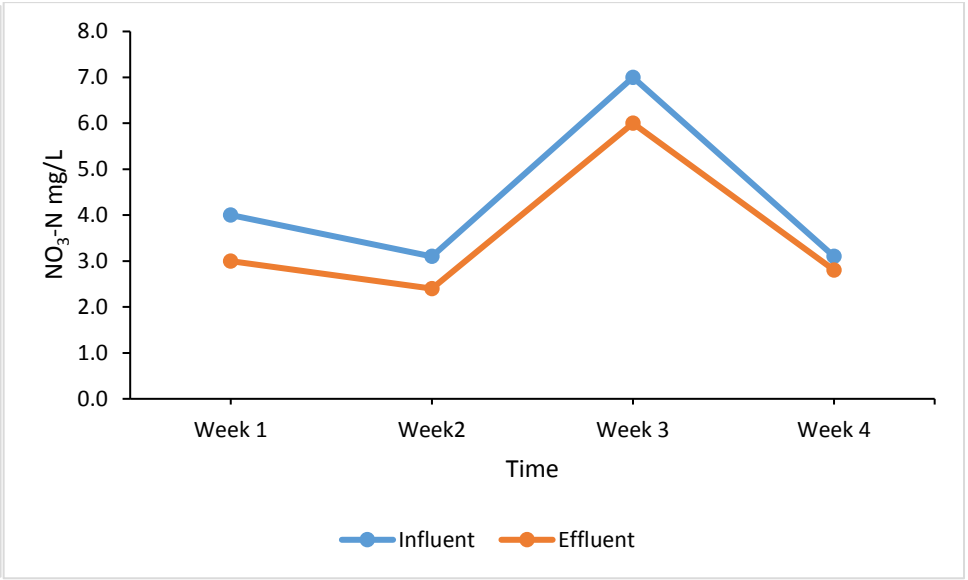


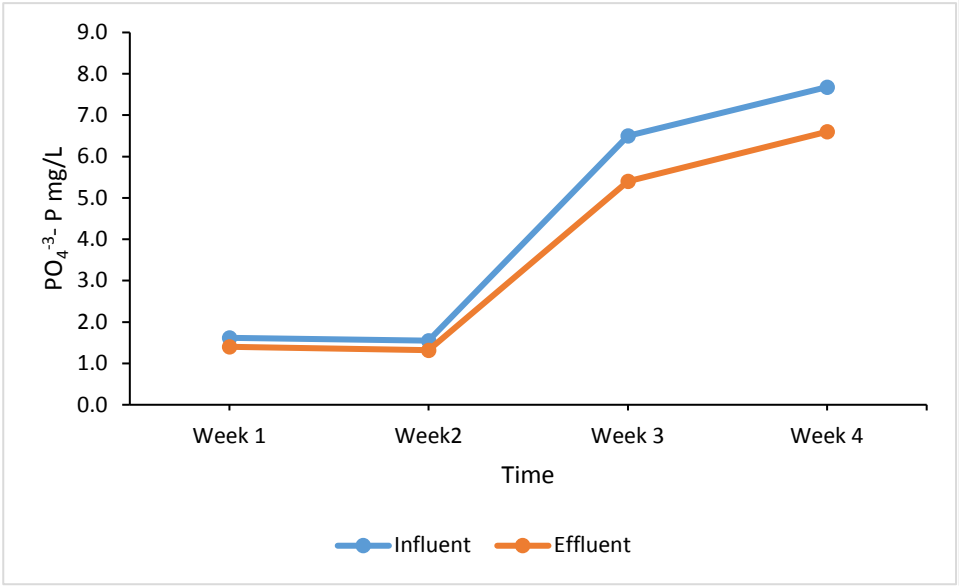


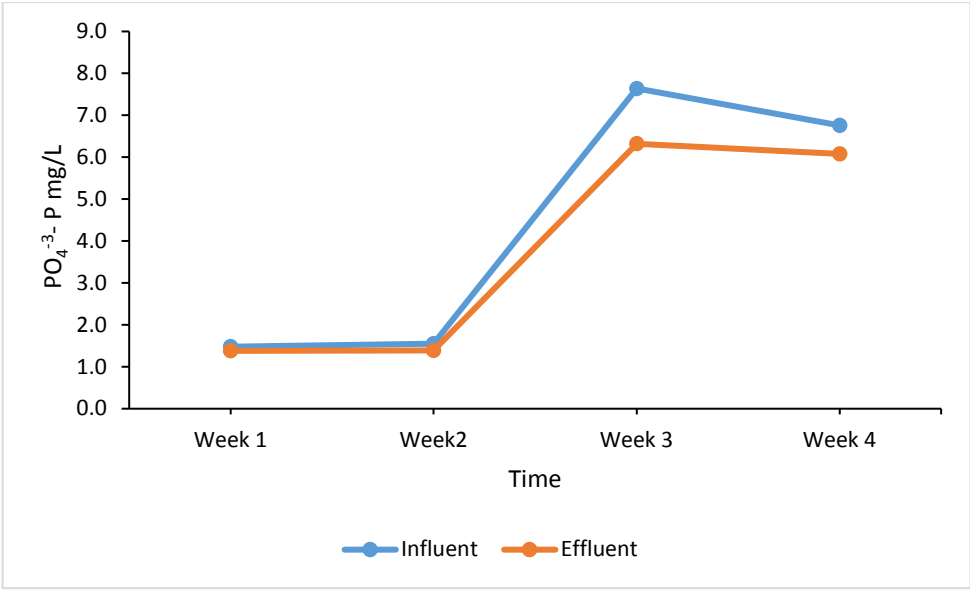


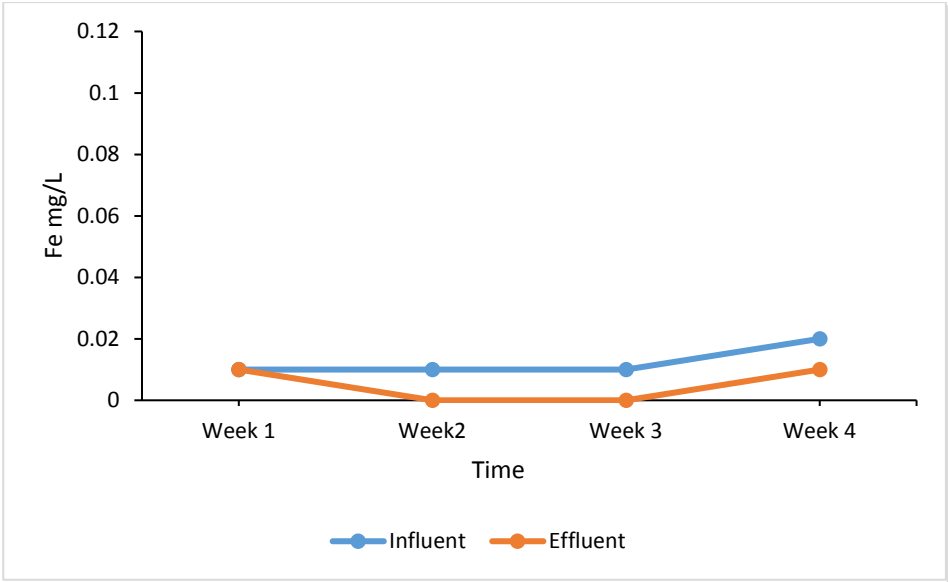


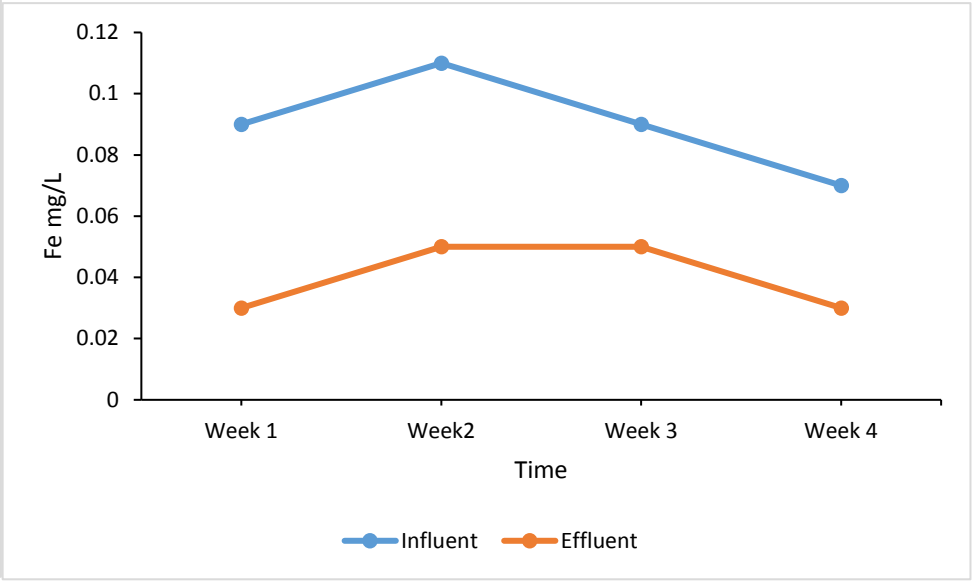


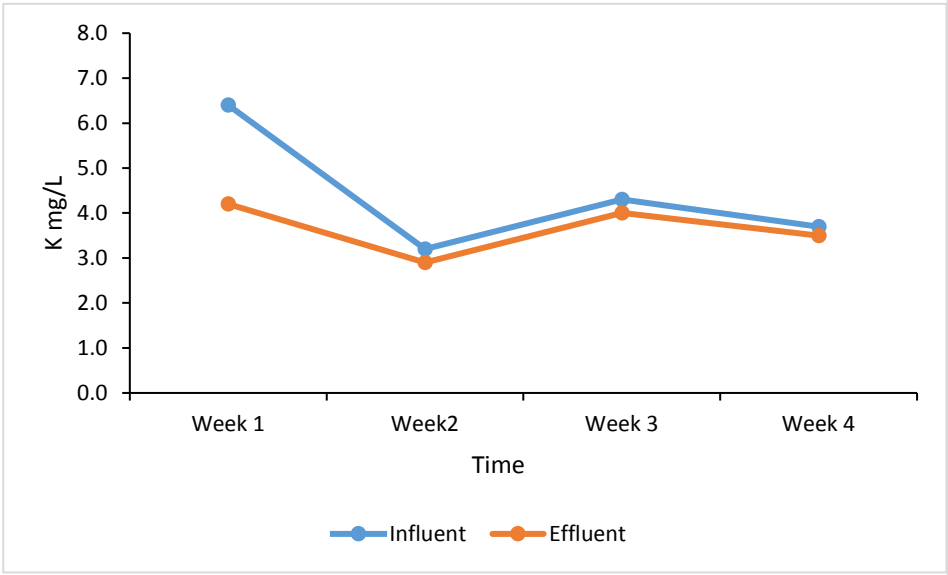


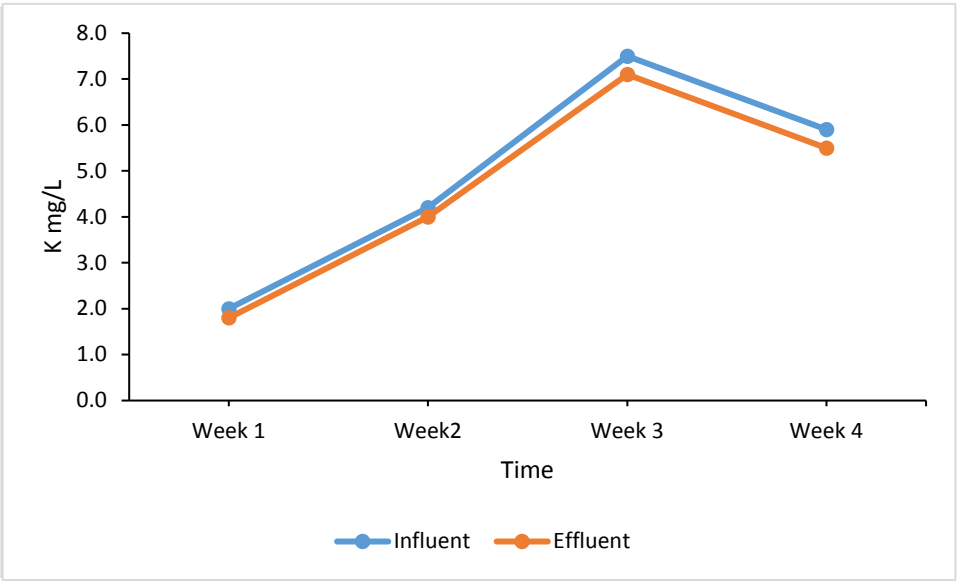












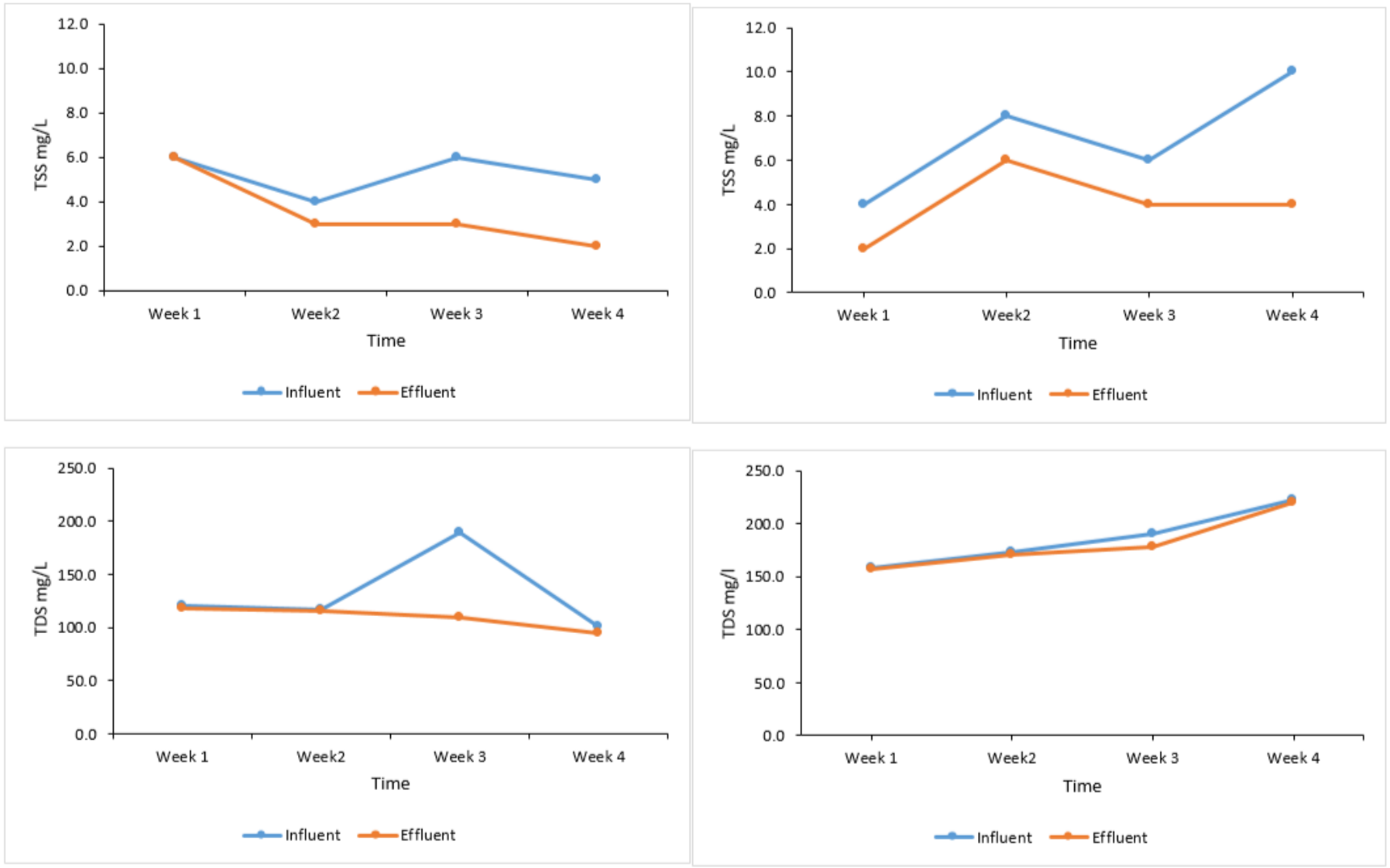
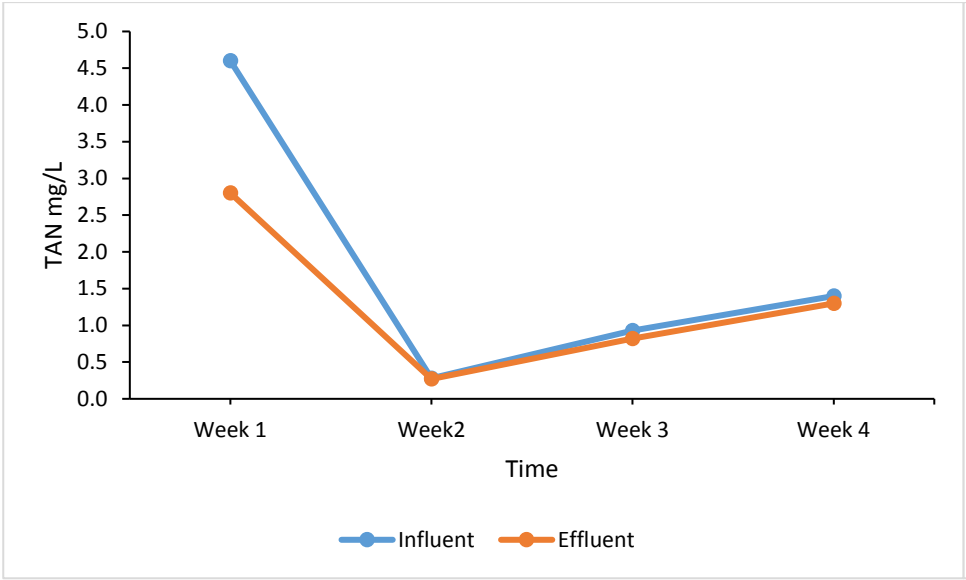
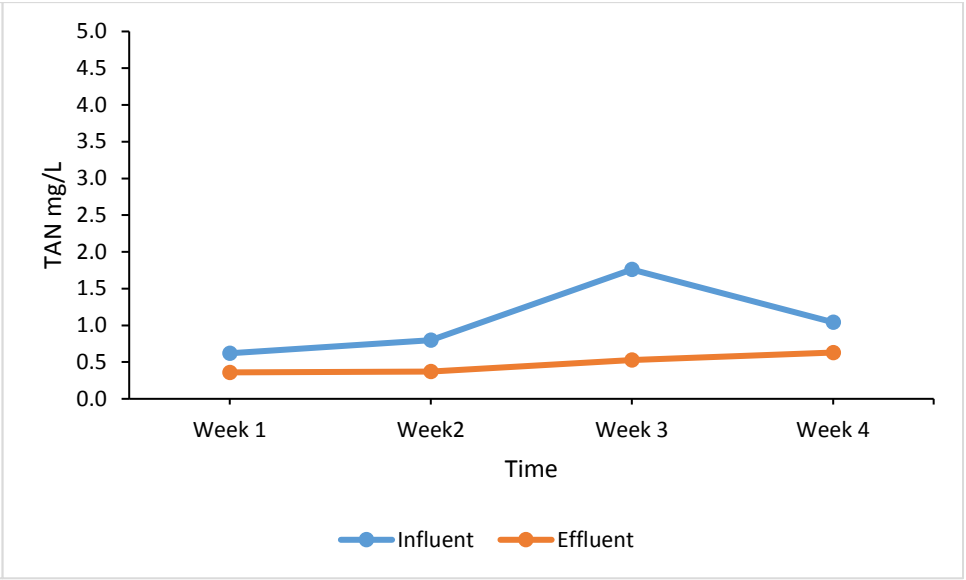
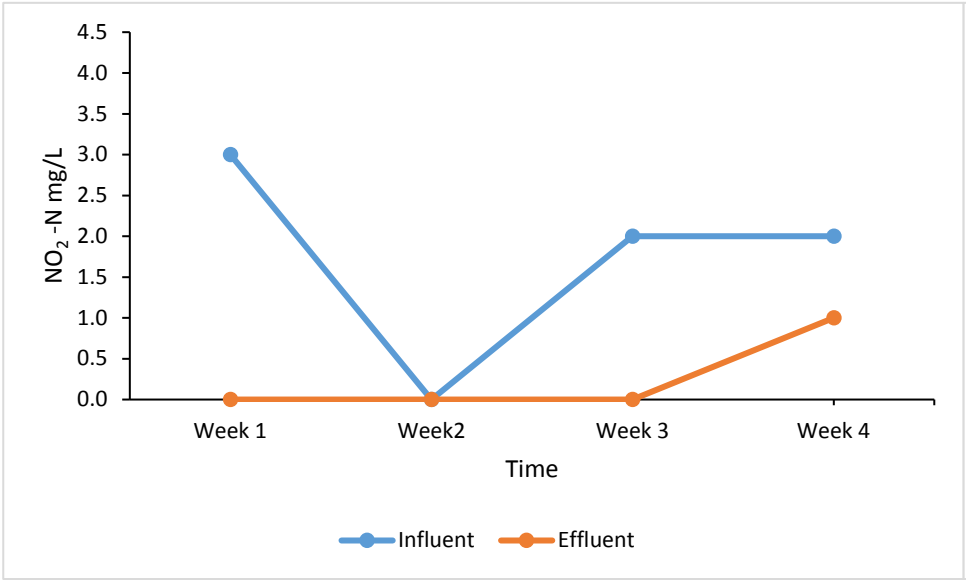
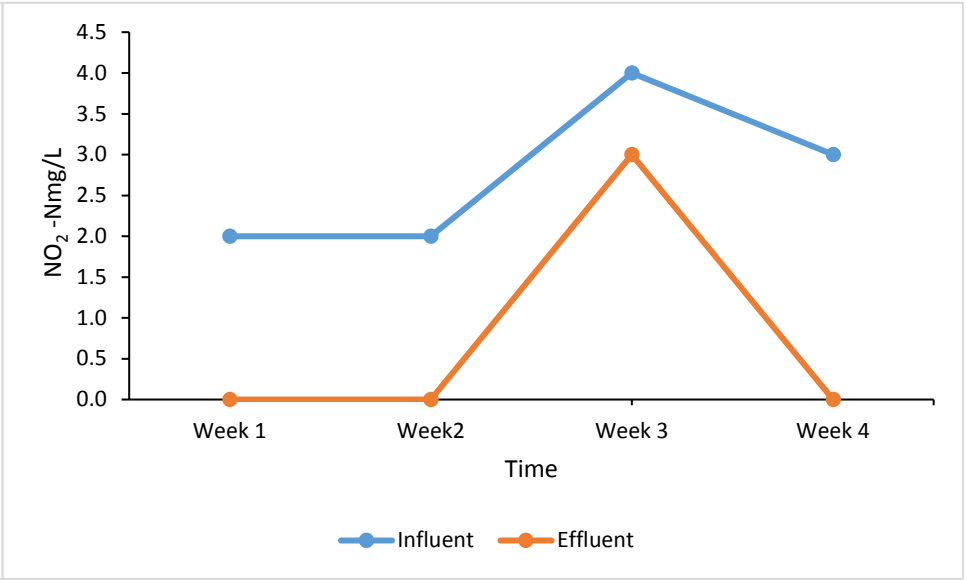


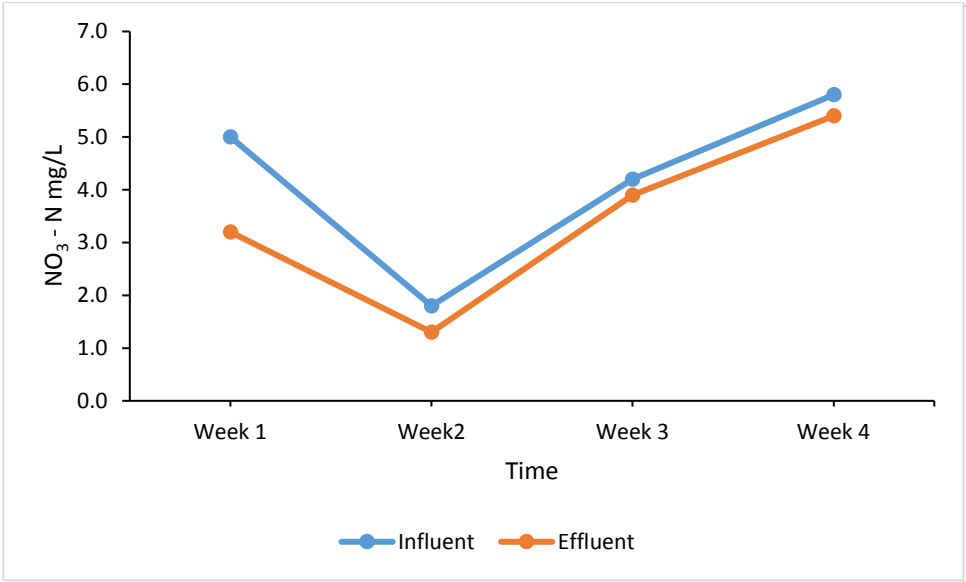
Figure 8-1 Influent and effluent in the plant growing beds in the integrated aquaponics system during the first cycle of production. Graphs on the left represent the control treatment, the right-hand side is the $FeSO_4$ treatment.

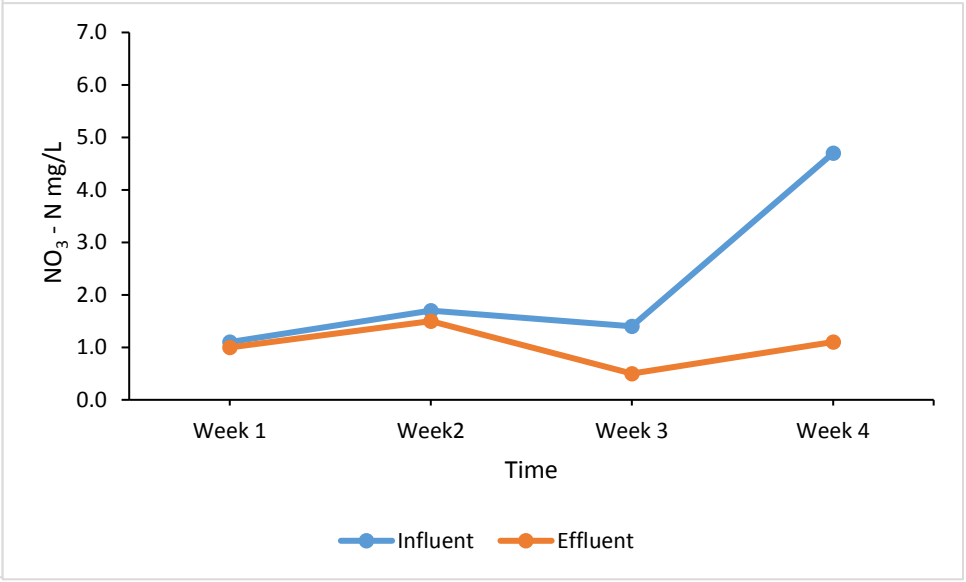


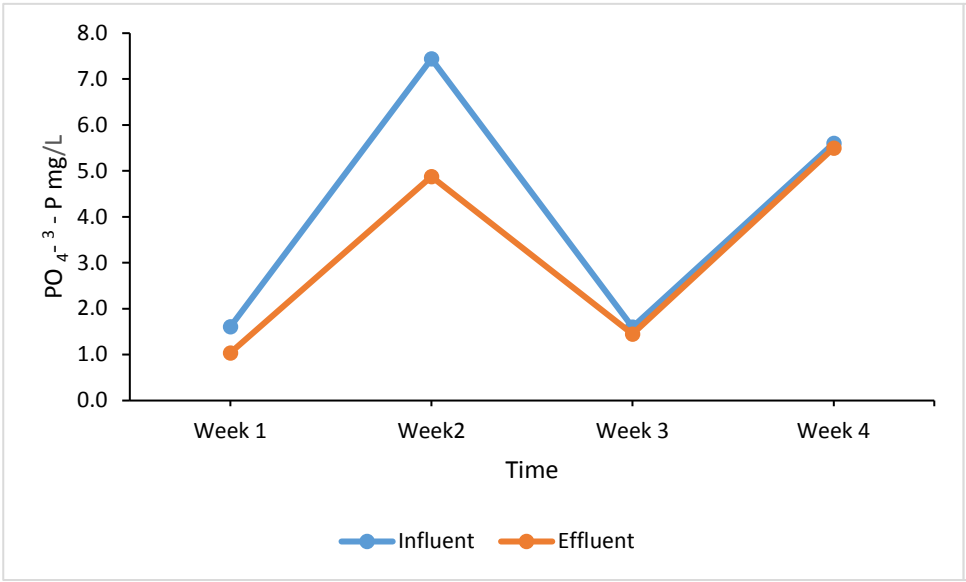


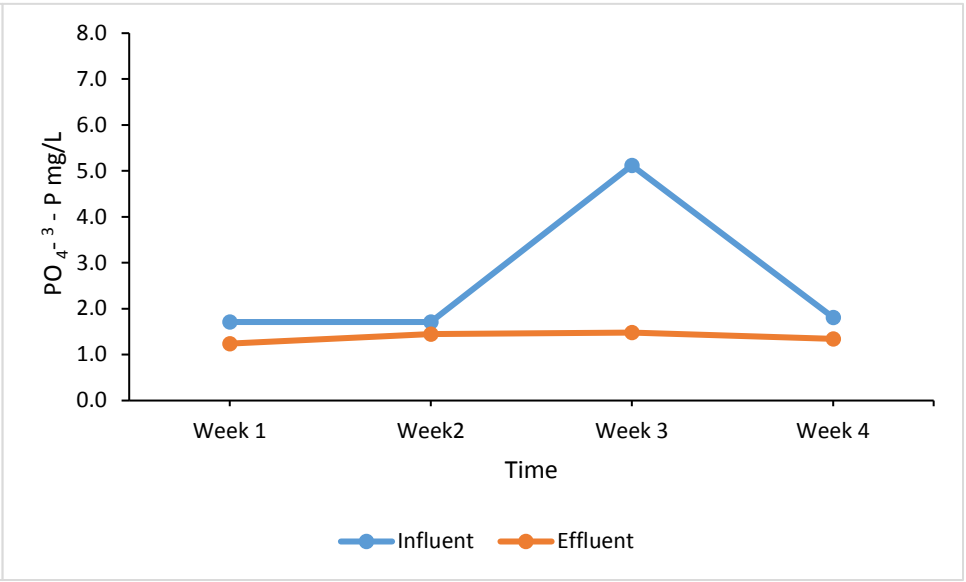


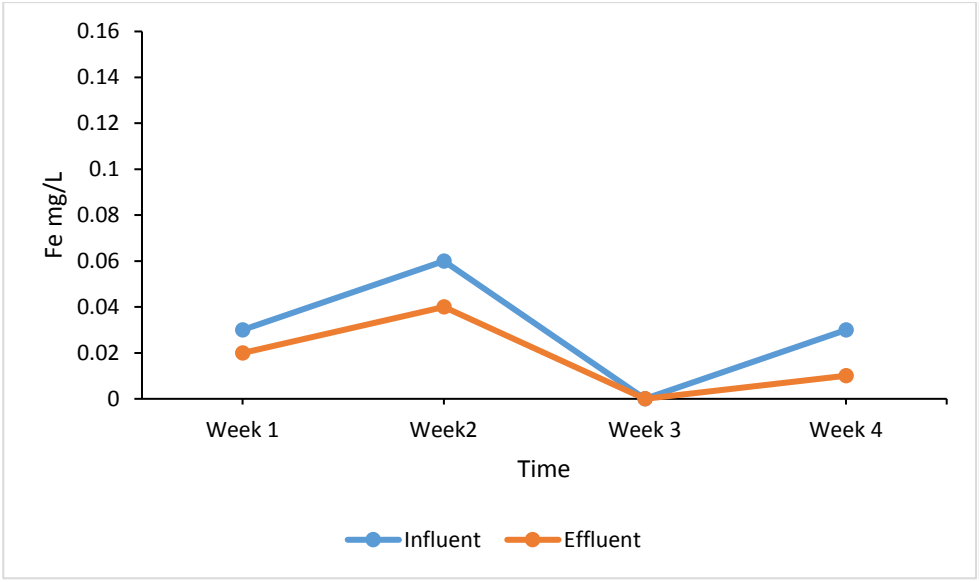


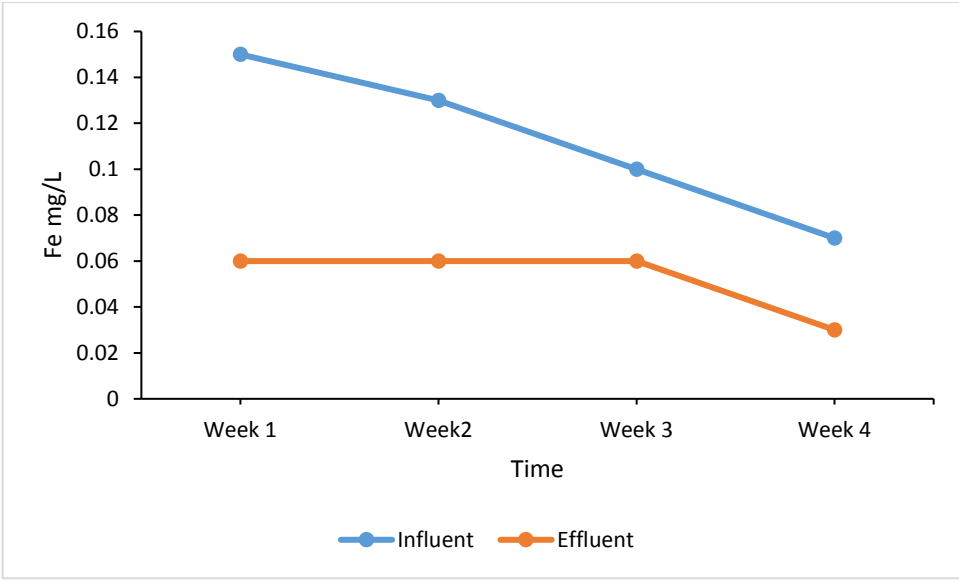


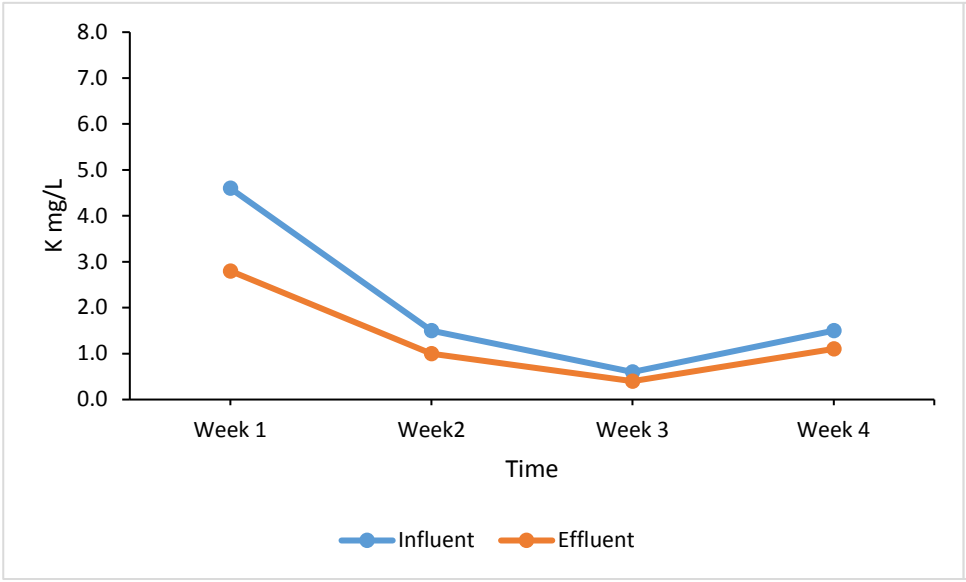


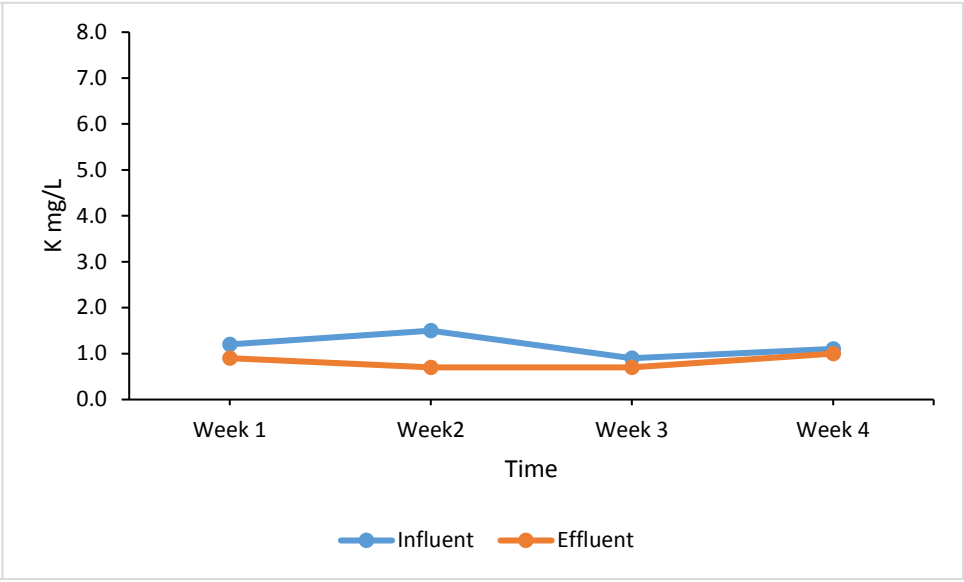












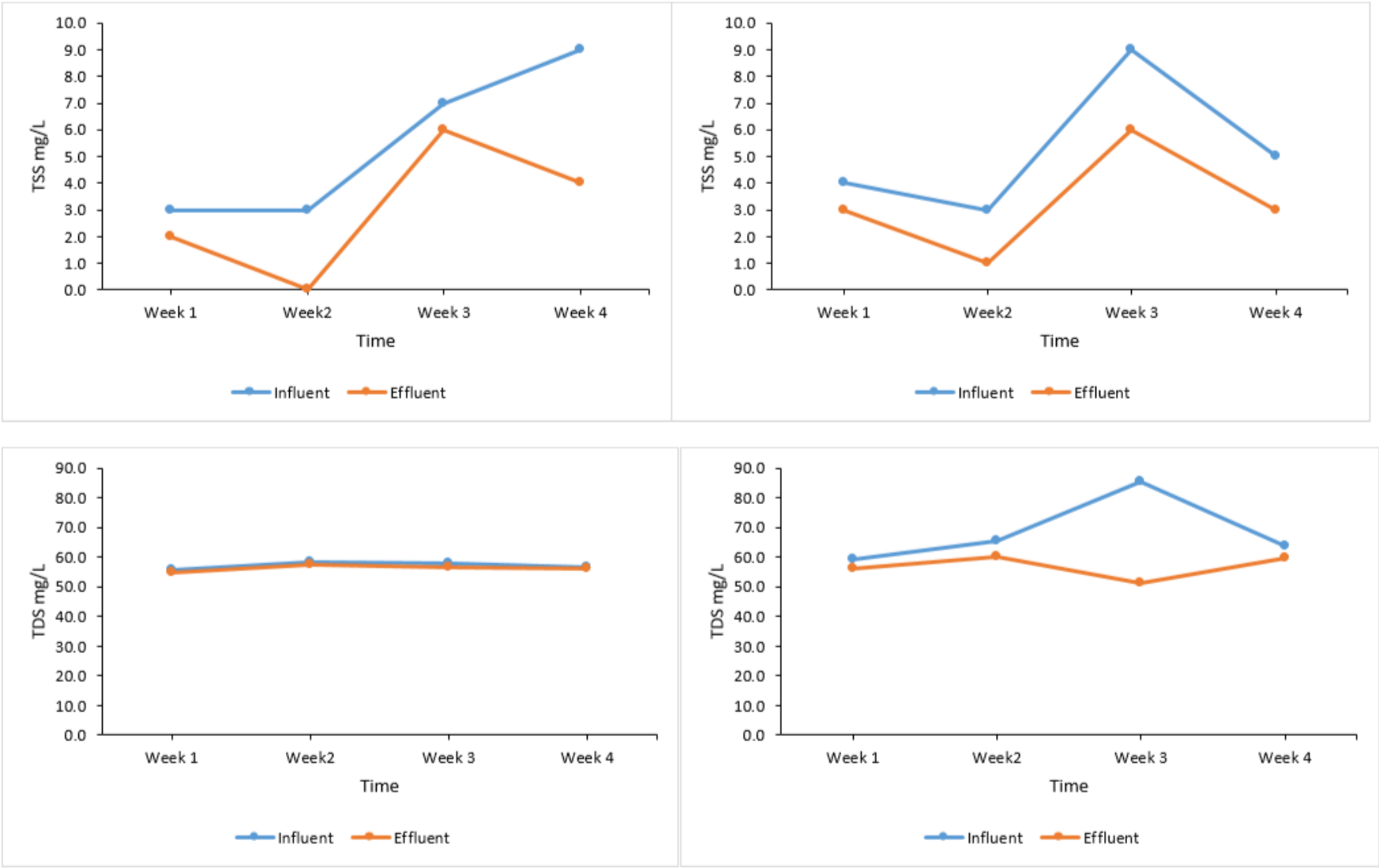
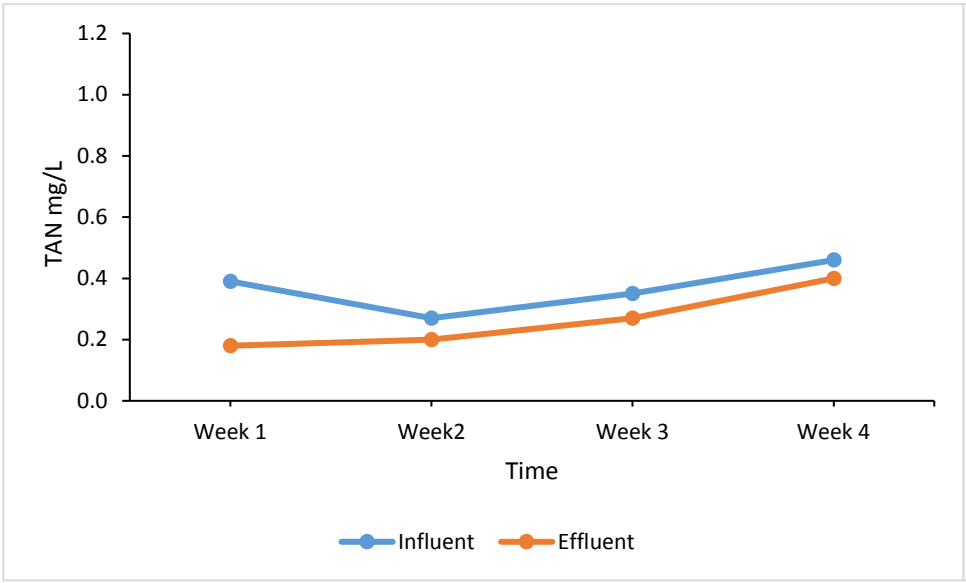
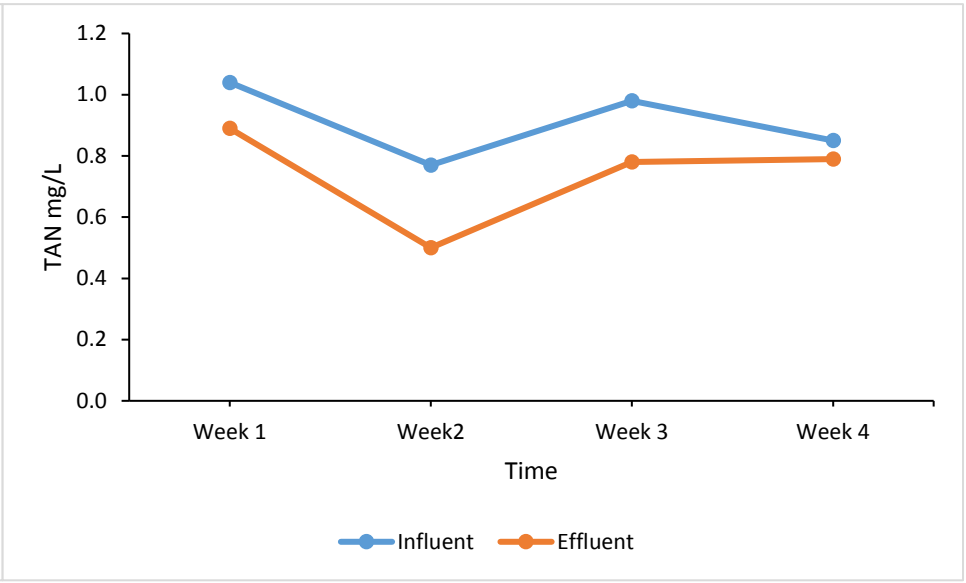
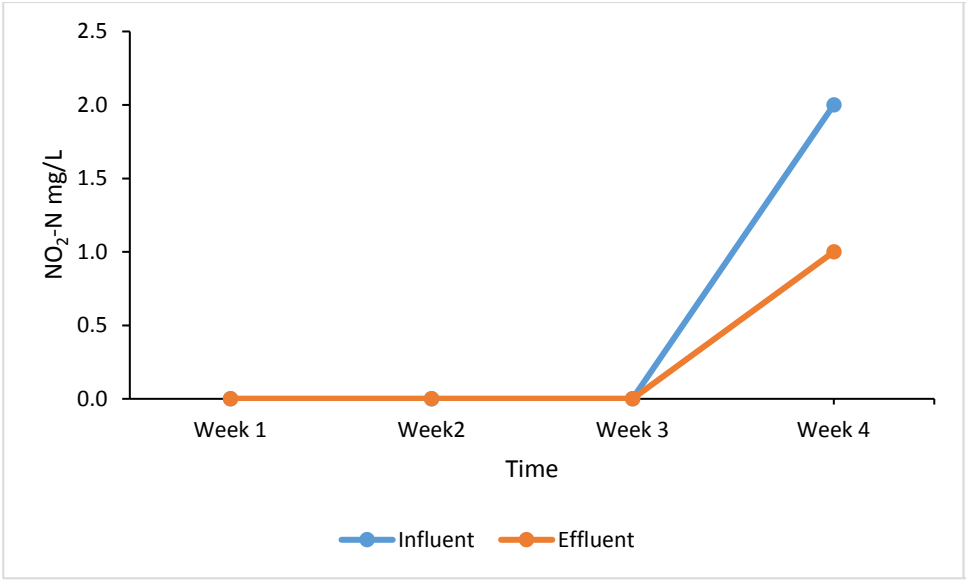
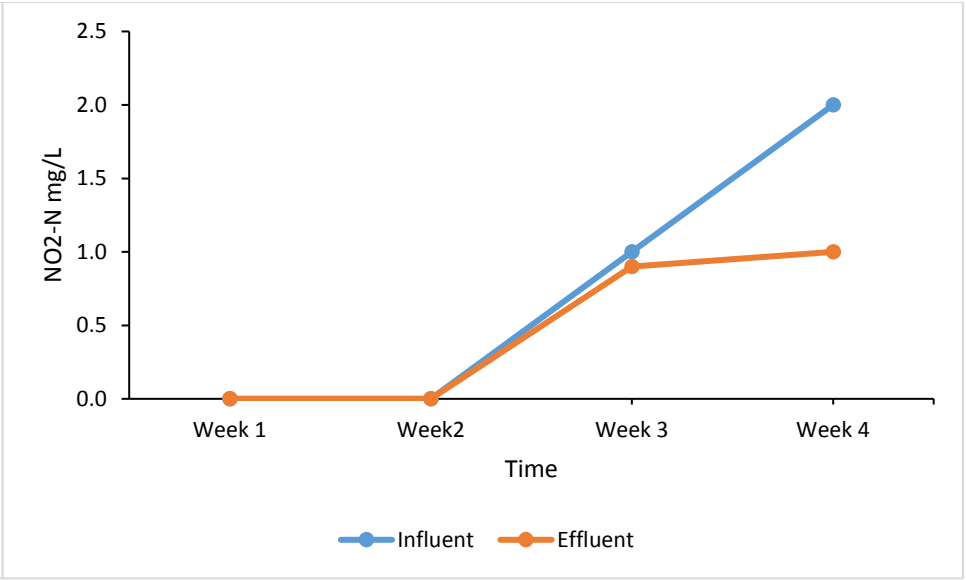


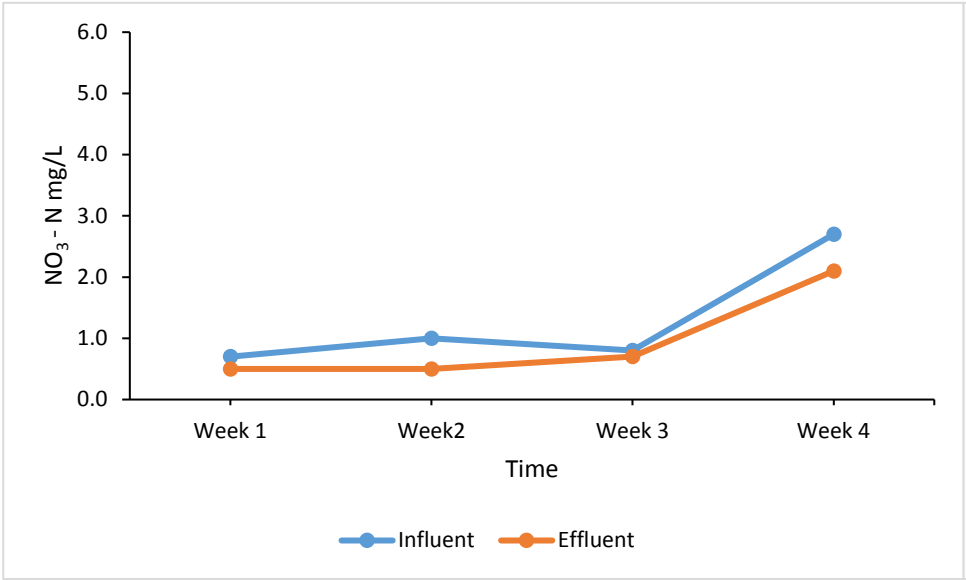
Figure 8-2 Influent and effluent in the plant growing beds in the integrated aquaponics system during the second cycle of production. Graphs on the left represent the control treatment, the right-hand side is the $FeSO_4$ treatment.

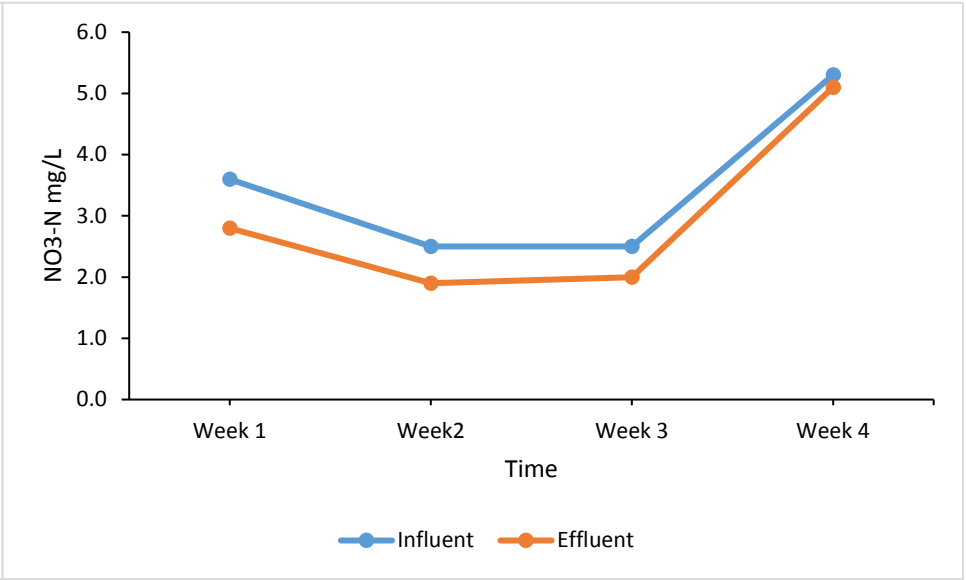


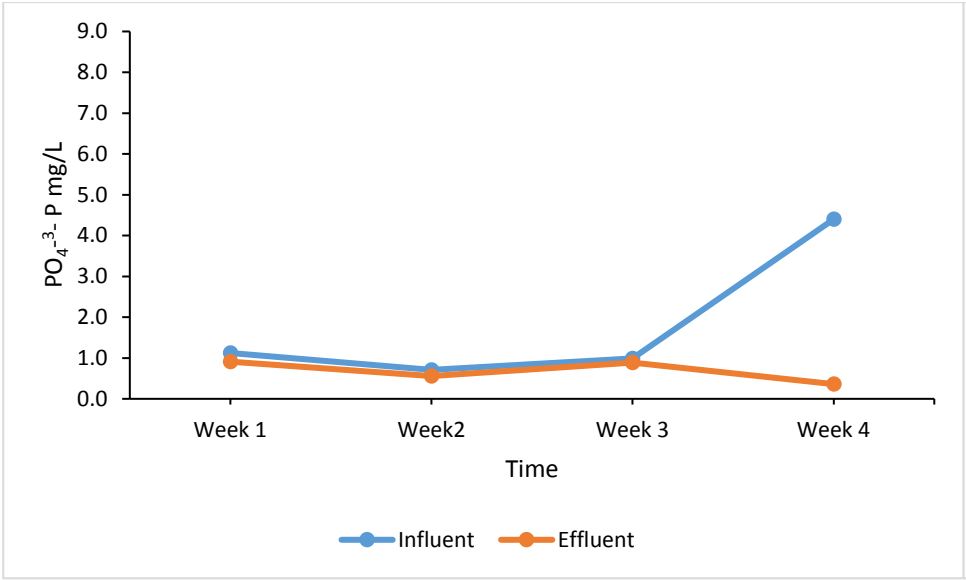


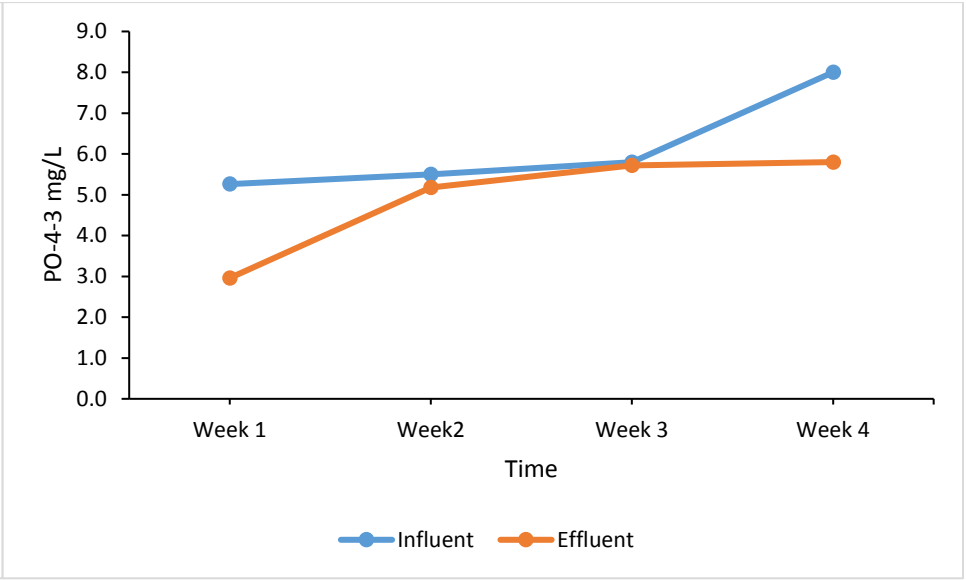


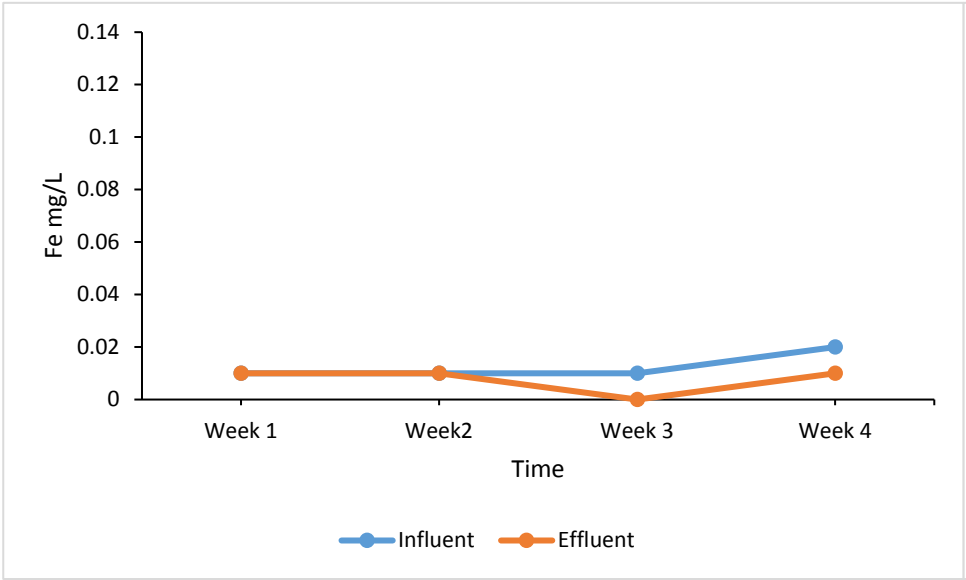


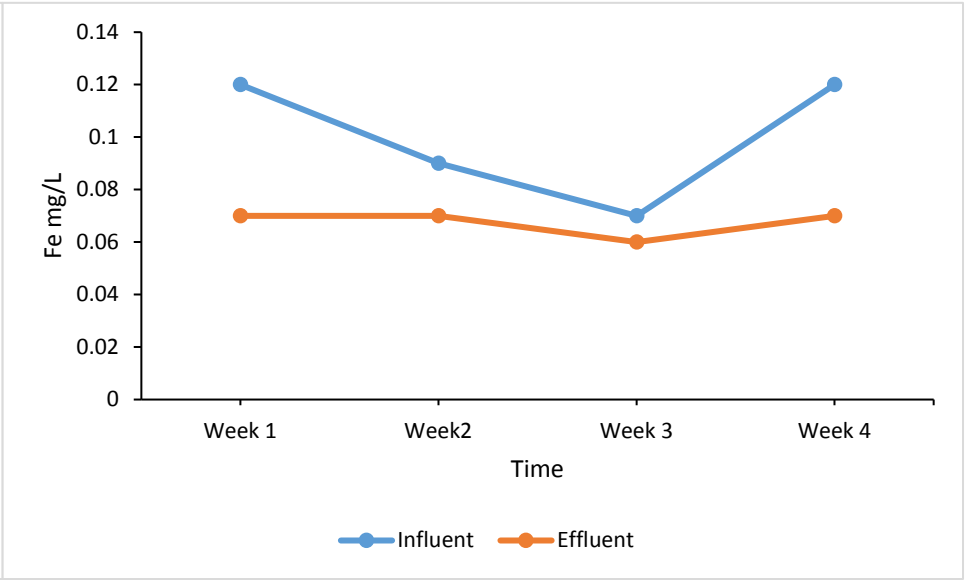


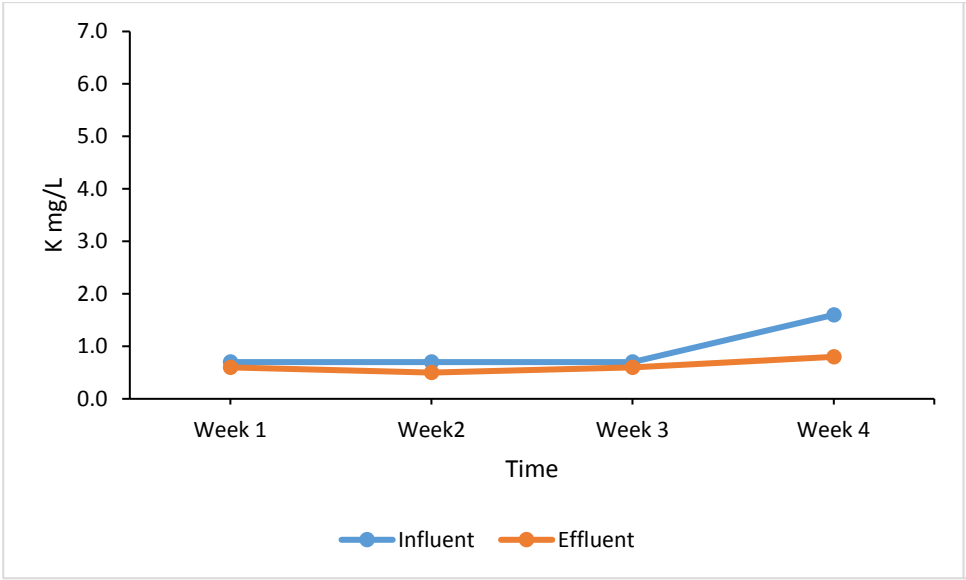


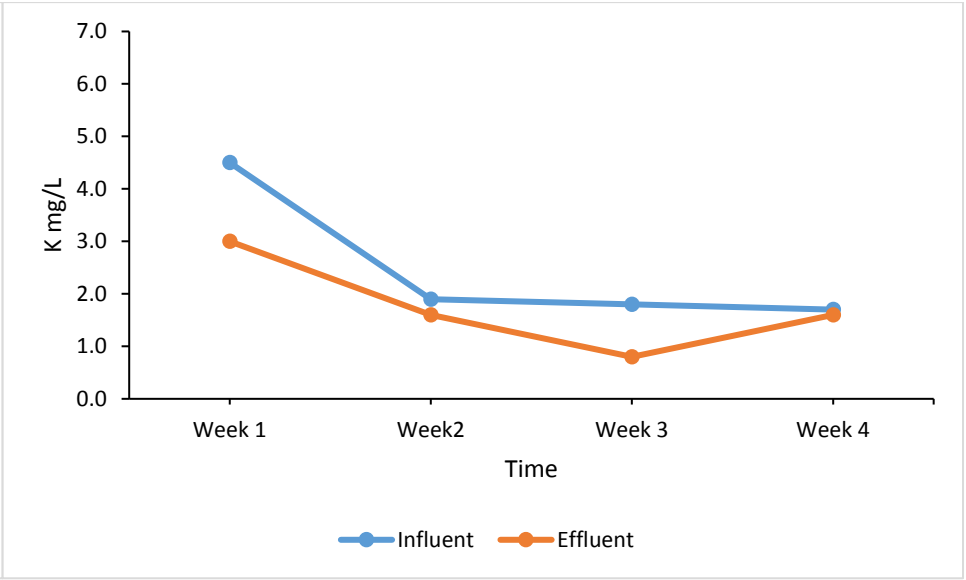












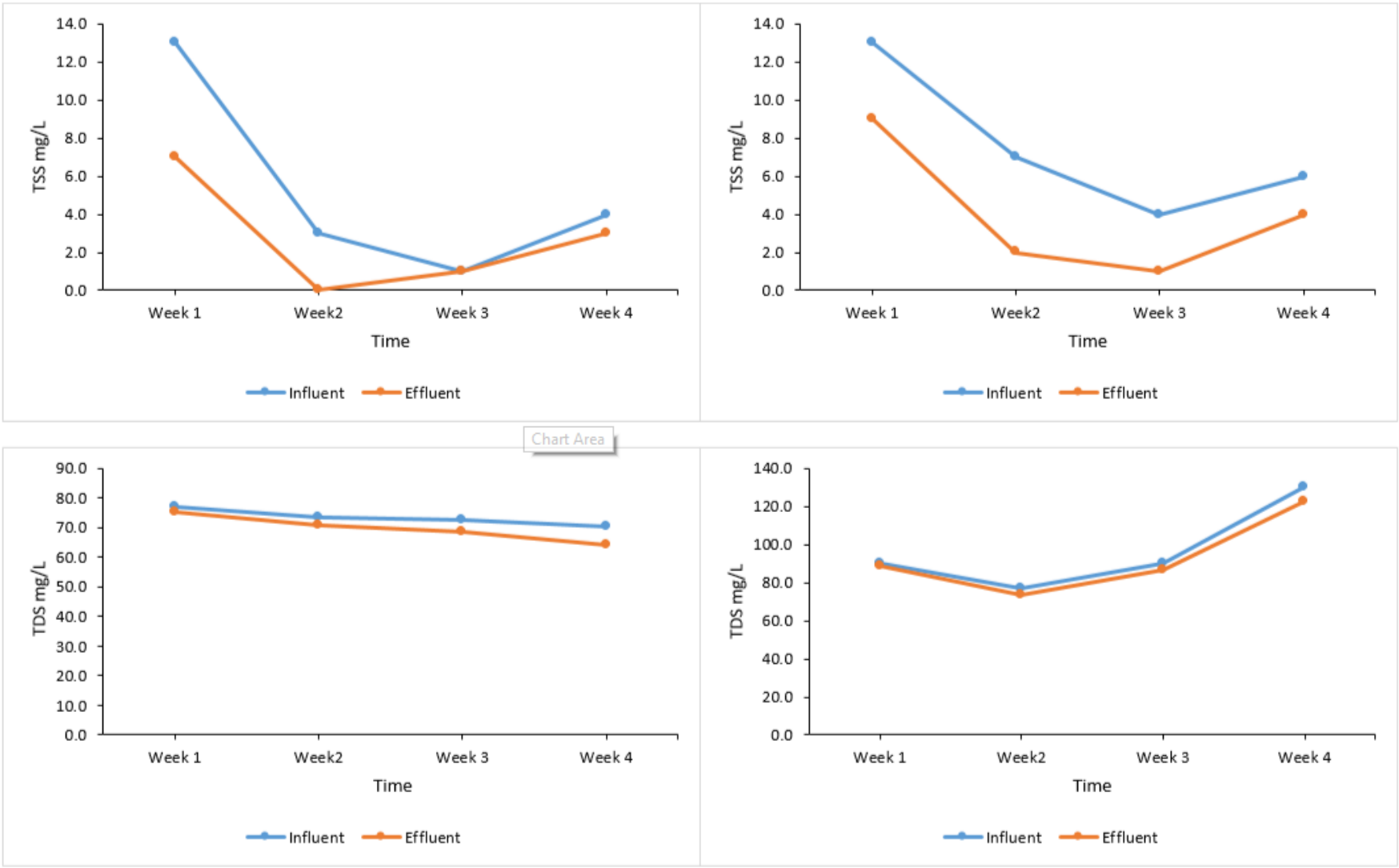


Figure 8-3 Influent and effluent in the plant growing beds in the integrated aquaponics system during the second cycle of production. Graphs on the left represent the control treatment, the right-hand side is the $FeSO_4$ treatment.

Chapter 9 Conclusions and recommendations

9.1 Conclusions

The aquaponics industry relies on the use of nutrient supplementation for optimum plant production, because using fish feed as the sole nutrient input into the system does not result in sufficient concentrations of nutrients in the circulating water to allow optimal plant production. This research proved that fish feed designed in this study can perform a dual role: provide optimal nutrition to fish and improve plant production in integrated aquaponics systems. This was achieved by supplementing the fish feed, which is the primary source of nutrients in aquaponics of the minerals potassium and iron. The supplementation of minerals to the fish feed was to ensure that upon excretion, the nutrients excreted by fish met the requirements of plants.

Mineral feed additives are known to differ in terms of bio-availability depending on their source. It was therefore important to evaluate different sources of minerals to ensure that the right source was used in the fish feed. Minerals from organic and inorganic sources were evaluated during the feeding trials. Along with the evaluation of minerals, the right inclusion level had to be determined, to ensure that the inclusion level would provide optimum fish production, haematology, and excrete the highest concentration of potassium and iron for use by plants. There has been no research to investigate the effect of different feed additives on the production of the African catfish and plants in aquaponics systems. This research details the use of organic and inorganic sources of minerals at different inclusion levels in this particular species. The feed additives that were used during the study acted as water soluble substances and were excreted either through the gills or urine, resulting in their availability for plants through the wastewater. The effect of these additives as water or fat soluble was not tested, this effect can only be deduced based on the excretion of the Fe and K to the water.

In the feeding trial evaluating potassium additives (Chapter 5), it was anticipated that the organic source of potassium would affect catfish production positively, however, unlike in other aquaculture species, this was not observed. The supplementation of potassium through different additives had no effects on the production of the African catfish, neither did it impact whole-body proximate composition. The haematological profile of the catfish was significantly improved by the addition of potassium from KDF at inclusion level 9 gkg^{-1}, while the

treatments had no effect on non-specific immunity. The effect of the potassium additives on the apparent digestibility coefficient of potassium could not be conclusively proven due to lack of replicates, however, there was an indication of high digestibility of potassium by the African catfish from both sources. Mineral analysis of the African catfish revealed no differences in the mineral concentration of the filet and vertebrae. However, there was no discernible explanation for the high iron concentration in the liver of the African catfish. The wastewater obtained from the inclusion of potassium at 9 gkg^{-1} had a high concentration of potassium. These results were an indication that KDF can be used a mineral additive to enhance the production of both fish and plants.

The evaluation of iron from different dietary sources and inclusion levels showed no significant effect on the production of the African catfish, while whole-body proximate composition was affected (Chapter 6). The inorganic source of iron performed better than the organic sources when it was included in the diet. Although non-specific immunity was not affected, the haematological profile was significantly improved as a result of including iron from the inorganic source, $FeSO_4$ at an inclusion level of 30 $mgkg^{-1}$. The mineral concentrations in the tissues of the fish did not exhibit differences as a result of the additives, and unlike the case of potassium feed additives, there were no excessive levels of iron in the liver as a result of the supplementation of iron to fish feed. The apparent digestibility coefficient of iron was high, although these results could not be statistically analysed. Along with improving the haematological profile of the catfish, the $FeSO_4$ treatment excreted wastewater with increased iron concentrations, indicating that it may be a good source of iron in integrated aquaponics systems.

The improved haematological profile of the African catfish and excretion of potassium and iron at increased concentrations in the wastewater as a result of including KDF at 9 gkg^{-1} (Chapter 5) and $FeSO_4$ at 30 $mgkg^{-1}$ (Chapter 6) showed its potential use in integrated aquaponics systems. These results indicated that these feed additives could benefit both fish and plants when included in the fish feed. When evaluating these feed additives in combination with lettuce (Chapter 7 and 8), they proved beneficial to the production of lettuce. The wastewater from the KDF treatment had a higher concentration of potassium and total dissolved solids than the control. The excreted wastewater from the $FeSO_4$ treatment also showed a higher concentration of iron and total dissolved solids compared to the control.

These water quality parameters resulted in increased biomass and shoot weight of the lettuce grown with KDF and $FeSO_4$ treatments.

Plant growing beds did not affect the growth of lettuce during the trials. Seasonal temperature, however, played a role in the growth of lettuce, and poor growth was observed at high temperatures in all the treatments investigated. The individual nutrient removal efficiency showed that lettuce absorbed the nutrients excreted by the fish in the systems. The inclusion of the minerals directly to the fish feed has proven its potential to the need to use nutrient fertiliser in aquaponics systems. This addition can lower production costs for aquaponics farmers and minimise the labour involved with constantly supplementing minerals in the system.

In summary, the study set out to enhance the overall production of the aquaponics system by improving the production of both fish and plants in the system. It aimed to accomplish this by supplementing fish feed with dietary feed additives. The aims were achieved in this study by use of KDF and $FeSO_4$. The results proved that in aquaponics systems, fish feed can be manipulated to provide the required nutrients to optimise fish and plant growth. The increased potassium and iron concentration in wastewater from KDF and $FeSO_4$ increased the average biomass and shoot weight of lettuce when used in an aquaponics system. These are crucial findings in aquaponics because lack of nutrients such as iron and potassium in aquaponics systems have resulted in the use of nutrient fertilizers. The feed from this study can eliminate the need to supplement iron and potassium in aquaponics systems, minimising management and cost efforts in aquaponics. The findings further showed that the inclusion of dietary feed additives can improve the health status of the African catfish through its haematological profile. A better haematological profile in fish translates to a better immune system that responds against diseases and infections in fish. The results of this study can be valuable to aquafeed and aquaponics production sectors.

9.2 Recommendations

It is recommended that investigations into the haematology of the African catfish are performed to establish baseline information using different dietary feed additives. This will contribute to the understanding of fish health in production systems.

Investigations into apparent digestibility coefficient of minerals are necessary. Information on the digestibility of minerals used as fish feed additives may play a role in the improvement of

feed formulation for both aquaculture and aquaponics systems. These studies should be carried out over longer trial periods and an increased number of replications. This because the length of the feeding trial and the number of replicates were limited in this study, resulting in the inability to perform statistical analysis.

Investigations into the effects of organic feed additives over longer trial periods and an increased number of replicates are still necessary for the African catfish. This is necessary because such experiments may improve its production performance. The effects on production, haematology, and non-specific immunity may be more evident under increased replicates. In this study, space was a limiting factor in the number of replicates used.

Research into the production costs of aquaponics when using minerals supplemented through feed additives versus nutrient supplementation through fertilizers could benefit aquaponics producers. This research may improve the cost-effectiveness of mineral supplementation through the inclusion of feed additives.

Appendix A: Ethical clearance

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Approved Protocol

Date: 30 – June – 2017

PI Name: Oyama O Siqwepu

Protocol #: SU-ACUD17-00015

Title: Optimization of nutrient input to integrated aquaponics systems through mineral supplementation by way of fish feed additives using the African catfish.

Dear Oyama O Siqwepu, initial application, was reviewed on 23-June-2017 by the Research Ethics Committee: Animal Care and Use via committee review procedures and was approved. Please note that this clearance is only valid for a period of twelve months. Ethics clearance of protocols spanning more than one year must be renewed annually through submission of a progress report, up to a maximum of three years.

Applicants are reminded that they are expected to comply with accepted standards for the use of animals in research and teaching as reflected in the South African National Standards 10386: 2008. The SANS 10386: 2008 document is available on the Division for Research Developments website www.sun.ac.za/research.

As provided for in the Veterinary and Para-Veterinary Professions Act, 1982. It is the principal investigator's responsibility to ensure that all study participants are registered with or have been authorised by the South African Veterinary Council (SAVC) to perform the procedures on animals, or will be performing the procedures under the direct and continuous supervision of a SAVC-registered veterinary professional or SAVC-registered para-veterinary professional, who are acting within the scope of practice for their profession.

Please remember to use your protocol number, SU-ACUD17-00015 on any documents or correspondence with the REC: ACU concerning your research protocol. Please note that the REC: ACU has the prerogative and authority to ask further questions, seek additional information, require further modifications or monitor the conduct of your research. Any event not consistent with routine expected outcomes that results in any unexpected animal welfare issue (death, disease, or prolonged distress) or human health risks (zoonotic disease or exposure, injuries) must be reported to the committee, by creating an Adverse Event submission within the system.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact the REC: ACU secretariat at wabeukes@sun.ac.za or 021 808 9008.

Sincerely, REC: ACU Secretariat

Research Ethics Committee: Animal Care and Use

Appendix B: Atmospheric and water temperature, Dissolved oxygen and pH data

Supplementary Table B1: Production cycle 1, 14 November – 15 December 2018

Date	Atmospheric(°C)	Water (°C)			Dissolved Oxygen (mgl^{-1})			pH		
		Control	KDF	$FeSO_4$	Control	KDF	$FeSO_4$	Control	KDF	$FeSO_4$
Wed14	16 - 27	18.6	18.7	18.70	7.1	7.2	7.2	7.21	7.20	7.22
Thur 15	15 - 26	18.5	18.5	18.5	7.1	7.1	7.2	6.93	6.95	6.90
Frid 16	15 – 24	18.6	18.5	18.5	7.1	7.1	7.2	6.44	6.50	6.43
Sat 17	11 – 22	16.9	16.9	16.9	7.1	76.9	7.1	6.40	6.51	6.40
Sun 18	15 - 27	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T
Mon 19	15 – 29	18.8	18.8	19.0	6.6	7.0	6.6	6.33	6.32	6.15
Tues 20	15 – 21	16.8	16.7	16.9	7.2	7.1	7.2	6.03	6.10	5.85
Wed 21	14 – 23	17.0	16.9	17.1	6.9	7.0	6.9	6.0	6.10	5.80
Thur 22	12 – 28	18.2	18.1	18.2	6.6	6.9	6.6	5.92	5.93	5.80
Frid 23	12 – 27	18.1	18.1	18.1	6.6	6.9	6.6	5.91	5.92	5.81
Sat 24	12 – 28	18.0	19.9	18.1	6.6	6.8	6.6	5.80	5.82	5.80
Sun 25	13 – 24	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T
Mon 26	14 – 25	17.1	17.1	17.2	6.8	6.9	6.6	5.77	5.75	5.76
Tues 27	13 – 19	16.1	16.0	16.0	6.8	6.8	6.7	5.76	5.75	5.76
Wed 28	11 – 21	16.5	16.5	16.5	6.8	6.8	6.8	5.77	5.76	5.76
Thur 29	9 - 29	18.7	18.8	18.8	6.6	6.7	6.6	5.70	5.69	5.72
Frid 30	15 – 34	20	20.1	20.1	6.6	6.7	6.6	5.72	5.70	5.71
Sat 1	16 – 28	18.5	18.6	18.5	6.6	6.8	6.6	5.72	5.71	5.73
Sun 2	15 – 25	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T
Mon 3	13 - 21	16.5	16.5	16.5	6.6	6.7	6.6	5.73	5.72	5.73
Tues 4	13 – 22	16.6	16.7	16.6	6.7	6.7	6.6	5.70	5.71	5.73
Wed 5	15 – 26	17.3	17.2	17.3	6.9	6.8	6.9	5.70	5.70	5.72
Thur 6	14 – 23	16.8	16.6	16.9	7.0	6.8	7.1	5.71	5.71	5.71
Frid 7	14 – 22	17.0	16.9	17.0	7.0	6.9	7.1	5.72	5.71	5.72
Sat 8	11 – 22	17.1	17.1	17.2	7.2	6.9	7.2	5.71	5.72	5.71
Sun 9	12 - 26	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T
Mon 10	12 - 28	18.3	18.4	18.3	6.6	6.8	6.5	5.73	5.72	5.74
Tues 11	15 - 29	18.5	18.5	18.5	6.5	6.9	6.5	5.74	5.73	5.74
Wed 12	16 – 24	17.8	17.9	17.9	6.7	6.8	6.6	5.74	5.74	5.75
Thur 13	12 – 22	16.8	16.7	16.8	7.1	6.9	7.0	5.74	5.74	5.73
Frid 14	11 - 26	17.6	17.7	17.7	6.8	6.9	6.7	5.74	5.73	5.74
Sat 15	13 - 27	18.3	18.2	18.2	6.6	6.9	6.5	5.75	5.73	5.74

Supplementary Table B2: Production cycle 2, 29 December 2018 – 29 January 2019

Date	Atmospheric(°C)	Water (°C)			Dissolved Oxygen (mgl^{-1})			pH		
		Control	KDF		Control	KDF		Control	KDF	
Sat 29	12 – 28	18.5	18.4	18.5	6.9	6.8	6.7	6.49	6.51	6.50
Sun 30	14 - 26	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T
Mon 31	14 - 33	22	22.1	22.1	6.4	6.6	6.6	6.49	6.51	6.50
Tues 1	17 – 26	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T
Wed 2	17 – 25	18.0	18.1	18.0	6.9	7.0	6.8	6.50	6.51	6.50
Thur 3	19 – 31	22.5	22.5	22.5	5.9	6.5	6.1	6.48	6.50	6.50
Frid 4	15 – 27	18.3	18.2	18.2	6.8	6.5	6.8	6.46	6.51	6.51
Sat 5	16 – 26	18.2	18.1	18.2	6.9	6.7	6.9	6.48	6.50	6.50
Sun 6	13 – 30	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T
Mon 7	15 - 28	21.0	21.1	21.0	5.8	6.5	6.1	5.70	5.91	5.80
Tues 8	15 – 26	18.2	18.1	18.2	6.6	6.4	6.5	5.71	5.89	5.79
Wed 9	18 – 29	20.2	20.1	20.2	6.2	6.7	6.5	5.72	5.89	5.74
Thur 10	15 – 24	17.8	17.8	17.8	6.9	6.8	6.8	5.71	5.88	5.74
Frid 11	14 – 25	18.9	18.8	18.9	6.9	6.8	6.9	5.72	5.89	5.73
Sat 12	11 – 23	17.0	17.1	17.0	6.9	7.2	7.0	5.74	5.85	5.74
Sun 13	16 - 26	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T
Mon 14	13 - 26	21.3	21.3	21.4	6.3	6.7	6.5	5.80	5.84	5.75
Tues 15	14 - 26	21.2	21.1	21.2	6.0	6.8	6.4	5.79	5.85	5.76
Wed 16	19 - 32	21.6	21.5	21.6	6.0	6.6	6.3	5.78	5.80	5.75
Thur 17	17 – 29	20.5	20.4	20.4	6.1	6.5	6.3	5.77	5.81	5.75
Frid 18	14 – 22	17.5	17.5	17.5	7.1	6.8	6.9	5.78	5.80	5.78
Sat 19	12 – 21	17.2	17.3	17.2	7.0	6.9	7.0	5.80	5.80	5.80
Sun 20	10 – 30	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T
Mon 21	13 – 30	22.0	22.1	22.1	6.8	6.9	6.9	5.79	5.80	5.78
Tues 22	16 – 25	19.0	19.2	19.0	6.9	7.0	6.9	5.79	5.81	5.79
Wed 23	13 - 22	17.4	17.5	17.5	7.2	7.0	7.1	5.78	5.79	5.80
Thur 24	14 – 28	20.2	20.5	20.2	6.2	6.8	6.6	5.77	5.80	5.78
Frid 25	14 – 29	20.5	20.5	20.5	6.0	6.5	6.3	5.77	5.80	5.77
Sat 26	19 – 34	22.2	22.3	22.3	6.1	6.3	6.3	5.76	5.81	5.76
Sun 27	18 – 33	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T
Mon 28	21 – 36	22.5	22.5	22.5	5.8	6.3	5.9	5.77	5.80	5.76
Tues 29	17 - 30	21.5	21.6	21.5	5.9	6.3	5.9	5.77	5.80	5.76

Supplementary Table B3: Production cycle 3, 04 February – 06 March 2019

Date	Atmospheric(°C)	Water (°C)			Dissolved Oxygen (mgl^{-1})			pH		
		Control	KDF	$FeSO_4$	Control	KDF	$FeSO_4$	Control	KDF	$FeSO_4$
Mon 4	13 - 26	18.3	18.3	18.2	6.4	6.5	6.4	7.0	7.1	6.90
Tues 5	14 - 30	22.2	22.2	22.3	6.0	6.1	6.2	7.0	7.0	6.85
Wed 6	15 - 35	23.2	23.1	23.2	5.8	5.8	6.0	6.90	7.0	6.88
Thur 7	19 - 39	24.0	24.1	24.1	5.8	5.8	6.1	7.1	6.90	6.89
Frid 8	17 - 28	19.8	19.9	19.9	6.7	6.7	6.3	6.90	6.89	6.88
Sat 9	16 – 29	20.1	20.0	20.0	6.8	6.7	6.4	6.90	6.89	6.89
Sun 10	17 – 28	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T
Mon 11	17 - 28	20.0	19.9	19.9	6.7	6.6	6.8	6.89	6.89	6.88
Tues 12	19 - 30	22.4	22.2	22.3	6.8	6.6	6.8	6.88	6.88	6.87
Wed 13	16 - 32	22.5	22.4	22.5	6.6	6.7	6.7	6.87	6.87	6.88
Thur 14	20 – 39	24.5	24.3	24.5	5.8	5.9	5.9	6.86	6.86	6.87
Frid 15	17 – 32	23.5	23.5	23.4	6.1	5.9	6.0	6.86	6.85	6.86
Sat 16	17 - 24	18.0	17.9	18.0	6.9	6.8	6.8	6.85	6.88	6.85
Sun 17	14 - 34	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T
Mon 18	16 - 34	23.1	23.2	23.0	6.8	6.9	6.8	6.66	6.80	6.80
Tues 19	17 - 32	22.8	22.8	22.9	6.2	6.3	6.4	6.65	6.80	6.75
Wed 20	17 - 26	19.0	19.1	19.1	6.8	7.0	6.6	6.66	6.79	6.76
Thur 21	16 – 31	22.1	22.1	22.0	6.9	7.0	6.8	6.54	6.79	6.74
Frid 22	18 – 33	23.0	23.1	22.9	6.0	6.2	6.2	6.54	6.78	6.56
Sat 23	15 – 29	20.0	20.0	20.0	6.1	6.0	6.2	6.54	6.75	6.55
Sun 24	17 - 26	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T	N/T
Mon 25	15 - 30	22.4	22.3	22.3	6.2	6.3	6.3	5.60	5.66	5.50
Tues 26	15 - 22	17.5	17.5	17.6	7.0	6.9	6.9	5.54	5.56	5.50
Wed 27	13 - 23	17.2	17.3	17.2	6.6	6.6	6.7	5.52	5.55	5.50
Thur 28	16 - 25	18.1	18.0	18.0	6.8	6.7	6.7	5.53	5.53	5.52
Frid 01	18 - 26	18.5	18.5	18.5	6.8	6.7	6.7	5.50	5.53	5.51
Sat 02	17 - 25	18.2	18.1	18.3	6.6	6.7	6.8	5.51	5.51	5.52
Sun 03	16 - 28	20.1	20.0	20.0	6.1	6.3	6.5	5.50	5.51	5.51
Mon 04	16 - 23	18.0	18.0	18.1	6.9	6.8	6.8	5.52	5.50	5.50
Tues 05	12 - 27	19.0	19.1	19.1	7.0	6.8	6.8	5.51	5.51	5.50
Wed 06	16 - 30	22.0	22.1	22.1	5.8	6.8	6.8	5.50	5.51	5.52

N/T: Not taken