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# GROWTH PERFORMANCE OF SPINACH (SPINACIA OLERACEA)ON DIETS SUPPLEMENTED WITH IRON-AMINO ACID COMPLEX IN AN AQUAPONIC SYSTEM IN KENYA

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# Abstract

Aquaponic is an environmental-friendly production system involving reuse of waste and nutrients in production of fish and vegetables. Currently the system experiences unbalance in pH and nutrients deficiency in plants. This study investigated the effect of iron amino acid chelate supplement in fish feeds on growth performance of spinach (*Spinacia oleracea*) in aquaponic system. The experimental research was conducted at the University of Eldoret from August-December 2016. A complete randomized design was used in triplicate treatments. The supplementation quantity in fish diets constituted 30 Fe kg<sup>-1</sup>, 20 Fe kg<sup>-1</sup> and 0 Fe kg<sup>-1</sup> of iron amino acid chelate respectively. At 30 Fe kg<sup>-1</sup>treatments spinach indicated a significant growth at (p < 0.05) than other treatments with final mean height (52.44  $\pm$  0.798cm) and 19.33 leaves. The least growth of spinach was at 0 Fe kg<sup>-1</sup> treatments with final mean (25.36  $\pm$  0.72 cm, 9.704) height andleaves respectively.30 Fe kg<sup>-1</sup> exhibited highest nutrients and improved water quality as compared to other treatments. The results revealed that 30 Fe kg<sup>-1</sup> iron amino acid chelate supplementation had significant nutritional attributes as feedstuff in aquaponic system for spinach growth than other dietary treatment tested.

# Introduction

Aquaponics is an integrated multi-trophic system that combines elements of recirculating aquaculture and hydroponics (Connolly and Trebic, 2010, wherein the water from the fish tanks that is enriched in nutrients is used for plant growth. The plants are grown in raised beds and this reduces the surface area needed for them to grow. Toxic waste products from fish are removed by vegetables and aerobic micro-organism. This allows the recirculating aquaponic system to raise large quantities of fish in relatively small volumes (Ezekiel, 2015). Plants can grow very fast when they utilize dissolved nutrients derived from fish excretions and the microbial breakdown of wastes in the system. The bacteria in the water and in the growing medium then convert ammonia to nitrite and then to nitrate. The nitrate is the most preferred form of nutrient for growing plants in aquaponic system because it is relatively harmless to fish (Rakocy *et al.*, 2015).

Feed formulations account for more than 50% of the total production cost in modern intensive aquaculture (Ibrahem *et al.*, 2010). Increasing feed efficiency especially by improving the metabolic assimilation of dietary nutrients, is of high priority in contemporary animal production (Ibrahem *et al.*, 2010). The aqua-farm industry dependmost on the fishmeal, which is the main source of protein for fish feed owing to excellent amino acid and fatty acid and minerals traces elements profile. Limited supply, high cost and stagnant production level restrict its use for sustainable fish farming (Baruah *et al.*, 2004). Macro and micronutrients transfers from aquaculture to plants component support efficient nutrient recycling, while water recirculation in the system reduces the water lost (Ezekiel, 2015). High production in the plant component in aquaponic systems require a specific amount of macro- and micronutrients from industrial and mining origin, leading to high energy and finite resources use. It is known that fish feed ingredient cannot provide all the essential growths nutrients to plants in an aquaponic system (Sarker *et al.*, 2007) but there are some number of micro- nutrient supplement



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with suitable amino acid profiles used to improve fish growth performance as well as the plants growth. According to (Apines-Amaret, 2004) chelated amino acids can be used as a growth promoter but the mechanism of action of amino acid chelates with traces of micronutrients as a growth promoter is not yet adequately researched. Chelated amino acid iron could have the ability to increase bioavailability of nutrients in plant.

This study was therefore conducted to assess the effect of iron amino acid chelate supplement in fish feeds on growth performance of spinach (*Spinacia oleracea*) in a recirculating aquaponic system at the University of Eldoret Fish Farm. The Experiments were used to elaborate on the theoretical, practical and application potential of gravel based aquaponic systems that can be adopted by local farmers

# Materials and methods

### Study Area

The experimental research was conducted at University of Eldoret hatchery of the Fisheries Department for a period of 119 days. The university is situated 9Km north east of Eldoret Municipality, on the Eldoret - Ziwa road. Global position of 0°35°N and 35°N-12°E at an altitude of 2180 m above sea level. Temperatures 8.4 °C - 25 °C, 2 rainy seasons – 900mm to 1,200mm per annum, the hatchery is 500m from 2.6 acre university fish farm. The farm has 42 earthen ponds, and rears Nile tilapia, goldfish and African catfish.

### **Experimental Design**

The experiment were conducted in an aquaponic system, which was consist of 12 rectangular indoor aquaria fish tank (45cm by 35cm by 35cm each) and 12 rectangular plastic plant components (1m by 0.5m) of gravel substrate area each. Water was continuously recirculated from the rearing tanks through sump and pumped through to the filter plants and gravels then back to the fish culture. Fish with the same initial average wet weight and average length was be selected randomly and stocked with the same stocking densities (30) pieces in each aquaponic system; with plants treatments carries 9 plants each. All the treatments were replicated three times in a completely randomized design layout. Each treatment diets with supplement 10 Fe kg<sup>-1</sup>, 20 Fe kg<sup>-1</sup> and 30 Fe kg<sup>-1</sup> iron amino acid chelate and control (0 Fe kg<sup>-1</sup>) iron amino acid chelate was administered respectively for the experiment.

#### Sampling

#### Water sampling

Water quality parameters which were measured include Dissolved oxygen, temperature, pH, Ammonia, nitrite and nitrate. Where water temperature, dissolved oxygen, conductivity and pH were measured using thermometer, an Oxymeter (YSI 200 model) and a portable field pH meter respectively three times a week in fish and plants components. While ammonia  $(NH_4^+)$ , nitrate  $(NO_3^-)$  and iron were measured weekly using YSI 9500 photometer

#### Plants sampling

Each week, 9 Spinachwereselected randomly from each tank and individually measured only for height and leaves number counts while wet and dry weights were only measured at initial and harvesting stage. Vernier caliper (westward, 0.01mm) were used to measure height and stem diameter, a digital scale (Ohaus, 0.01g) were used to record wet weight, dry weight and leaves number.

# Feeds

#### **Feed Ingredients**

Commercial diets were locally purchased and formulated at the same crude protein (30% CP) but with different supplement level of iron amino acid chelate (10 Fe kg<sup>-1</sup>, 20 Fe kg<sup>-1</sup> and 30 Fe kg<sup>-1</sup>) respectively which were commercially bought. Example in Diet 1, Diet 2, Diet 3 and Diet 4 in figure 1.



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# Feed formulation and proximate analysis

Feed ingredients were subjected to proximate analysis before diet formulation to determine the crude protein, moisture content, lipid, crude fiber, ash and digestible carbohydrate. Experimental diets were formulated using the Winfeed (version 2.8) computer program at 30% crude protein content. The ingredients was weighed and ground to small particle size and thoroughly mixed with water to obtain a 30% moisture level. Corn Oil, Vitamins and minerals mixture were added to the diets. Iron amino acid chelate was included in diet formulations to serve as traces iron and amino acid supplements.

Table 1:	Feed ingredient required for	or formulation of exp	erimental Diets	
Ingredients	Diet 1 (10gkg <sup>-1</sup> )	Diet 2(20gkg <sup>-1</sup> )	Diet 3 (30gkg <sup>-</sup> 1)	Diet 4(0gkg <sup>-1</sup> )
<u> </u>	CP (32%)	CP (32%)	CP (32%)	CP (32%)
Yellow corn	48	48	48	48
Soy bean meal (44%)	18.5	18.5	18.5	18.5
Fishmeal (72%)	26.0	26.0	26.0	26.0
Corn oil	5	5	5	5
Vitamin premix	1.5	1.5	1.5	1.5
Starch	1	1	1	1
Iron amino acid chelates	10gkg <sup>-1</sup>	20gkg <sup>-1</sup>	10gkg <sup>-1</sup>	0gkg <sup>-1</sup>

Composition	Experimental	Experimental diets				
	<b>0</b> Fe kg <sup>-1</sup>	<b>10 Fe kg</b> <sup>-1</sup>	<b>20 Fe kg</b> <sup>-1</sup>	<b>30 Fe kg</b> <sup>-1</sup>		
Dry matter (%)	91.2±0.012	90.75±0.024	90.55±0.014	91.02±0.241		
Ash content (%)	$12.9{\pm}0.97$	12.9±0.0423	12.425±0.166	14.142±0.119		
Crude lipids (%)	$14.2\pm0.66$	13.208±0.377	14.067±0.156	12.458±0.313		
Crude protein (%)	$33.47{\pm}0.39$	34.492±0.546	35.454±0.553	35.60±1.33		
Moisture content (%)	$0.642 \pm 0.35$	$0.487 {\pm} 0.001$	$0.542 \pm 0.275$	$0.452 \pm 0.124$		
Crude fibre (%)	3.21	2.98	2.95	2.82		
NFE	36.219	36.345	35.104	34.98		

# Data Analysis

All data were subjected to normality test using Kolmogorov–Smirnova. One way analysis of variance (ANOVA) followed by Tukey's multiple-comparison post hoc test were applied to determine differences among all treatment in plants growth: dry weights, wet weights, plants heights while Kruskal Wallis were used to determined difference on number of spinach leaves. The comparison of regression lines procedure was designed to compare the regression lines relating the growth rate in terms of log number of leaves and plant heights of spinach in aquaponic system using single factor classification for multiple slopes implemented in Statgraphics Ver. 16 (StatPoint Technologies, 2010).

# Results

# Plants Growth in Aquaponic System

The results of *Spinacia oleracea* in the different treatments have been represented in Table 3. The mean final spinach wet weight (113.6  $\pm$  9.46g), number of leaves (19.33) and plant height (52.44  $\pm$  0.798 cm) was highest in the 30g Fe kg<sup>-1</sup> treatment whereas the 0g Fe kg<sup>-1</sup> treatment had the lowest mean wet weight (30.65  $\pm$  2.15g), number of leaves (9.704  $\pm$  0.225) and plant height (25.36  $\pm$  0.723cm).

There was a significant (p < 0.05) difference in mean *S. oleracea* wet weight. The 30g Fe kg<sup>-1</sup> treatment gave the highest mean final wet weight (113.6  $\pm$  9.46g) and mean final dry weight (32.973  $\pm$  0.253g) as compared



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with other treatments which had  $107.39 \pm 9.48$  g,  $59.75 \pm 2.8$  g,  $30.65 \pm 2.15$  g wet weight and  $23.796 \pm 0.215$  g,  $6.7422 \pm 0.0445$ g,  $4.1704 \pm 0.0816$  g mean dry weight (20g Fe kg<sup>-1</sup>, 10g Fe kg<sup>-1</sup> and 0g Fe kg<sup>-1</sup>) respectively. Mean number of leaves was significantly (p < 0.05) among the treatments.

The highest mean number of leaves was recorded in 30g Fe kg<sup>-1</sup> (19.33  $\pm$  0.392) and the lowest of the same was recorded in 20g Fe kg<sup>-1</sup> (15.70  $\pm$  0.509) (Fig. 1). Kruskal-Wallis non-parametric One-Way ANOVA showed that the number of leaves counted in all treatment was significantly varied (H = 37.79, DF = 3, p-value = 0.001). 30g Fe kg<sup>-1</sup> gave the highest (19.33  $\pm$  0.392) and 0 Fe kg<sup>-1</sup> gave the lowest (9.704  $\pm$  0.225).

Treatments				
0g Fe kg <sup>-1</sup>	<b>10g Fe kg</b> <sup>-1</sup>	<b>20g Fe kg</b> <sup>-1</sup>	<b>30g Fe kg</b> <sup>-1</sup>	
2±0.131 <sup>a</sup>	2±0.131 <sup>a</sup>	2±0.131 <sup>a</sup>	2±0.131 <sup>a</sup>	
9.704±0.225ª	12.85±0.16 <sup>b</sup>	15.70±0.509°	$19.33 \pm 0.392^{d}$	
3±0.131 <sup>a</sup>	3±0.131 <sup>a</sup>	3±0.131 <sup>a</sup>	3±0.131 <sup>a</sup>	
25.36±0.723ª	33.33±1.37 <sup>b</sup>	41.52±0.633°	$52.44 \pm 0.798^{d}$	
$0.592 \pm 0.523^{a}$	$0.592 \pm 0.523^{a}$	$0.592 \pm 0.523^{a}$	0.592±0.523 <sup>a</sup>	
$30.65 \pm 2.15^{a}$	$59.75 \pm 2.8^{b}$	107.39±9.48°	113.6±9.46°	
$0.197{\pm}0.017^a$	$0.197{\pm}0.017^{a}$	$0.197{\pm}0.017^a$	$0.197 \pm 0.017^{a}$	
$4.170 \pm 0.082^{a}$	$6.742 \pm 0.0445^{b}$	$23.796 \pm 0.22^{\circ}$	$32.973\pm0.25^d$	
$30.058{\pm}1.627^{a}$	59.16±2.277 <sup>b</sup>	106.8 ±8.957°	$113.01{\pm}8.94^{c}$	
	$\begin{array}{c} \hline \mathbf{0g \ Fe \ kg^{-1}} \\ \hline \mathbf{0g \ Fe \ kg^{-1}} \\ \hline 2\pm 0.131^{a} \\ 9.704\pm 0.225^{a} \\ 3\pm 0.131^{a} \\ 25.36\pm 0.723^{a} \\ 0.592\pm 0.523^{a} \\ 30.65\pm 2.15^{a} \\ 0.197\pm 0.017^{a} \\ 4.170\pm 0.082^{a} \end{array}$	$0g Fe kg^{-1}$ $10g Fe kg^{-1}$ $2\pm 0.131^a$ $2\pm 0.131^a$ $9.704\pm 0.225^a$ $12.85\pm 0.16^b$ $3\pm 0.131^a$ $3\pm 0.131^a$ $25.36\pm 0.723^a$ $33.33\pm 1.37^b$ $0.592\pm 0.523^a$ $0.592\pm 0.523^a$ $30.65\pm 2.15^a$ $59.75\pm 2.8^b$ $0.197\pm 0.017^a$ $0.197\pm 0.017^a$ $4.170\pm 0.082^a$ $6.742\pm 0.0445^b$	$0g Fe kg^{-1}$ $10g Fe kg^{-1}$ $20g Fe kg^{-1}$ $2 \pm 0.131^a$ $2 \pm 0.131^a$ $2 \pm 0.131^a$ $9.704 \pm 0.225^a$ $12.85 \pm 0.16^b$ $15.70 \pm 0.509^c$ $3 \pm 0.131^a$ $3 \pm 0.131^a$ $3 \pm 0.131^a$ $25.36 \pm 0.723^a$ $33.33 \pm 1.37^b$ $41.52 \pm 0.633^c$ $0.592 \pm 0.523^a$ $0.592 \pm 0.523^a$ $0.592 \pm 0.523^a$ $30.65 \pm 2.15^a$ $59.75 \pm 2.8^b$ $107.39 \pm 9.48^c$ $0.197 \pm 0.017^a$ $0.197 \pm 0.017^a$ $0.197 \pm 0.017^a$ $4.170 \pm 0.082^a$ $6.742 \pm 0.0445^b$ $23.796 \pm 0.22^c$	

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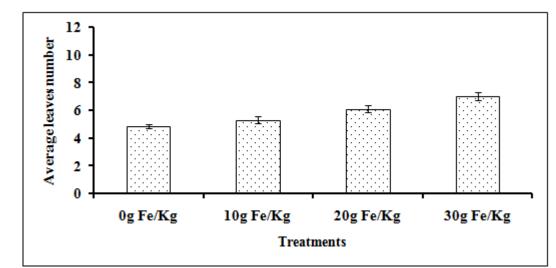


Figure 1: Mean ± SE number of leaves of Spinacia oleracea in an aquaponic system from four different iron amino acid chelate supplementation treatments

#### Comparison regression of spinach heights in an aquaponic system

Higher slope spinach heightsvalue was recorded for Diet 4 treatment(0.430084) and the lowest for diet 1 treatment(0.233699). In the first days the spinach heights were slightly the same in all treatments but after twentieth day spinach heights show significant (p < 0.05) difference up to the entire period of 119 day (Fig 2). Since the P-value is less than 0.05, there is an indication of possible serial correlation at the 95.0% confidence level. Because the P-value for the slopes is less than 0.01, there are statistically significant differences among the slopes for the various values of Treatment at the 99% confidence level (Fig 2).

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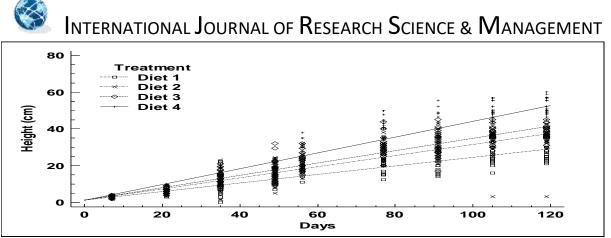


Figure 2: Linear regression model describing the relationship between Height (cm) of Spinacia oleracea, sampled period (days) and four treatments in aquaponic system

# comparison regression of spinach number of leaves in an aquaponic system

There was higher slope spinach number of leavesvalue was recorded in Diet 4 treatment(0.00733283) and the lowest for diet 1 treatment(0.00513697). In the first days the number of leaves were slightly the same in all treatments but after twentieth day spinach leaves show significantly (p < 0.05) difference up to the entire period of 119 day (Fig 3). The differences among the treatments were found to be significant (p < 0.05) difference where diets 4 treatments performed better than the other three treatments. The highest mean number of leaves was recorded in Diet 4 treatments and the lowest was recorded in Diet 1 treatment (Fig 3).

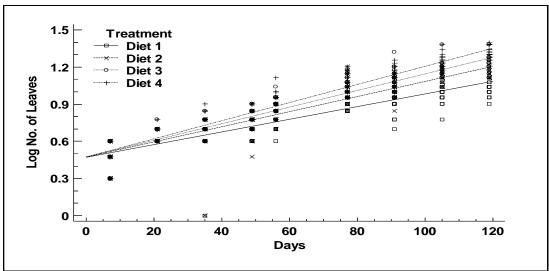


Figure 3: Linear regression model describing the relationship between Spinacia oleracea number of leaves, sampled period (days) and four treatments in aquaponic system

#### Dry and wet weights of spinach growth in an aquaponic

The results of *Spinacia oleracea* dry and wet weights in different treatments in the aquaponic system have been represented in (Fig 4 and 5). There was a significant (p < 0.05) difference in mean *S. oleracea* wet weights and dry weights. The 30g Fe kg<sup>-1</sup> treatment gave the highest mean final wet weight (113.6  $\pm$  9.46g) and mean final dry weight (32.973  $\pm$  0.253g) as compared with other treatments which had 107.39  $\pm$  9.48 g, 59.75  $\pm$  2.8 g, 30.65  $\pm$  2.15 g wet weight and 23.796  $\pm$  0.215 g, 6.7422  $\pm$  0.0445 g, 4.1704  $\pm$  0.0816 g mean dry weight (20g Fe kg<sup>-1</sup> and 0g Fe kg<sup>-1</sup>) respectively. The parametric One-Way ANOVA in both the dry and wet weight of *S. oleracea* in all treatments differed significantly difference (Dry: F(3,104) = 6432.221, p-value = 0.0000) (Fig 64) and (Wet: F(3,104) = 32.9886, p- value = 0.000) respectively (Fig. 5).



[Rono \* et al., 5(7): July, 2018]

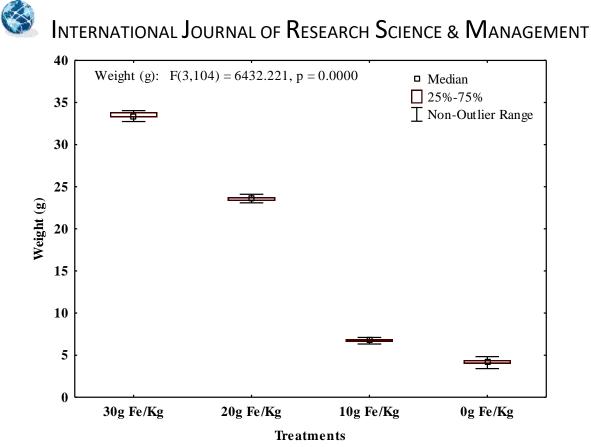


Figure 4:Dry weights of Spinacia oleracea in an aquaponic system from four different iron amino acid supplementation treatments



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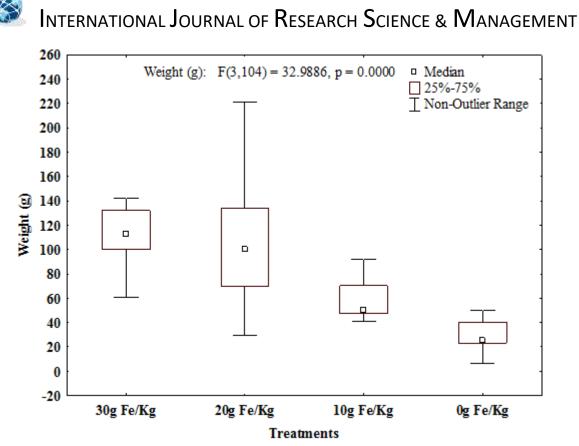


Figure 5: Wet weights of Spinacia oleracea in an aquaponic system from four different iron amino acid supplementation treatments

# Water quality in aquaponic system

The Physico- chemical parameters of aquaponic water have been represented in Table 4. Results of one-way ANOVA test indicated a significant difference in ammonia, nitrate, iron, pH and conductivity ( $F_{0.05, 3}$  = 8.63; p-value = 0.0001), ( $F_{0.05, 3}$  = 11.34; p-value = 0.0001), ( $F_{0.05, 3}$  = 79.88; p-value = 0.0001), ( $F_{0.05, 3}$  = 89.95, p-value = 0.0001) and ( $F_{0.05, 3}$  = 30.74; p-value = 0.0001) respectively among the treatments. However, there was no significant difference ( $F_{0.05, 3}$  = 0.5; p-value = 0.679) in temperature between treatments respectively (Table 4).

Table 4: Mean water chemistry parameters for	the four treatments (0, 10, 20 and 30 Fe kg <sup>-1</sup> ) in aquaponic system			

Parameter -	Plants component				
	<b>0</b> Fe kg <sup>-1</sup>	<b>10 Fe kg</b> <sup>-1</sup>	<b>20 Fe kg</b> <sup>-1</sup>	<b>30 Fe kg</b> <sup>-1</sup>	•
Ammonia (mg L <sup>-1</sup> )	$0.5522 \pm 0.088^{a}$	$0.19 \pm 0.034^{b}$	$0.297 \pm 0.05^{\circ}$	$0.21 \pm 0.04^{d}$	
Nitrate (mg L <sup>-1</sup> )	$1.397{\pm}0.154^{a}$	$1.677 \pm 0.18^{b}$	2.373±0.22°	$2.95{\pm}0.26^d$	
Iron (mg L <sup>-1</sup> )	0.039±0.01ª	$1.083 \pm 0.12^{b}$	2.237±0.21°	$2.89 \pm 0.153^{d}$	
pH	$7.86{\pm}0.035^{a}$	$7.75 \pm 0.029^{b}$	7.29±0.037°	$7.14 \pm 0.04^{d}$	
Conductivity ( $\mu$ S cm <sup>-1</sup> )	$427.3\pm25.7^{a}$	794.6±53.5 <sup>b</sup>	$1108 \pm 70.4^{\circ}$	972.4±53°	
Temperature (°C)	22.650±0.24ª	23.014±0.2 <sup>a</sup>	22.903±0.2 <sup>a</sup>	$22.858{\pm}0.2^{a}$	

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# Discussion

#### Growth of Spinach (Spinacia oleracea) in aquaponic system

Iron is one of the most important micronutrients for plant growth and productivity and its availability affects many plant morphological, physiological and biochemical parameters (Steer and Hocking 1984). Plants require iron traces element amount, thus it is often the growth limiting micronutrient. From the results it is concluded that iron amino chelate and some nitrogen deficiency significantly decreased growth parameters of spinach cultivated in aquaponic system, such as biomass accumulation, plants heights and the number of leaves, in agreement with earlier findings with plants like salvia (*Salvia splendens* L.) (Kang and Van Iersel, 2004), dill (*Anethumgraveolens* L.), thyme (*Thymus vulgaris* L.) and lettuce (*Lactuca sativa* L.) (Marsic and Osvald, 2002). According to Golcz *et al.*, (2006) and Olfati *et al.*, (2012), nitrogen fertilization has been also shown to directly correlate with the growth, yield, and essential oil content of basil plants, in contrast with Jacimovic *et al.*, (2010).

Both plant species of the all treatment showed poor growth at first week, which might be due to the sensitivity of the root system on nutrients characteristics present in the system (Licamele et al., 2009). Growth in terms of plants means final height, number of leaves, wet weight, weight gain and dry weight of S. oleracea was significantly different in all treatments, higher in 30 Fe kg<sup>-1</sup> treatment where iron amino acid chelate supplementation was high compared to treatments 20 Fe kg<sup>-1</sup>, 10 Fe kg<sup>-1</sup> and 0 Fe kg<sup>-1</sup> although the same plants was applied at an equal ratio in all the plants treatments. The causes might include high nutrients due to higher iron amino acid supplementation which stabilizes pH and allows the present of nitrate and iron bioavailability. Similar with findings of Danaher, 2013 who reported better growth in terms of heights, number of leaves, diameter, dry and weight in supplementation of iron amino acid chelate in aquaponic system also further indicated that supplementation of iron amino acid chelate stabilized pH and provide adequate nutrients for the plants growth. The number of leaves was significantly difference where 30 Fe kg<sup>-1</sup> had the highest and 0 Fe kg<sup>-1</sup> had the lowest this might be probably because of nutrient reflection with the increment of iron supplementation (Hamid and Simin, 2012) reported that when the amount of nutrients does not suffice, the growth of leaves and then, leaf areas index can be limited due to either the low level of photosynthesis or insufficient cell elongation. The cells of leaves are smaller in plants suffering from nitrogen deficiency (Hamid and Simin, 2012). These effects arise from the decrease in water conductance which results in water deficiency in the sheaths of growing leaves (Hamid and Simin, 2012). Yield on the dry and wet weights was statistically significant difference among all the treatment 0 Fe kg<sup>-1</sup> treatment had the lowest as compared with 10 Fe kg<sup>-1</sup>, 20 Fe kg<sup>-1</sup> and 30 Fe kg<sup>-1</sup> <sup>1</sup>, these give an indication that supplementation of iron amino acid chelated promote the accumulation of K and N which decreases longitudinal growth and increases the formation of auxiliary roots (Hamid and Simin, 2012) reported that K and N deficiencies increase longitudinal growth and reduce the formation of auxiliary roots.

#### Water quality in aquaponic system

Physico- chemical parameters played a significant role in the maintenance of a healthy aquatic environment and production of natural food organism (Monir*et al.*, 2013). Aquaculture waste nutrients should ideally meet the requirements of plants co-cultured in aquaponic systems (Trang *et al.*, 2010). In fish and plants component temperature and dissolved oxygen showed no significant (p > 0.05) difference among the treatments. Water temperature for all treatment varied with the average value of (23°c) was within the normal range for the survival of spinach.

The observed and recorded pH values were within the accepted levels for Spinach plants growth, significant difference (p < 0.05) were recorded among all the treatments. High pH was recorded in 0 Fe kg<sup>-1</sup> followed by 10 Fe kg<sup>-1</sup>, 20 Fe kg<sup>-1</sup> and low in 30 Fe kg<sup>-1</sup> iron amino acids chelates. Supplementation of iron amino acid reflects reduction in pH that is likely caused by the respiration of fish and bacteria that produce carbon dioxide. The presence of CO<sub>2</sub> will shift the equilibrium carbonate reaction, produces H<sup>+</sup> ions, and lowers the pH. Decrease in the pH was presumably associated with the oxidation process undertaken by bacteria in the system. According to Princic *et al.* (1998), in environments with high inputs such as ammonia from aquaculture wastewater, oxidation of this compound produces CO<sub>2</sub> and lowers the pH. The present result agrees with the findings of



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Hefni *et al.*, 2016 who reported that treatments with supplementation of micronutrients result to decrements of pH in relation to increment of the micronutrient in the diets and vice versa in aquaponic system.

However, high ammonia content was recorded in aquaponic system with treatments of 0 Fe kg<sup>-1</sup> iron amino acid supplement which might be due to poor utilization of the diets by the fish resulting to high accumulation of waste in the system also might be because of high pH more than 7.5 which cannot support survival of bacteria which can easier nitrification process to occur these findings are in conformity with the findings of Kohinoor et al. (2009) and Simeonidou et al., (2012). Nitrate concentration also increased during the experiment, and the concentration at the end is greater than the beginning of the experiment. Ammonia (NH<sub>4</sub>) assimilation occurs relatively rapidly by plants and metabolic reactions are more efficient than NO<sub>3</sub>. The low NO<sub>3</sub> removal by lettuce has been documented in other aquaponic systems (Buzby and Lin, 2014). During the experiment, the concentration of NO<sub>3</sub> was still supportive for the life of spinach in aquaponic system. According to Watson and Hill (2006), NO<sub>3</sub> should be maintained below 100 mg L<sup>-1</sup>. Nitrate concentration was highest in treatment 30 Fe kg<sup>-1</sup> and lowest in 0 Fe kg<sup>-1</sup>. The possible cause might be the amount concentration of oxygen supply. At 30Fe kg<sup>-1</sup> treatments oxygen supply was adequate for NO<sub>3</sub> oxidation process and offered favorable condition for bacteria to convert ammonia level to nitrate. However, in plants component ammonia and nitrate varied among all the treatments, ammonia was high in 0 Fe kg<sup>-1</sup> treatment as compared with other treatments probably due to poor nitrification of ammonia by bacteria to nitrate which was also reflected to be low nitrate at 0 Fe kg<sup>-1</sup> treatment. Lower nitrate removal rate and higher ammonia concentration rate which were accounted for in 0 Fe  $kg^{-1}$  treatments were in accordance with the findings of Endut *et al.*, (2009) in a study of aquaculture effluent treatments under different hydraulic loading rates using Ipomoea aquatica. Supplementation of iron amino acid chelated indicated that it affect ammonia and nitrate concentration in aquaponic system, this might be also due to the influence of iron amino acid chelate on lowering the pH and thus influence the nitrification process by bacteria. In plants components iron concentration varied among the treatments 30 Fe kg<sup>-1</sup> had the highest and 0 Fe kg<sup>-1</sup> had the lowest. There was an increase of iron concentration in aquaponic system in relation with the increment supplementation of iron amino acid chelated. The increase of iron concentration might be due to the present of iron traces in the diet.

# Conclusion

The present study on Spinach (*Spinacia oleracea*) growth has confirmed that iron chelate amino acids supplementation have positive effect on the growth index such as wet weight, dry weight, heights of the plants and the number of leaves of treated compared to the non-supplemented diets. According to the obtained results supplementation of iron amino acids chelate at 30 Fe kg<sup>-1</sup> exhibited best spinach (*Spinacia oleracea*) growth in terms of wet weights, dry weights, height of the plant and the number of leaves than the non-supplemented control diet with lower spinach growth parameters. Water quality parameters in iron amino acids chelate supplementation treatments in fish component indicated significance differences in plants component where 30 Fe kg<sup>-1</sup> treatment resulted in high nitrate, conductivity, iron concentration, oxygen with low ammonia and pH levels compared to the other treatments in plant components.

The experiment therefore demonstrated the ability of aquaponic systems to produce spinach (*Spinacia oleracea*) using iron amino acid chelate supplementation in fish diets as the nutrient sources. Generally our study reports that the growth of spinach greatly depend on the amount of iron amino acid chelates supplemented in the fish diet.

# Recommendation

The present findings indicated that supplementation of iron amino acid chelate in aquaponic system has potential of provide essential dissolved nutrient in aquaponic system hence improving the growth of spinach in the system.

The study recommends 30 Fe kg<sup>-1</sup> iron amino acid supplementation for spinach growth pertaining to the improved results in growth parameters in plant aquaponic system.



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