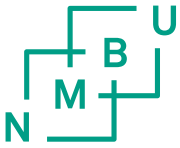
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Abstract

Abstract

The overall aim of the study was to estimate the digestibility of maggots by modifying diet replacement method, checking if fermentation activity occurs in mid to last part of intestine in Nile tilapia, and to evaluate the maggots potential as source of protein and lipid by conducting a growth trial. An eight day digestibility and 56 day growth trial was conducted to evaluate the digestibility of Asian latrine fly, *Cryosoma megacephala* maggots and, the growth performance parameters of Nile tilapia *Oreochromis niloticus* fed with maggots as the only source of protein and lipid. For digestibility trial, basal diet was replaced with one third and two third maggots. Feces were collected by dissecting the fish. Apparent digestibility coefficient for mid and distal parts of intestine were evaluated separately for energy, nitrogen and minerals, Ca, k, Mg, P and Zn. ADC of energy for two third maggot diet was significantly ($P<0.05$) high than one third replaced maggot diet and basal diet made of all plant protein sources. ADC of minerals showed high variations because of water borne mineral uptake by the fish. Cumulative feed intake (g), weight gain (g), final weight (g) and feed conversion ratio did not shown any significant differences for the fish fed with the maggot diet and the fish fed with the plant diet. High inclusion level of maggots in the maggot feed altered the growth performance negatively in the 2nd month of growth trial. So it is suggested that inclusion of maggots in the diet should be done preferably with less inclusion level and after having complete knowledge of the adverse effects of them.

Preface

The studies presented in this thesis has been a spiritual and fascinating journey which has given me great self-satisfaction and confidence, which is in fact the result of efforts and encouragement by many great people.

First of all I would like to thank God Almighty who given me another life when I was so quick to come to world (after seven months and with very little weight).

Special thanks to my Mom, who kept on raising me despite my rebelling attitude.

And yes my Late Dad, you did a great job by always letting me think freely. You was my great friend.

Trond storrebakken (my supervisor), finally you made chicken man to fish man. Thanks a lot Guru!

I would like to acknowledge Mr. Bjørn Reidar and Mr. Frank Sundby for their help and support in practical issues in the experiments.

Keke Zheng, thanks for your motivation and great help in experiments (sorry, I forget to mention great Chinese food). You are just great.

Thanks for all those unmentioned people who helped me out with their actions and thoughts.

Zulkernain Akhter

May, 2015

Abbreviations

(Systematic names of mineral elements not included)

ADC = Apparent digestibility coefficient

AM = before noon (Ante Meridiem)

ANOVA = Analysis of variance

BW = Body weight

CFI = Cumulative feed intake

CP = Crude protein

DFI = Daily feed intake

DM = Dry matter

DMI= Dry matter intake

FAO = Food and Agriculture Organization

FCR = Feed conversion ratio

GIFT = Genetically Improved Farmed Tilapia

h = Hour

ICP = Inductively-coupled plasma analysis

IPM = Institutt for plante- og miljøvitenskap
IHA = Institutt for husdyr og akvakulturskap

IW = Initial average fish weight

Kj = Kilojoules

l = liter

mg = milligram

min = minute

mm = milimeter

MS = Microsoft

n-6 = Polyunsaturated fatty acids with the first double bond in 6 position from the methyl end

N = nitrogen

NMBU = Norges miljø og biovitenskaplige universitet

NH_4-N = Ammonium-N

NO_{2-}N = Nitrite-N

N = Nitrogen

SAS = Statistical Analysis system

WG = Weight Gain

Contents

1. Introduction

1.1 Importance for feed in Tilapia Production

Responsible trend setting in aquaculture feeds is vital for the concrete solutions in place (Diana et al., 2013). Ensuring long-term production of the feed ingredients is key to sustainability. Plant protein ingredients e.g. soybeans, peas, lupins, canola, rapeseed along with their concentrates have played great role with coping the demands of the feed industry, but the nutritional short comings including phytic acid are still in place (Naylor et al., 2009). With weak amino acid profiles and lower energy and protein contents in the likes of high non-starch polysaccharides represents the cautions (Naylor et al., 2009).

Global Nile tilapia production is increasing 10% per annum. If this trend continues, the production will increase at the rate of 3 to 5 million tons per year until it will be doubled by 2030 (Bruinsma, 2003). The affordable price of the tilapias is one reason to believe that it is potential food for the rising world population. On the other hand, tilapias are not only popular in China where it is representing the second most eaten fish after carps but they are also finding their way in markets of Europe and North America other than its home ground in Africa (Bruinsma, 2003). Fish meal and fish oil, which are vitally used, but limited ingredients in the present day fish diets will exhibit hike in their prices by 2030. So as compared to salmonids, tilapias are more reliable with market perspective (Zarro, 2014). Being omnivorous, tilapias can be fed wide range of feed ingredients. They can be efficiently produced with the low cost diets based on the food by products. Thus, ensuring feed efficient production of Nile tilapia could be a major contribution to the future food supply of the world (Liang et al., 2011).

1.2 Potential of insect larvae in feed

Researchers have seen the great potential of using larvae based protein source as future feed ingredients for mono gastric animals both from sustainability point of view and as high nutritional value sources (Akpodiete et al., 1997; Bamgbose, 1999b; Bondari and Sheppard, 1981; Ebenso and Udo, 2004; Madu and Ufodike, 2004; Ogunji et al., 2008; Ogunji et al., 2007; Rumpold and Schlüter, 2013; Sing et al., 2014; van Huis, 2013; Zuidhof et al., 2003). The crude protein can be as high as 63% and crude fat can range from 12.5 to 36% in dry matter. Insect larvae have a balanced protein composition (Makkar et al., 2014; Ogunji et al., 2008). High palatability of the insect larvae meal also make them suitable feed ingredients (Makkar et al., 2014). No oxidative stress and negative metabolic effects has been reported while using maggots as feed ingredient in Nile tilapia diets (Ogunji et al., 2007).

Maggots can be produced rapidly and efficiently by composting animal wastes e.g. from pig and poultry manure. Two-stage composting has shown high production of maggots in short period of time (Zhu et al., 2012).

Maggots can also be reared by food waste from hospitals and schools (Sherman and Wyle, 1996). There are several factors involved to ensure the high production of maggots e.g. high egg production, high hatchability and the short duration of larval stage (Rumpold and Schlüter, 2013). There are other environmental factors which are important to consider while growing the maggots, e.g. temperature, light, humidity level and microbial contamination (Rumpold and Schlüter, 2013). Maggots can even be successfully turned into high value protein by using human soar (Lalander et al., 2013). Thus, their production can also be used to solve problems related to waste management.

A flow diagram is shown in Figure 1 to account the different stages of a maggot production process from rearing of larvae to end product (Rumpold and Schlüter, 2013).

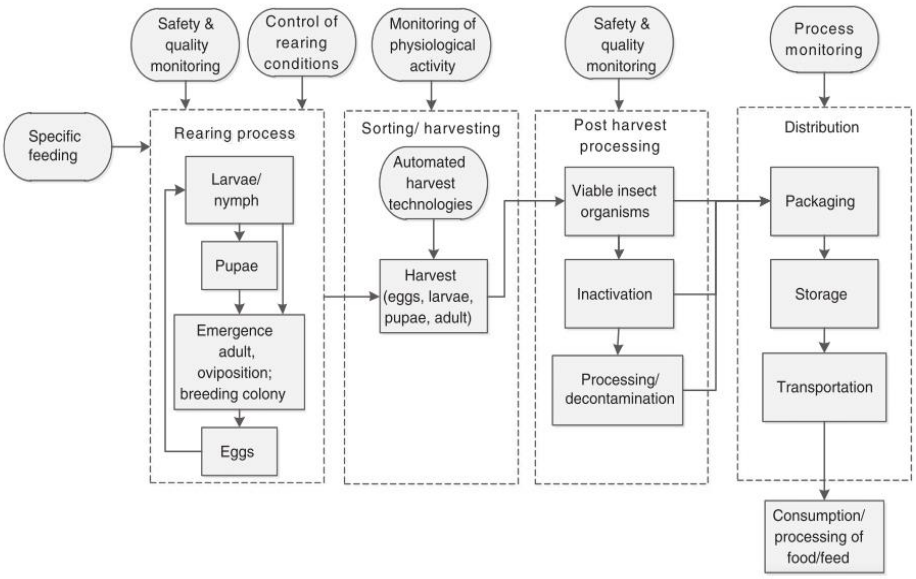

Figure 1. Schematic overview of different stages from a production point of view.

1.3 Digestibility assessment

As a first step on the way to understand the nutritional value of any feed ingredient in the feeds, digestibility studies play a vital role. Some queries are required to address properly before evaluating the ingredient, like what should be the inclusion level of the particular ingredient in the feed? How palatable is the ingredient for the fish and what is the economic standing of the ingredient (Ngo et al., 2015). Digestive physiology of fish and functional properties of the test

ingredient and its response to immune system of the fish species is also important to understand and therefore wide range of feeding strategies and formulation designs can be applied to evaluate the ingredient (Glencross et al., 2007). Exposure to extreme inclusion levels of a specific ingredient in the formulated diets in digestibility experiments could be vital to understand, and threshold limits may give good understanding of the ingredient digestibility and palatability.

The method used for collection of feces is key factor in digestibility experiments with fish (Glencross et al., 2007). Scientists have tried various ways to collect the feces to conduct the digestibility trials. The indirect method which depends on the use of an inert marker (Austreng, 1978) is much more reliable than assessment of total feed intake and feces production, due to leaching of nutrients to the water from feed and feces (Storebakken et al., 1998a).

Mechanical sieving methods for collecting the feces are still being practiced even they mostly give over estimates of digestibility (Gominho-Rosa et al., 2015; Mugo-Bundi et al., 2015). Feces collection by the Guelph system (Cho and Slinger, 1979) is one example of such a method. The advantage of this method is that it offers a high quantity of feces with small efforts, without posing stress to the fish. *In vitro*- digestibility assessment methods has also been tested and applied but its dependence on the collection of specific digestive enzymes makes it complicated and the correlation to *in vivo*- digestibility results maybe questionable (Yasumaru and Lemos, 2014).

The stripping method, employing careful abdominal pressure at the distal part of the belly (Austreng, 1978) has been seen as useful tool to collect the feces (Ngo et al., 2015) but frequent handling poses stress to the fish (Storebakken et al., 1998a). Unfortunately, the method can only be successfully applied to carnivore fish with short and simple intestine. Nile tilapia has a long intestine, up to 12 times its body length, and the intestine is highly twisted inside the body cavity (Glencross et al., 2007). Hence, till now the method involving dissection of the last part of the distal intestine and careful removal of its contents (Austreng, 1978) seems to be the most reliable method to collect feces of Nile tilapia. But at the same time cautions has to be take into account about the contamination and the intestinal tissues while collection of the feces.

1.3.1 Ingredient inclusion methods

The assessment of digestibility of a feed ingredient is mostly done by two methods, with the diet replacement method (DRM) or the ingredient replacement method (IRM) (Aksnes et al., 1996). In most cases when using the ingredient replacement method, the test ingredient is replacing a standard diet ingredient at levels ranging from 25 to100 % (Austreng et al., 2000; Mugo-Bundi et al., 2015; Storebakken et al., 1998a; Xavier et al., 2014). The diet replacement method which involve adding the test ingredient to replace a portion of the reference diet to make a test diet (Glencross et al., 2007) has also been applied but mostly researchers have done replacement of reference diet at one-third (Cho and Slinger, 1979; Guimarães et al., 2014; Ngo et al., 2015;

Sugiura et al., 1998). In the DRM method, digestibility values are determined for reference and test diet and digestibility of the specific ingredient is calculated based on the proportionality factors. In this method, the share of the reference diet within test diet should be fully representative of the complete reference diet (Glencross et al., 2007).

1.4 Microbial fermentation and mineral regulation in intestine

The impact of the microbial fermentation in the fish intestine has been studied by several researchers (Amirkolaie et al., 2006; Buddington and Diamond, 1987; Escaffre et al., 2007; German and Bittong, 2009; Kihara and Sakata, 1997; Pavan and Santini, 2002). In most studies (Austreng, 1978; Mugo-Bundi et al., 2015; Ngo et al., 2015) the contents of distal intestine are considered for the apparent digestibility evaluation. Based on the fermentation activity, the apparent digestibility in the distal and ileal intestine may differ. The difference of the digestibility between the last and middle part of intestine in the pig has been seen different (Skrede et al., 1998). This is because the post-ileal fermentation activity is higher than what is occurring in pre-ileum (Skrede et al., 1998). The distal intestine is observed as the important for excretion of excess cationic elements in the fish (Kraugerud et al., 2007; Storebakken et al., 1998b). The mineral regulation in the gastrointestinal tract of fish is important as it regulates the phosphorus absorption in the fish as cationic minerals may form chelates with phosphorus (Avila et al., 2000; Khanal and Nemere, 2008; Peter and Rejitha, 2011).

1.5 Aims of the study

In this study two experiments were conducted. Experiment 1 was a digestibility trial and experiment 2 was conducted to evaluate the growth of Nile tilapia fed with a mixed plant and fishmeal diet with a diet composed of Asian latrine fly (*Cryosoma megachepala*) maggots as the only source of protein and lipid.

The 1st aim of the experiment 1 was to see that are there components in the feed that can violate the assumed way in the DRM method by two different replacement levels? The 2nd aim was to evaluate the digestibilies obtained by replacing one and or third of a test diet with Asian latrine fly maggots results in similar apparent digestibility estimates? The 3rd aim was to assess the changes in apparent digestibility of nitrogen and energy and the apparent absorption of cationic minerals from the mid to the distal sections of the intestine. The overall aim of the experiment 2 was to evaluate growth responses in Nile tilapia fed a diet with maggots from the Asian latrine fly (*Cryosoma megachepala*) as the only source of protein and lipid.

2. Materials and methods

2.1 Experiment 1

2.1.1 Feed formulation and Preparation

Three diets were formulated. (1) Control diet with only plant protein ingredients. Ingredients were soybean meal, corn gluten meal and potato protein concentrate. Na-alginate (2 %) was added in the diet as binder. (2) The control diet was replaced 1/3 with maggot meal thus making the second diet. (3) The third diet was prepared by replacing the control mix with 2/3 of maggot meal. Additional Na-alginate (2 %) was added also in diet 2 and diet 3 to achieve satisfactory physical quality. Vitamin and mineral premix along with other necessary micro nutrients were added in the diets. Yttrium oxide (0.1 g/kg) was added in the diets as the digestibility marker. The formulation of the feed in Table 1 and the chemical composition of the three diets are shown in the Table 2.

Table 1. Feed formulation and chemical composition of the control diet.

Control diet Ingredients	g/kg Dry matter
Soybean Meal[a]	222.0
Corn gluten Meal[b]	298.0
Potato protein Concentrate[c]	301.0
Mono calcium phosphate[d]	20.0
Rapeseed Oil[e]	49.0
Premix[f]	9.0
Yttrium Oxide[g]	0.1
Lysine[h]	3.0
Threonine[i]	1.0
Methionine[j]	4.0
Phenylalanine[k]	1.0
Taurine[l]	1.0
Arginine[m]	1.0
Sodium Alginate[n]	18.0

[a]Soybean meal, Denosoy, Denofa, Fredristad, Norway. [b]Corn gluten, Cargill 13864. [c]Potato protein concentrate, Potet star, Sweden. [d]MCP Bolifor, Yara, Norway. [e]Food grade Eldorado, Oslo, Norway. [f] see (Nemati Shizari, 2014); Trouw Nutrition, LA Putten, The Netherlands. [g]Metal Rare Earth Limited, Jiaxing, China. [h]L-lysine, Ajinomoto, Japan. [i]Threonine, Ajinomoto, Japan. [j]Methionine,Adisseo Brasil Nutricao Animal Ltd., Sao Paulo, Brazil. [k]Sigma Aldrich Co, St. Louis, USA. [l]Taurine-JP8, Qianjiang Yongan Pharmaceutical Co., Ltd., Hubei, China. [m]Arginine, Ajinomoto, Japan

Table 2. Chemical composition of the three diets.

Chemical composition	Diet 1	Diet 2	Diet 3
Gross energy KJ/g	21	22	23
Crude protein (N×6.25), g/kg	606	536	513
Minerals, g/kg			
Ca	4.6	5.2	6.8
K	8.8	9.0	10.3
Mg	1.8	2.0	2.9
P	9.2	7.9	10.7
Zn	0.027	0.30	0.36
Y	0.11	0.087	0.078

2.1.2 Ingredients and feed production

Maggots of Asian latrine fly (*Cryosoma megachepala)* were obtained from China (Hefei Dayan Biological Technology Co., Ltd) and were tested for the *Salmonella* and *Campylobacter* by

Table 3. Chemical composition and amino acid profile of Asian latrine fly maggots.

Chemical composition	
Crude protein (N×6.25), g/kg	587
Gross energy KJ/kg	23
Lipid, g/kg	190
Ash, g/kg	60
Starch, g/kg	30
Moisture, g/kg	70
Amino acids, g/100 g	
Histidine	1.7
Isoleucine	2.1
Leucine	3.5
Lysine	4.4
Methionine	1.4
Phenylalanine	3.8
Threonine	2.4
Tryptophan	0.8
Valine	2.9
Alanine	3.2
Arginine	2.9
Asparagine	5.9
Aspartic Acid	5.9
Cysteine	0.4
Glutamic Acid	8.2
Glycine	2.5
Serine	2.3
Proline	2.4
Taurine mg/100g	88.0

Eurofins Food Lab, Moss, Norway. According to the producer the larvae were fed with only wheat bran and water during their rearing in China. The chemical composition analysis and amino acid profile for the maggots were done in the same lab. The results are shown in the Table 3.

Maggots were stored in the freezer at -80^{o}C till they were frozen and then were ground with a grinder from Retsch Technology GmbH., Haan, Germany with the screen size of 4 mm. Soybeans were ground with the screen size of 1 mm. Lysine and monocalcium phosphate were grounded finely with the small laboratory grinder from Barun, Mexico. Micro ingredients were separately weighed and then were mixed thoroughly together with a laboratory mixer (Moretti Forni Grain, Italy) until the mix was homogenized.

The ingredients were mixed with the dough mixer (Moretti Forni Grain, Italy) for at least 20 minutes. The mix of micro ingredients were also added during the mixing. Rapeseed oil and 40 % tap water was added during the mixing. Both diets were then passed five times through the pasta extruder (P55DV, Italgy, Carasco, Italy). The size of the die was 3 mm during the production of feed. The knife speed of the extruder was adjusted to produce 3 mm of pellet length. The temperature of the die was 50^{o}C. The five times passing of the mix through the extruder was done to ensure the conditioning and physical quality of the feed. After production, the feeds were dried in the hot air oven at 55^{o}C for 6 h. The moisture levels in the feeds were less than 6 %. Homogenous samples of the feeds were obtained for the proximate analysis.

2.1.3 Fish and experimental setup

An eight days digestibility trial was conducted in the fish laboratory at NMBU, Ås, Norway. Nile tilapias raised in the fish laboratory were distributed in nine tanks with number ranging from 27-46 fish with the biomass 9 ± 0.3 kg (means ± s.e.m). The tanks were filled with 270 l of water. Tilapias were previously fed commercial feed (Aller Aqua, Denmark). The brood stock was from generation 12th of selection, donated by Genomar AS (Norway). The average water temperature was 26^{o}C. The light was provided 24 h in the facility. The feed was supplied in each tank with electrically driven automatic belt feeders unrestrictedly 24 h.

Before weighing and putting the fish in the tanks fish were anesthetized by using the anesthetic agent MS-222, Western Chemical, USA. Half a table spoon of agent/10 l of water was used. The same anesthetic approach was adopted before slaughtering and dissecting the fish for the feces collection.

After anesthetizing, each the fish was killed with the help of sharp knife by cutting the neck and then the fish was dissected to take out the intestine. The intestine was carefully placed on a clean surface and the feces were collected by gentle squeezing the last 15 cm of the distal intestine. The feces were also collected from the middle part of the intestine. The feces were collected

separately for middle and last part of intestine and were placed in aluminum boxes allocated for each tank.

Feces were frozen pooled by tank and were frozen immediately and subsequently freeze dried for three days at feed analysis laboratories, NMBU. The dried feces were grounded fine with the help of pestle and mortar and transferred in small plastic bags.

2.2 Experiment 2

2.2.1 Feeds and feed production

Two diets were formulated, diet 1 (plant diet) and diet 2 (maggot diet). Both feeds were formulated to have similar chemical composition. Diet 1 was formulated with mainly plant ingredients as sources of protein and only 10 % of fish meal was added. Rapeseed oil was added for the source of lipid and also as the source of *n-6* fatty acids. Maggots were used as the only source of protein and fat in the Diet 2. Because of the sufficient quantity of the tryptophan in maggots, no additional amount of it was added in Diet 2 to balance it with diet 1. Potato starch was used in the both diets as the source of starch and binder. Na-alginate (2 %) was added in both diets as a binding agent. Formulations of the both diets is shown in Table 4. Chemical composition of the diets is shown in Table 5.

Table 4. Feed formulation of Diet 1 (plant diet) and Diet 2 (maggot diet).

Ingredients, g/kg dry matter	Diet 1	Diet 2
Fish meal[a]	90	-
Wheat Gluten[b]	103	-
Soybean Meal[c]	71	-
Maggots[d]	-	486
Corn Gluten[e]	146	-
Potato Starch[f]	331	365
Mono calcium Phosphate[g]	20	20
Rapeseed Oil[h]	81	-
Premix[i]	5.0	5.0
Y_2O_3[j]	0.1	0.1
Lysine[k]	13.0	4.0
Threonine[l]	8.0	5.0
Tryptophan[m]	2.0	-
Methionine[n]	8.0	6.0
Phenylalanine[o]	11.0	7.0
Taurine[p]	1.0	1.0
Arginine[q]	3.0	1.0
Sodium Alginate[r]	18.0	18.0

[a]Low temperature dried fish meal (Norse LT-94). [b] Wheat gluten, Cargill 13864. [d]Dried maggots, Hefei Dayan Biological Technology Co., Ltd. For rest of superscripts refer Table 1.

Table 5. Chemical composition of plant and maggots diet.

Chemical composition	Plant diet	Maggots diet
Gross energy, KJ/g	21	21
Crude protein (N×6.25), g/kg	347	300
Crude fat, g/kg	89	93
Ash, g/kg	48	55

The ingredients for both feeds were subjected to same procedures except few changes. The diets were passed through the pasta extruder two times (conditioning) using the 4 mm die size. In the third turn, the diets were produced using 2.5 mm die size. The amount of water used during homogenized mixing was 45 %. The moisture level in the diets was checked till it was dried with the final moisture level of 5 %. The diets were then passed through standard sieve size of 2 mm. After taking samples of more than 0.1 kg from both diets, diets were stored in the refrigerator until fed to the tilapias.

2.2.2 Fish and experimental setup

A 56 day Nile tilapia experiment was conducted in the fish laboratories of NMBU, Norway. The juveniles hatched in the same lab were fed with the commercial diet (Aller Aqua, Denmark) were raised to approximately 25 g. The genetic background of the fish were the same as in Experiment 1.A total of six tanks each with capacity of 70 cm × 50 cm × 50 cm were used in the experiment. Nine fish with mean weight ± SEM at, 224.3 ± 2.4 g were distributed into each tank. Air stones into each tank were provided the fish oxygen for maximum feed intake (Dam and Pauly, 1995). The light was provided 24 h in the facilities. The average water temperature during the trial was 24.2 ± 0.5. The average dissolved oxygen was 7.1 mg/l. The pH was 7.5.

Ad libitum feed was offered two times every day at 8:00 a.m. and 16:00 p.m. for 35 min each meal. Uneaten feed was collected during the feeding time and continued for 30 more min after that. The tanks were also flushed after the last meal to collect the feed from the bottom of the each tank. The uneaten feed was stored in the freezer at -20 ^{o}C between the two feedings. Later the uneaten feeds were kept in the hot air oven overnight at 105 ^{o}C for drying. The weights of the dried feeds were recorded to follow up the feed intake every day for each tank. Fish were anesthetized and weighed after 28th day and 56th day after keeping the fish off feed for 24 h.

2.2.3 Ammonium and nitrite measurement

Amount of ammonium (NH_4-N) and nitrite (NO_2-N) in outlet water of the tanks were measured at 7:00, 8:00 a.m. (two times before the meal) and then after every 2 h (10:00 a.m., 12:00, 14:00, 16:00 p.m.) till the last meal and once after that (18:00 p.m.). Measurements were carried out at 28th and 56th day of the trial using Spectroquant NOVA 60. For detailed method description please see (Hoque and Bajgai, 2014).

2.2.4 Proximate analysis of feeds and feces

The feces and feeds from experiment 1 were analyzed for minerals including yttrium oxide (digestibility marker) by ICP-mineral analyzer (Perkin Elmer, Optima 5300 DV, California, USA) at IPM, NMBU, by using the method described by (McQuaker et al., 1979). Nitrogen in feces was determined by Dumas method in the same Department. Gross energy in the diets and feces were analyzed using Bomb Calorimeter (Bomb calorimeter Parr 1281, Moline, Illinois, USA) at IHA, NMBU.

The experimental feeds from both experiments were subjected to proximate analysis. Nitrogen was analyzed by Kjeldhal-N method by Kjeltec auto 1035/1038 system (Tecator, Sweden). For crude protein (N × 6.25). Crude fat was determined by method prescribed by Commission dir. 98/64/EC (Faithfull, 2005). Ash was measured by heating the samples at 500^{o}C in muffle furnace for 12 h.

2.2.5 Calculations

Digestibility trial: The apparent digestibility coefficient (ADC) of nitrogen, energy and minerals were calculated by using the following equation (Maynard and Loosli, 1969):

$$\text{Apparent digestibility} = 100 - 100 * (\frac{\text{nutrient in faeces (\%)}}{\text{nutrient in feed (\%)}} * \frac{\text{Yttrium in feed (\%)}}{\text{Yttrium in faeces (\%)}})$$

Where nutrient and yttrium in diets and feces are analyzed parameters.

Growth trial:

Daily feed intake (DFI), Weight gain (WG) and feed conversion ratio (FCR) were calculated using the following equations (Nemati Shizari, 2014).

DFI=DM of daily feed (g)-(DM of daily uneaten feed (g).

WG = Final mean fish weight (FW, g)-initial mean fish weight (IW, g).

FCR was calculated as: FCR=CFI/WG

2.2.6 Statistical analysis

For the digestibility trial, the one-way and two-way analysis of variance (ANOVA) were done to analyze significant (P<0.05) differences by using MS-Excel and SAS 9.4 for windows. Least square means and standard errors were also analyzed using SAS. All the parameters of growth trial are presented as means and s.e.m. Significant differences were ranked by least-square differences in SAS.

3. Results

3.1 Digestibility trial

The apparent digestibility coefficients (ADC) for the diets and minerals are shown in Table 6. The ADC of energy was significantly high ($P<0.05$) for 2/3 maggots diet than control and 1/3 maggot diet. Ca, K, Mg, P and Zn indicated higher digestibility values for 2/3 maggot diet. No significant difference was recorded for nitrogen among the three diets. Distal intestine showed significantly higher digestibility for the nitrogen than middle intestine. K and Mg indicated significantly high absorption in the distal intestine. Rest of the minerals did not depicted any significant difference in absorption at middle or distal part of intestine.

Table 6. Apparent digestibility coefficients (ADC) of energy, nitrogen and mineral elements.

ADCs of	Main effect for Diet			P(Diet)[p]	Main effect for position		P(Position)[p]	P(interaction)[p]	Pooled
	Control	1/3 maggot	2/3 maggot		Distal intestine	Middle intestine			s.e.m[1]
Energy	70.6[a]	73.1[a]	81.4[b]	0.02	79.8[y]	70.8[x]	0.010	0.09	2.2[2]
Nitrogen	98.3	98.1	98.6	0.20	98.6	98.1	0.030	0.80	0.1
Elements									
Ca	-57.6[a]	-16.3[b]	17.4[c]	<.0001	-19.5	-18.1	0.70	0.04	7.7
K	80.4[a]	83.1[b]	89.5[c]	0.0008	86.4[y]	82.2[x]	0.02	0.50	1.2
Mg	47.5[a]	59.8[b]	69.7[c]	0.0004	64.4[y]	53.3[x]	0.004	0.20	3.0
P	58.1[a]	56.9[b]	75.0[c]	<.0001	63.6	63.1	0.80	0.70	2.3
Zn	-1534[a]	8.9[b]	1.1[c]	<.0001	-524.8	-503.0	0.60	0.70	176.0

[p]Probability level for main effects of diet, position and interaction. [abc] Indicate the significant differences ($P<0.05$) among diets. [xy] Indicates the significant differences among the positions. [1]Standard error of means. [2]one replicate from control and 1/3 maggots had insufficient material.

3.2 Growth trial

The growth performance parameters (Table 7) showed significant ($P<0.05$) differences for cumulative feed intake (CFI) and feed conversion ratio (FCR) in the period of 0 to 28 days of trial. No significant difference were observed in weight gain or final weight. FCR recorded for the plant diet was 1.1, while that found for the maggot diet was 1.3. The final weight of the tilapias fed the maggot diet was 54 g, while that for the tilapias fed the plant diet was 47 g.

For the overall period (0 to 56 days) of trial as shown in (Table 8) no significant differences were observed for any of the growth parameters considered. FCR recorded for the plant and maggot diets was 2.8 and 1.8, respectively. Final weight for tilapias fed the plant diet was 74 g and it was 63 g for the fish fed the maggot diet. Daily feed intake graphs with standard error bars for the fish fed with plant diet and maggot diet are shown in Figure 2 and Figure 3 respectively.

3.3 Mortality

Only one fish died during the period of 0 to 28 days of the growth trial. The overall mortality for 56 days was 6 out of 27 tilapias fed the plant diet and 3 out of 27 fed the maggot diet. Four of the mortalities occurred in one tank with fish fed the plant diet, after 28th days, and is ascribed to sampling and weighing stress.

Table 7. Growth performance parameters for 0-28 days

Growth parameters	Plant diet	Maggots diet	P-value^{p}	s.e.m^{1} pooled
Cumulative feed intake, g/ tank	303.0	271.0	0.60	26.0
Weight gain, g/fish	29.0^{a}	22.0^{b}	0.01	1.80
Final weight, g/fish	54.0^{a}	47.0^{b}	0.02	1.80
FCR, g DM intake/g gain	1.1	1.3	0.40	0.10

^{p}Probability levels for growth results of diets. ^{ab}Different letters indicate significant (P<0.05) differences among plant diet and maggot diet.

^{1}Standard error of means.

Table 8. Growth performance parameters for 0-56 days

Growth parameters	Plant diet	Maggots diet	P-value^{p}	s.e.m^{1} pooled
Cumulative feed intake, g/ tank	628.0	512.0	0.30	52.0
Weight gain, g/fish	49.0	38.0	0.40	6.40
Final weight, g/fish	74.0	63.0	0.40	6.20
FCR, g DM intake/g gain	2.8	1.8	0.50	0.70

^{p}Probability levels for growth results of diets. ^{1}Standard error of means.

3.4 Ammonium excretion and nitrite concentration in the water

The (NH_4-N) loss at 4, 6 and 8 h of meal on 28th day (Figure 5) was significantly (P<0.05) less for the tilapias fed with maggot diet, while (NH_4-N) loss was also significantly less (Figure 6) for the tilapias fed with maggot diet after 4 and 6 h of meal at 56th day. No significant differences were recorded for nitrite loss on 28th day (Figure 6) and 56th day (Figure 7) at different time intervals for the fish fed with plant and maggot diets.

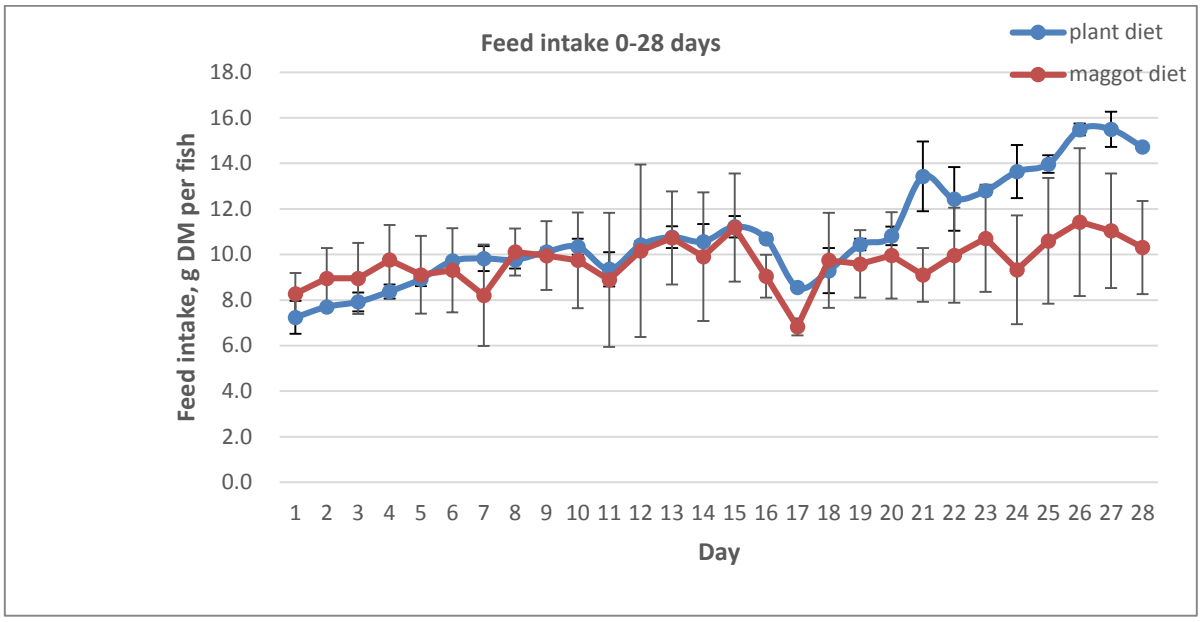

Figure 2. Daily dry matter feed intake for Nile tilapia fed plant or maggot diet during period of 0 to 28 days of growth trial.

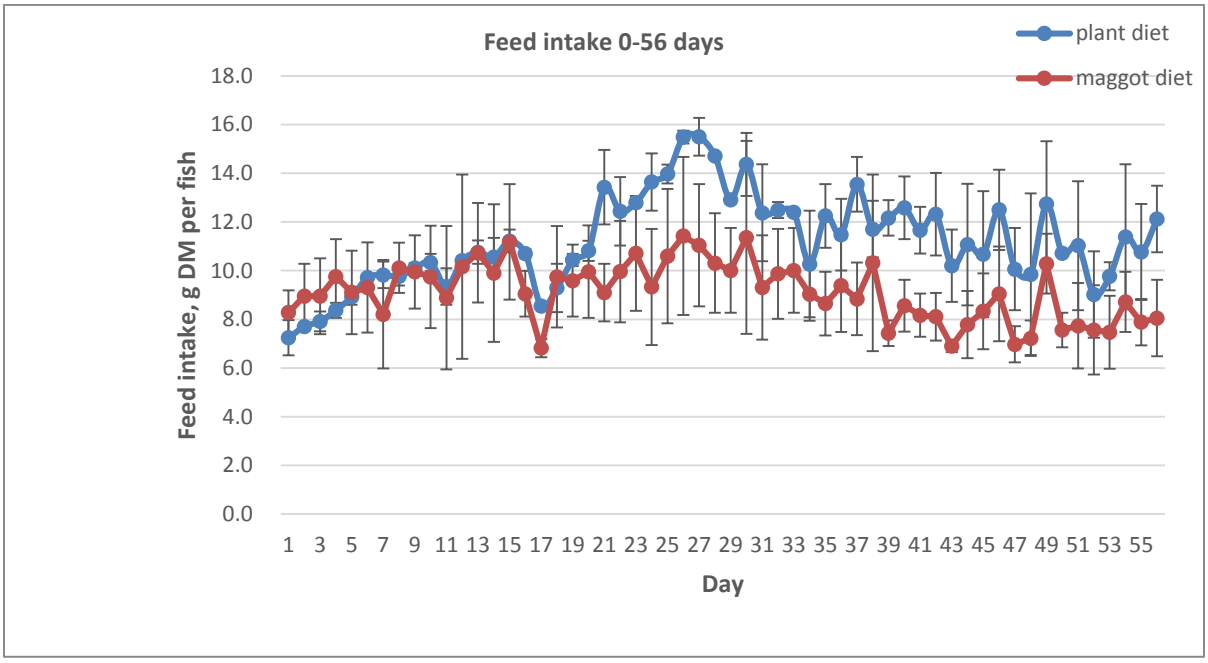

Figure 3. Daily dry matter feed intake for Nile tilapia fed plant or maggot diet during 0 to 56 days of growth trial.

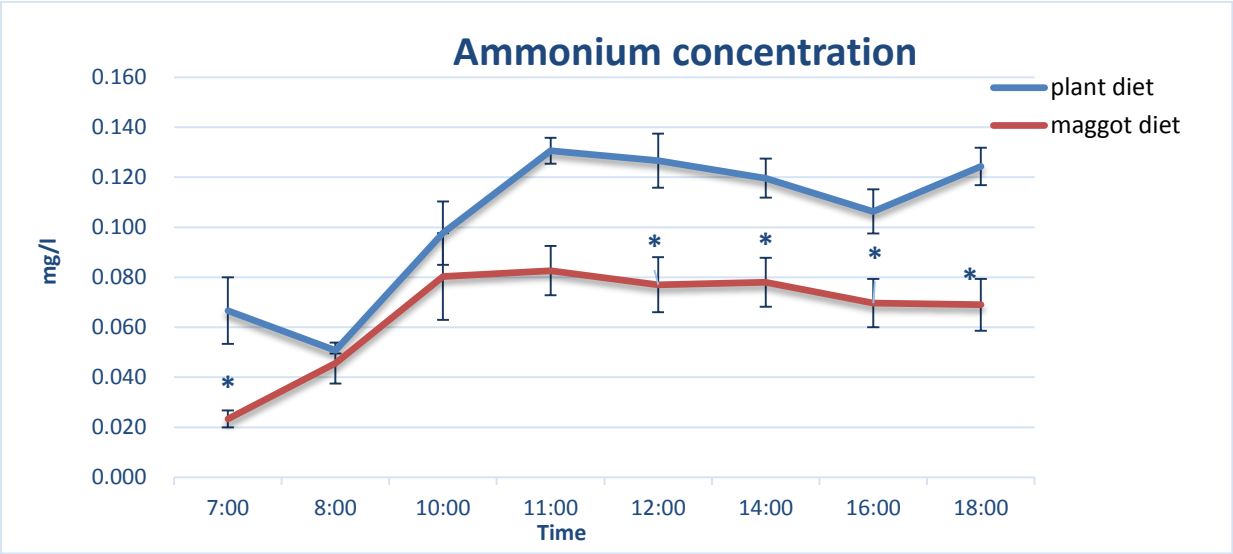

Figure 4. Ammonium excreted in mg/l measured at different times from the water outlet on day 28th. *Indicates significant (P<0.05) differences between fish fed diets with plant ingredients or maggots.

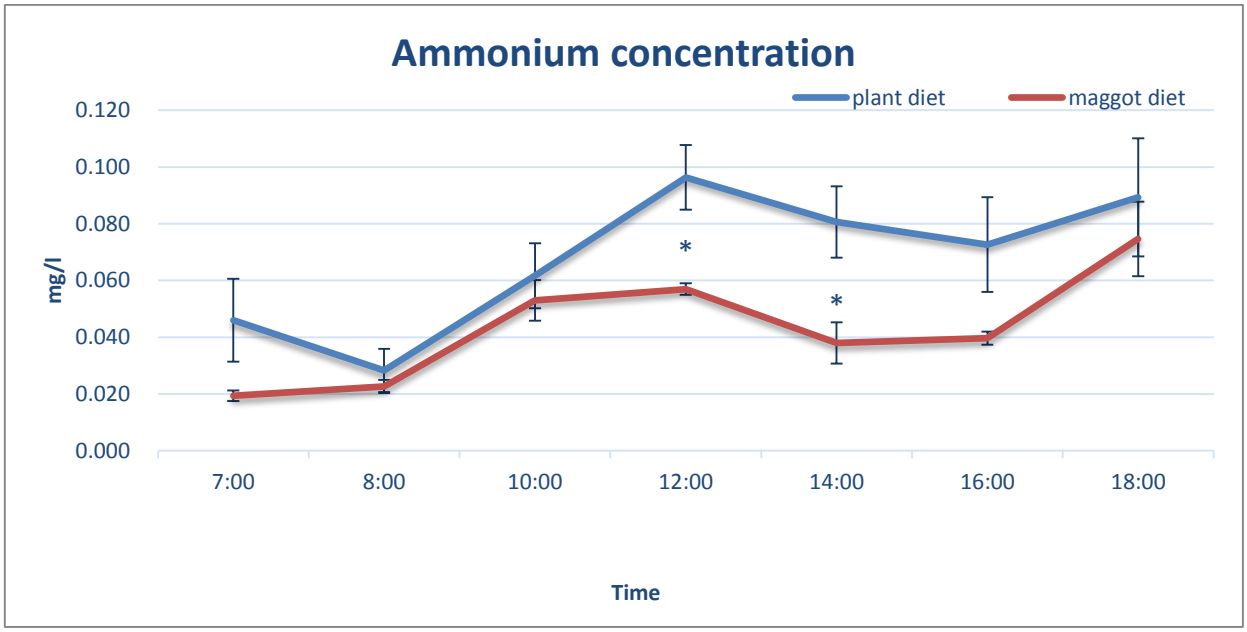

Figure 5. Ammonium excreted in mg/l measured at different times from the water outlet on day 56th. *Indicates the significant (*P*<0.05) differences of the ammonium loss among diets.

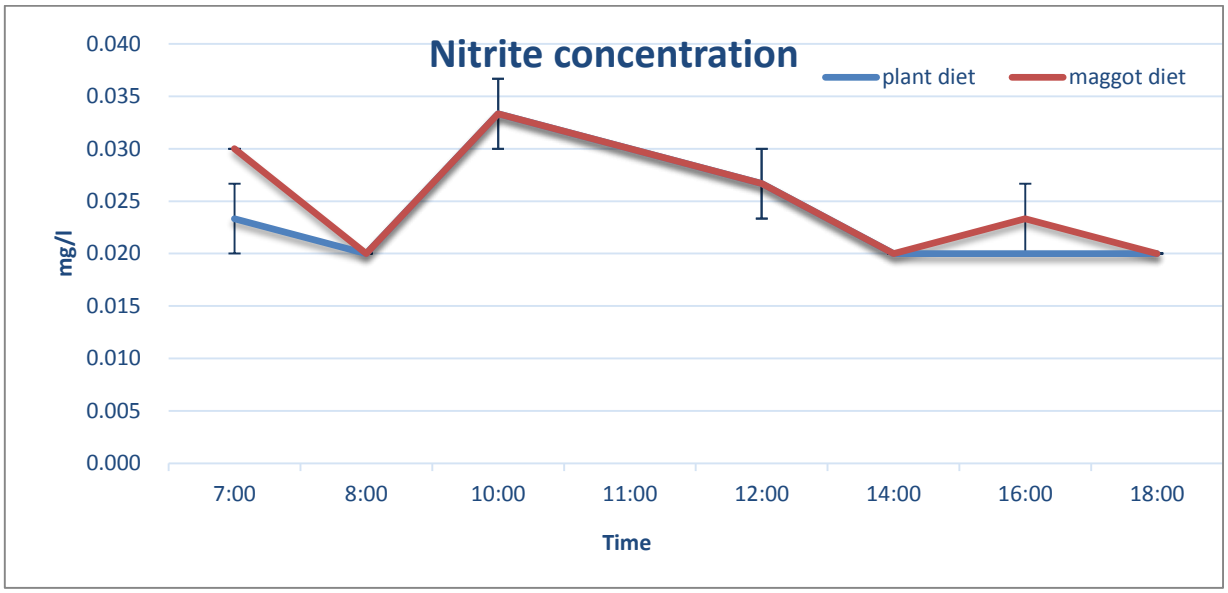

Figure 6. Nitrite concentration in mg/l measured at different times from the outlet water on day 28th.

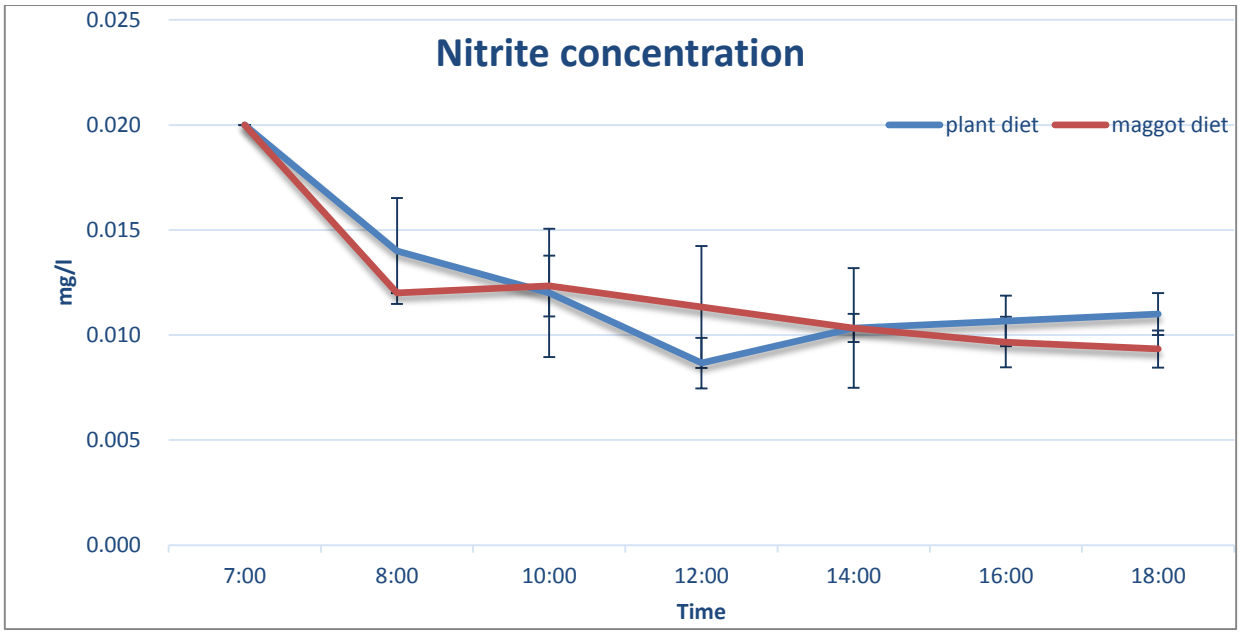

Figure 7. Nitrite concentration in mg/l measured at different times from the outlet water on day 56th.

4. Discussion

4.1 Digestibility trial

The method used for feces collection in this study involved slaughtering of tilapias. The number of fish was high, the procedure was time consuming, and the amount of feces collected was only sufficient to analyze nitrogen, energy and minerals. In view of this, methods to asses apparent digestibility coefficients *in vitro* (Yasumaru and Lemos, 2014) or by feces collection methods from water (Cho and Slinger, 1979; Gominho-Rosa et al., 2015; Xavier et al., 2014) may seem tempting. However, nutrients were not lost to the water by this procedure, avoiding over estimation of nutrient digestibilities. Furthermore, this resource consuming procedure facilitated comparison between estimates obtained from the mid and distal parts of the intestine.

4.1.1 ADC of energy and nitrogen

The basal diet formulated for the trial all consisted of high concentration of plant protein (60.6 %). The ADC of energy was comparable with other studies conducted on Nile tilapia fed with animal protein sources (Mugo-Bundi et al., 2015; Xavier et al., 2014) and for nitrogen it was higher than (Mugo-Bundi et al., 2015; Xavier et al., 2014). Tilapias fed with housefly maggots meal had lower digestibilies for energy and nitrogen (Ogunji et al., 2008). Tilapias fed with "super worm meal" showed lower ADC of nitrogen than present study (Jabir et al., 2012). Nile tilapia fed with ostrich offal meal resulted lower digestibilies for nitrogen and energy than current results (Sales et al., 2012) ADC of both energy and nitrogen were recorded lower for the tilapias fed industrial wastes of fish and poultry as compared to this study (Boscolo et al., 2008; Goddard et al., 2008; Hisano and Pietro, 2013; Meurer et al., 2003).

The high ADC for the energy in the last part of intestine maybe attributed to the high fermentation activity from the mid to the last part of intestine which is not the case with studies on salmonids (Austreng, 1978) where effect of microbial activity is less. Hence, feces in salmonids are collected only from the distal intestine. The higher digestibility for the energy may also be connected to greater intestinal coefficient (longer intestine, up to 12 times of body length) of Nile tilapia (Gominho-Rosa et al., 2015).

4.1.2 ADC of minerals

The negative digestibilies for the calcium (Ca) in this study could be attributed to its intake from the water in addition to what is supplied by the feed. This is in alliance with the other studies conducted on mineral absorption (Storebakken, 1985; Storebakken et al., 1998b; Sugiura et al., 1998). So it is important to consider water borne element presence while interpreting the results of ADC for minerals, as suggested by (Storebakken et al., 1998b). The positive value of digestibility for the calcium in two third maggot replaced diet may also be connected with the high amount of calcium in the feed than the other diets For Nile tilapia gills are the most active

site for the calcium regulation so the intake of the calcium remains doubtful as suggested by (Lall, 2002) . Apparent digestibility of the potassium (K) which has shown high absorption for the three diets could be compared with (Dabrowski and Schwarz, 1986) and (Sugiura et al., 1998). The fish mostly relied for the K intake from the diet and its absorption may have not be affected from the water borne potassium. The absorption levels for the magnesium are well in accordance with (Sugiura et al., 1998).

ADC for the phosphorus was high and is comparable with (Sugiura et al., 1998). The digestibility values for the mid and last part of intestine indicates the high absorption of P in the mid and lower parts of intestine. The highly negative digestibility values for Zinc could be attributed to the water borne Zinc uptake by fish in access which is also indicated by (Storebakken et al., 1998b)

Generally, in this study for the ADC of minerals present in reference diet appears to be less reliable than the estimates for nitrogen and energy, as can be seen from the current digestibility values. Digestibility values are regardless of amount of minerals present in the feed. There should be a developed method to quantify the amount of mineral elements taken up by the fish from water, in order to obtain correct estimates of absorption from the feed. It may also be preferable that the studies should be conducted separately to evaluate the mineral absorption and, the diets should be preferably formulated with the right mineral balance. The ADC evaluation equations may also be revisited to understand the complexity of negative and uncertain digestibility values for minerals.

4.1.4 Ingredient digestibility: what may have gone wrong?

The diet replacement method was modified to have two third replacement of test ingredient but did not shown lower ADC for energy and nitrogen then diet with one third replacement. In current study ADC for the diets were also calculated to analyze the apparent digestibility of maggots but the recommended equations proposed by (Forster, 1999; Sugiura et al., 1998) did not evaluated the ingredient digestibility. This may have been the result of high apparent digestibility values of nitrogen in this study. This may have also happened because during calculation the nitrogen in the feces were analyzed by Dumas method and the nitrogen level in the diets were analyzed by Kjeldhal-N method.

4.2 Growth Trial

A daily dietary dry matter feed intake DMI % of body weight at 3.6 % was observed, slightly higher in the first two weeks then the maggot diet fed fish (DMI % = 3.4). The feed intake stabilized for both feed groups later during the third and fourth week at 3 % DMI. As the amount of maggots in the maggot diet represented 48.6 % of dietary dry matter, so the acceptability of the maggots in this much high quantity may be the reason of drop in feed intake. While formulating the maggot diet, the maggots were relied completely upon as source of protein and lipid (18.5 %

lipid in maggots) and, the overall lipid level in the diet was 9.3 %, which was quite high. Juvenile tilapias shows good growth performance with the lipid level of 6 % in the diet (Abdel-Tawwab et al., 2010).

The FCR observed in the first 28 days, is in alignment with the other studies conducted where animal protein sources were used (Mugo-Bundi et al., 2015). The DMI % kept on going down with the passage of time till it dropped and stabilized to 2 % for the plant diet fed fish and 1.7 % for maggot diet and in the second month of the experiment. This declining trend in the feed intake may be attributed to the exposure to high level of lipid in both diets. The overall growth performance for the 56 days indicates that the fish was not able to coup with high lipid and protein coming from maggots. This is alliance with the studies conducted on tilapia where soldier fly maggots were used as the only protein and fat source for diet (Bondari and Sheppard, 1987) and turbot fed black soldier fly dried maggots, where the inclusion level of maggots was high (Kroeckel et al., 2012). High inclusion level of maggots resulted in poor growth performance in broiler chickens also (Atteh and Ologbenla, 1993; Bamgbose, 1999a). The present study, uneaten feed was not collected properly in one of the tank because some of the feed used to stay in the bottom of the tank during few days until the error was fixed. As the result of that the FCR was observed less in the one tank during first month. The mortality in one of the tank after 28th day as the result of sampling stress also contributed to the error.

So, if we consider taking out these two tanks out of the overall results then few changes could be observed. If we exclude the troubled tanks from the period of 0-28 day then the growth parameters are shown in Table 9. As it could be observed that even if we exclude the tank with low FCR, there remains no significant difference in growth parameters. However, the FCR show the slight difference for fish fed with plant or maggot diet. The final weight also remains almost the same.

Table 9. Excluding tank 3 and 5 from 0-28 day period

Growth parameters	Plant diet	Maggot diet	P-value	s.e.m pooled
Cumulative feed intake, g/ tank	288	222	0.20	25.0
Weight gain, g/fish	28	22	0.07	1.80
Final weight, g/fish	53	47	0.10	1.70
FCR, g DM intake/g gain	1.0	1.0	0.10	0.03

If we consider the 2nd month of the experiment 2 separately and exclude the tanks with errors (Table 10), then also we can see that there are no significant differences among the growth

parameters for both diet groups. The poor FCR (3.0) and lower CFI in the maggot diet fed fish could be seen. This is probably because the fish was reluctant to eat the feed. This could be attributed to the poor physical quality and poor palatability of the maggot diet. The rancidity of the feed could also be the potential reason of feed refusal thus resulting lower feed intake and poor FCR. (The confirmation of this would be made later by subjecting the feeds and maggots to the oxidation analysis).

Table 10. Growth parameters 29-56 days excluding tank 3 and 5.

Growth parameters	Plant diet	Maggot diet	P-value	s.e.m pooled
Cumulative feed intake, g/ tank	366	207	0.12	52.0
Weight gain, g/fish	32	21	0.026	4.8
Final weight, g/fish	84	68	0.360	6.1
FCR, g DM intake/g gain	1.8	3.0	0.501	0.7

If we consider removing the both tanks with errors from the whole experiment (Table 11). Then, it exhibits no significant difference in any of the growth parameters considered in this study. The difference between the FCR in this scenario could be seen still having less difference then in case considering all the tanks where the FCR for the plant diet fed fish was high. It is because of the mortality happened in one of the tank in the plant diet fed fish.

Table 11. Growth parameters 0-56 days excluding tank 3 and 5.

Growth parameters	Plant diet	Maggot feed	P-value	s.e.m pooled
Cumulative feed intake, g/ tank	654	429	0.13	75.0
Weight gain, g/fish	45	39	0.80	8.90
Final weight, g/fish	84	65	0.126	6.30
FCR, g DM intake/g gain	1.3	1.6	0.562	0.20

The high inclusion level of the maggots in the maggot diet may have resulted in poor growth by altering the digestive physiology of the fish fed with maggot diet for 56 days. During sampling for the metabolomic analysis, swelling and inflammation in the intestine of few a fish fed with

maggots was noticed. Gall stones and necrotic lesions on the liver were also observed in one of the fish fed with maggot diet (Personal discussion, K.Zheng, May 2015).

5. Conclusions

The digestibility estimates for the nitrogen and energy were considerably high in the digestibility trial. The two third replacement of test ingredient had not affected the digestibility values. ADC of energy has increased in the last intestine than the mid part of intestine which suggests that the microbial activity in the mid to the distal part of intestine may have played the role. Water borne uptake of elements by the fish may result in highly negative digestibility values. The method for assessment of mineral absorption used here seemed working here for Phosphorus only but for the other cationic elements it may not function. High inclusion level of maggots has affected the growth negatively and it has impaired the physiology of fish. More research needs to be conducted to understand the maggots behavior on the fish physiology. Moreover, feeds formulated for Nile tilapia involving animal protein and lipid sources should have less inclusion level.

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Note: The Endnote program used for handling citations did not allow editing, such as setting Latin names in italics, or changing capital to small letters.