EVALUATION OF GIANT AFRICAN SNAIL (*Achatina fulica* FERUSSAC, 1821), AS AN ALTERNATIVE DIETARY PROTEIN SOURCE FOR NILE TILAPIA (*Oreochromis niloticus* LINNAEUS, 1758)

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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author or institution.

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RECOMMENDATIONS

This is to recommend that the thesis entitled **"EVALUATION OF GIANT AFRICAN SNAIL (*Achatina fulica* FERUSSAC, 1821), AS AN ALTERNATIVE DIETARY PROTEIN SOURCE FOR NILE TILAPIA (*Oreochromis niloticus* LINNAEUS, 1758)"** has been carried out by Miss Karishma Chaudhary for the partial fulfillment of Master's Degree of Science in Zoology with special paper Fish and Fisheries, this is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institution.

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LETTER OF APPROVAL

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This thesis work submitted by Miss Karishma Chaudhary entitled **"EVALUATION OF GIANT AFRICAN SNAIL (*Achatina fulica* FERUSSAC, 1821), AS AN ALTERNATIVE DIETARY PROTEIN SOURCE FOR NILE TILAPIA (*Oreochromis niloticus* LINNAEUS, 1758)"** has been accepted as a partial fulfillment for the requirement of Master's degree of Science in Zoology with special paper Fish and Fisheries.

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ABSTRACT

Giant African snail (GAS), *Achatina fulica* is huge invasive agricultural pest rich in protein content which can be utilized as fish dietary protein source for Nile tilapia, *Oreochromis niloticus* as they are omnivorous hardy fishes having wide range of food items. Tilapia is second most cultured fish after carps throughout the world having good future in Nepal and the use of agricultural pest helps to reduce the agricultural loss due to GAS destruction and maintain sustainable management contributing low aquaculture expenses. This study was carried for 2 months to investigate the growth performance of Nile tilapia fed with diets containing soybean, soybean and GAS (50:50%) and GAS alone as alternative dietary protein source. Three diets were prepared with 45% crude protein content. Fingerlings were stocked in three tanks with three replication for each diet in Graeco Latin Square Design (GLSD). Average size 9±677 cm and weight 24±066 g were stocked in tanks of 1mx1mx0.8m. The experimental fishes were fed at 5% of its body weight twice a day, on morning at 8:00 am and on evening at 4:00 pm for two months continuously. Direct observation and measurement method was applied to collect primary data every 15 days. No significance difference ($p>0.05$) in average weight gain, apparent feed conversion ratio, daily weight gain, gross fish yield and net fish yield was observed. Nile tilapia showed slightly higher growth fed with soybean and GAS than soybean alone or GAS alone. The physic-chemical parameters like temperature, dissolved oxygen, pH, transparency etc. also favors the Nile tilapia growth positively with no mortality. Thus, the study revealed that Giant African snail (GAS) can be used partially or totally as an alternative dietary protein source for Nile tilapia.

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LIST OF ABBREVIATION

Abbreviated form	Details of abbreviation
AFCR	Apparent food conversion ratio
AGDP	Agriculture gross domestic product
ANOVA	Analysis of variance
CP	Crude protein
DFTQC	Department of Food Technology and Quality Control
DO	Dissolved oxygen
DOFD	Directorate of fisheries development
DWG	Daily weight gain
EDTA	Ethylene diamine tetraacetic acid
EOL	Encyclopedia of Life
FAO	Food and agriculture organization
FM	Fish meal
GAS	Giant African snail
GFY	Gross fish yield
IMP	Inosine monophosphate
KFM	Knife fish meal
GLSD	Graeco Latin square design
MIPOP	Massachusettes Introduced Pests Outreach Projects
NWG	Net weight gain
RARS	Regional agricultural research station
SBM	Soybean meal
SRG	Specific growth rate

1. INTRODUCTION

1.1 Background

Aquaculture is one of the emerging agricultural fields in Nepal as it has wide varieties of water bodies lying at altitudes ranging from 60 m to 8848 m which facilitate good commercial culture of varieties of fishes with little supplementary feeds. Fish is excellent source of protein essential for overall development of body. FAO estimated that about 1 billion people worldwide rely on fish and fish products as primary source of animal protein (FAO, 2000). But Nepal has comparatively least per capita fish consumption i.e. 2.45 kg (DOFD, 2014/015). Aqua culturists mainly use locally available feed materials such as rice brans, soybeans, wheat flour, mustard oilcakes etc. The use of harmful pests like *Achatina fulica* as fish feed decreases feeding expenses as well as it helps in sustainable management of such pests. Thus, its sustainable utilization can enhance health and socio-economic status of Nepalese people increasing nation economy.

1.2 *Achatina fulica*

Scientific classification

Phylum: Mollusca

Class: Gastropoda

Order: Stylommatophora

Family: Achatinidae

Superfamily: Achatinoidea

Genus: *Achatina*

Species: *A. fulica* (Ferussac, 1821)

Common name: Giant African Snail

Achatina fulica is largest land snail commonly known as Giant African land snail as it is native of Africa but now it is distributed worldwide. Nine exotic molluscs i.e. two species of slugs (*Deroceras leave* and *Laevicaulis alte*) and six species of snails (*Achatina fulica, Filopaludina sumatrensis, Pseudosuccinea columella, Planorbarious corneous* and *Gulllela bicolor*) are present in Nepal (Budha, 2015). Among which A. fulica acts as invasive pest. In Nepal it was introduced during 1930-1940s from India in eastern Nepal (Raut, 1999; Budha and Naggs, 2008) which is well established in almost all areas of Terai region and some parts of Hilly region. It is spreading mainly due to its beautiful shell (Budha and Naggs, 2008) and transportation with agro products.

These species are found in tropical, temperate and terrestrial habitats and have been adapted to cooler environment (Strokes, 2006). They prefer tropical, moist climate and are nocturnal in nature, also found active in morning. The typical adult shell is conical shape mostly of light to dark brown with vertical stripes of darker brown shades. When fully mature, it reaches up to10 cm in diameter and up to 30 cm in length (MIPOP, 2008). The average weight of snail is approximately 32 grams (Cooling, 2005). Its average life span is 5-6 years but are known to live up to 9 years. Predators and unfavorable climatic condition are the major causes of mortality (Barker and Raut, 2002).

They are mainly herbivorous consuming wide variety of plants, but also found feeding on dead moist leaves. It feeds on wide variety of plants, nearly 500 plants are known that has been attacked by it (White, 2011). Florida estimated annual loss of $11 million if *A. fulica* is left unchecked (USDA, 1982). Thus, is one of the burning problem for agriculturalists.

It also creates threats to native molluscs and acts as intermediate host of various diseases like Black disease, Abdominal angiostrongyliasis, Schistosomiasis etc. (EOL, 2015). *A. fulica* has high protein content (above 40%), low fat (less than 3%) and relatively a good source of micro minerals like calcium, phosphorous, magnesium, potassium and sodium. It is excellent source of iron but poor in phosphorous, potassium and magnesium (Abouo, 1990). Thus, it is also used as fish bait and production of fertilizer, chicken feed and biological compounds in laboratories (Strokes, 2006).

1.3 *Oreochromis niloticus*

Scientific classification

Phylum: Chordata

Subphylum: Vertebrata

Class: Actinopterygii

Order: Perciformes

Family: Cichlidae

Genus: *Oreochromis*

Species: O. *niloticus (*Linnaeus, 1758)

Common name: Nile tilapia

Tilapia is native of Africa and is common name for a large group of fish species within the family Cichlidae. It is classified into three genera: *Tilapia*, *Saratherodon* and *Oreochromis* based on its reproductive behavior (Teichert-Coddington et al. 1997). Tilapia is commercially cultured worldwide under genera Oreochromis. In Nepal Nile tilapia was introduced in 1985 from Thailand through aquaculture pathway (Bartley, 2006; FAO, 1997; Manandhar, 1995).

It is a deep-bodied generally laterally compressed to oval fish generally with white abdomen and nine longitudinal black stripes on body surface (Naca, 1989) as its distinguishing characteristics. Its body coloration varies depending on environmental, physiological and dietary factors. Coloration of body is also variable with relation to its distribution (Little and Macingtosh, 1995). Generally, Nile tilapia is primarily herbivorous, with aquatic microphyte, algae and diatoms comprising more than 90% of its diet and remainder includes aquatic insects, crustaceans, fish eggs and organic debris (Alne-na-ei and Khallaf, 1987). Fingerlings becomes entirely herbivorous feeding mainly on phytoplankton using mucus trap mechanism and its pharyngeal teeth (Moriarty and Moriarty, 1973; Moriarty et al., 1973). Generally ingestion occurs at day and digestion occurs at night (Trewaves, 1983) with its long digestive tract i.e. six times of its total body length (Opuszynski and Shireman, 1995).

Nile tilapia is considered as most suitable commercial aquaculture species in sub-tropical and tropical region at it has best protein digestibility at 25°c (Stickney, 1997). It is second most widely cultured worldwide species after carps because of its wide tolerance range to environmental factors (Pillay, 1999). Global tilapia production in 2012 was estimated at 4.5 million tons whose major production was from China (Fitzsimmon, 2000 and FAO, 2014).

1.4 Fish food

Fish consumes various sorts of food based on fish species and its feeding behavior. It may feed on foods of plant, animal or bio waste origins. In modern aquaculture system fishes are fed with supplementary foods along with natural food for maximum yield. The supplementary foods are made according to the species reared. Some of the by-products like poultry, animal husbandry, fish farm, waste food materials etc. has been utilized as alternative sources of fish food.

1.5 Common feed ingredients used in aquaculture

Various feed ingredients of plant, animal and bio waste origins are being used in aquaculture as a form of supplemental feed.

1.5.1 Ingredients of plant origin

Various plant origin ingredients has been used in fish feed researches in order to evaluate its efficiency to replace animal stuffs such as fodder plants, oilcake and oil meals, aquatic weeds (EI-Sayed, 1999), Luecaena leaf (Wee and Wang, 1987), legume seed (Keembiyehetty, 1993), roots and tubers (Tusche et al., 2013) and cereal and cereal byproducts (Santis et al., 2016). Some of the mostly used plant origin ingredients in aquaculture are mustard oil cake, soybean, rice brans, maize and wheat flour etc.

1.5.2 Ingredients of animal origin

Animal origin ingredients are comparatively rich in protein, essential amino acids especially lysine and methionine that are limiting in plant sources. These includes fresh and dry meat of various fish (Castaneres, 1990), blood, earthworms (Rawling et al., 2012; Gosh, 2004), pupae and worms (Hilton, 1983) and insects (Fasakin et al., 2003).

1.5.3 Ingredients of bio waste origin

The bio waste origin ingredients includes hydrolyzed poultry feathers (Abdel-warith et al., 2001), fish silages, milk byproducts, kitchen byproducts and slaughter house byproducts (Cheng et al., 2015) which are regarded as the waste are being used as supplemental feed in aquaculture to reduce the feed expenses and biological waste management practices.

1.5.4 Miscellaneous feed ingredients

It includes cane molasses, brewer's yeast, leaf protein concentrate, grain distiller byproduct, fats, algae, yeast (Ran and Zhou, 2016), bacterial protein etc.

1.6 Objectives of the study

1.6.1 General objective

To evaluate *Achatina fulica* as an alternative dietary protein source for *Oreochromis nilotica.*

1.6.2 Specific objectives

a. To investigate the growth performance.
b. To find the Apparent Food Conversion Ratio (AFCR) and Daily weight gain.
c. To compute gross and net fish yield and survival rate.
d. To determine physic-chemical parameters of the experimental feeding trial.

1.7 Rationale of the study

Giant African Snail, huge troublesome invasive agricultural pest which causes tremendous damage to agricultural products every year is rich in protein content like other molluscs such as *Pila globosa*, *Filopaludina* spp which could be utilized as food source for better aquaculture reducing the feeding expenses. It further helps sustainable and economical management of invasive pest enhancing socio-economic status of poor aquaculturalists, farmers and collectors. This rich protein source can be utilized as fish dietary protein source for *O. niloticus* as they are omnivorous hardy fishes having wide range of food items. Thus, this research work will explore to use Giant African snail as an alternative dietary protein source for Nile tilapia.

2. LITERATURE REVIEW

Nile tilapia ingests on wide variety of natural foods organisms, including planktons, aquatic microphyte, planktons and benthic invertebrates, larval fishes, detritus and decomposing matters. They are omnivorous showing herbivorous nature (Fitzsimmons, 2000) and filter feeding capacity (Turker et al. 2003). They show satisfactory growth consuming its natural foods which increases up to 30-50 percent with additional supplementary feeds. GAS is regarded as largest land invasive snail (Buddha and Naggs, 2008) in Nepal having high protein content i.e. above 40% (Abouo, 1990). Various alternative fish feeds has been used in trials to replace fish meal in aquaculture like fish by-products, terrestrial animal by-products, oilseed plants, aquatic plants, single cell proteins, grain legumes, plant protein concentrates and cereal by-products (EI-Sayed, 1999).

Nine experimental diets containing 25%, 50% and 100% dietary protein as plant protein with soaked, sun dried and commercial *Luecaena* leaf meal fed to Nile tilapia showed a trend of reduced growth performance and feed utilization efficiency with increase in *Luecaena* leaf meal incorporation for all treatments. Soaked leaf meal showed better growth than dried and commercial leaf meal (Wee and Wang, 1987).

Florida red Tilapia was tested for its growth rate, feed and protein utilization and carcass composition using three isocaloric diets containing 20%, 25% and 30% protein of equal quality. It showed higher protein efficiency with 20% protein with no significant differences among its survival, specific growth rate, daily weight gain, feed consumption and conversion, carcass composition and fish condition (Clark et al. 1990).

Nile tilapia fed with commercial pellet containing soybean, fish meal, rice bran, corn had high percentage weight gain than fish fed with only Azolla and mortality percentage was higher (10.03%) in commercial pellet fed fish as compared to only Azolla fed fish (Castaneres, 1990).

Legume seeds could replace about 33% of fish meal on trial of sixteen isocaloric diets containing one of three protein levels i.e. 20, 25 and 30% made by partial and total replacement of fish meal with legume seeds *Vigna catiang* (cowpea) and *Phaseolus mungo* (black gram) on Nile tilapia fingerlings without any adverse effect on growth rate (De-Silva and Keembiyehety, 1993).

Trial was conducted to determine the effect of continuous versus two meals per day feeding and 30% starch versus 30% glucose diets on carbohydrates utilization of white sturgeon and hybrid Tilapia which showed higher liver 6-phosphogluconate dehydrogenase and malic acid activities in continuous than two meal per day and higher specific growth rate, feed efficiency, protein efficiency ratio and liver glucose-6-phosphate, 6-phosphogluconate dehydrogenase and malic acid activities on tilapia fed with starch diet than glucose diet (Cui et al. 1997).

When poultry by-product meal at 20%, 40%, 60%, 80% and 100% with substitution of total protein content were compared with fish meal based control diet 100% fish diet, it indicated replacement up to 40% of protein content with poultry by-product in practical feed is significant (Abdel-warith et al, 2001).

Clarias gariepinus fed with full fat and defatted maggot meal, performed better with defatted maggot than full fat maggot (Ajayi et.al, 2003).

Nile tilapia fed with dehulled solvent extracted soybean meal and expeller pressed soybean meal as alternative of fishmeal showed no significant difference in final mean weight, survival rate and feed conversion ratio (Nguyen and Allendavis, 2009).

Air classified faba bean protein concentrate was effectively utilized as dietary protein source by Post smolt Atlantic salmon for good growth (Santis et al, 2016). When the effect of replacing 25%, 50% and 75% of fish meal with soybean flour was examined it showed 50% of fish meal protein is significant for practical catfish diet (Davies, 2001). Soybeans meal could be partly an alternative protein source if phosphorous supplied for fish by incorporation with microbial phytase or phosphorous in Persian sturgeon (Bagheri and Imanpoor, 2012).

Trout fed with fish meal containing freeze-dried worm as an alternative dietary protein source showed negative result (Hilton, 1983) whereas fish biomass from vermicompost fed pond showed an increasing trend compared to inorganic fertilizer treated pond (Gosh, 2004). Mirror carp fed tropical earthworm meal had significant enhancement of growth and feed utilization efficiency (Rawling et al, 2012).

Fish diet formulated by partial and total replacement of fish meal by potato protein concentrate with 20, 40,60,80 and 100% protein, showed negative effect on growth with 60% and above and supported growth below 60% in rainbow trout (*Oncorhynchus mykiss*) (Tusche et al,2013).

Dietary inclusion of soybean meal results higher feed bulk density and inclusion of copra and palm kernel meals results in lower feed bulk densities. The 30% inclusion of unrefined form of copra and palm kernel meal in Nile tilapia is usefully possible without any adverse effect on feed intake and pellet nutrient losses prior to ingestion (Obirikorang and Skov, 2015).

Maize can be totally replaced by glycerol in pelleted diet for Nile tilapia fingerlings without any effect on hepatocytes and morphology of the hepatopancreas (Moesch and Santos, 2016). Crude glycerin can be used as good and safe source of energy for Nile tilapia as it did not affects hepatosomatic, liposomatic and viscerosomatic indices, survival rate and hematological parameters (Cyrino and Goncalves, 2015).

Different food wastes and meat meal as a major source of protein to replace Fish meal used in high quality fish production were examined in various trophic level fishes like Bighead carp (*Hypophthamichthysnobilis*), Grass carp (*Ctenopharyngodonidella*) and mud carp (*Cirrhinus molitorella*) of omnivorous chain. It was found that grass carp and bighead carp fed with kitchen waste food are relatively free of polycyclic aromatic hydrocarbons so it was consumable (Cheng et al, 2015).

A high rearing density of Nile tilapia was investigated the effects of dietary live and heat-inactive baker's yeast on growth, gut health and disease resistance which showed decrease in growth, feed utilization, microvilli length and disease resistance against *A. hydrophila* concluding live baker's yeast alleviate negative effects on high density reared tilapia inducing crowding effects (Ran and Zhou, 2016).

Silvery black porgy showed positive result when fed with partial and total replacement of dietary fish oil with alternative lipid sources (Mozanzadeh et al, 2016). Partial replacement of

25% (w/w) of reference Fish meal with oilseed cakes as a byproduct from oil production is feasible in aquaculture (Tyapkova et al, 2016).

All life stages of *Aurelia* sps were accepted as source of food by Gill head sea bream *Sparus aurata* (Bouvier et al, 2016).

Three different levels of carbohydrates 0%, 30% and 50% dextrin were fed to Nile tilapia to find out molecular adaptation of glucose metabolism of carbohydrates. Feed with 30% dextrin showed higher growth while 50% showed worst. Further it showed increase in hepatic and muscle glycogen, hepatic somatic index and plasma metabolites with no hyperglycemia and no body composition change which were linked with increase of dietary carbohydrates. Thus it says that Nile tilapia can use high level of carbohydrates without any deregulation of glucose homeostasis (Boonanuntanasarn et al. 2018). Nonstarch polysaccharides digested by Nile tilapia are less well utilized for growth (Haidar and Schrama, 2016).

Three different fish pellet containing linseed oil cake (0, 50 and 100%) as mustard oil cake replacement were fed to Nile tilapia which showed no significant difference (p>0,05) in mean weight, final weight, average daily weight gain, survival rate and apparent feed conversion ratio in all three treatments (Ghimire, 2016).

An eight week feeding trial to determine the effect of sodium salts of ethylene diamine tetraacetic acid (EDTA) showed EDTA supplements at level of 13 g/kg diet improves fish growth performance and antioxidant activities (Tawwab and Shady, 2017). Dietary supplementation with a combination of formic acid, propionic acid and calcium propionate improves the performance of Nile tilapia (Reda and EI-Araby, 2016).

Locally available plant and animal ingredients can replace fish meal protein for striped catfish fingerlings without compromising growth performance, feed utilization and carcass traits (Chau et al, 2012). Appropriate proportion of different types of food waste can satisfy basic nutritional requirement of lower trophic level fishes like grass carp and tilapia (Wong et al, 2016).

A feeding trial of practical diet containing protein primarily from menhaden fish meal (FM) and soybean meal (SBM) on Nile tilapia juveniles showed highest weight gain, lower average daily feed intake, lowest feed conversion and greater specific growth rate with dietary protein based meal to soybean based meal ratio of 1.22 to 1.35 (Hyde and Koch, 2016).

Dietary supplementation with essential oils of clove basil and ginger was useful for improving growth, immune responses and disease resistance with significant differences in thrombocytes, total leucocytes, lymphocytes and neutrophils (Brum and Martins, 2017).

Six experimental diets containing different levels of supplemental glutamine and/or arginine fed to juvenile Nile tilapia showed significant effects on weight gain, feed intake, feed efficiency, protein efficiency and protein retention. Diet with combined supplement of glutamine and arginine at 1% gave more improved growth performance than diets supplemented individually with glutamine and arginine (Pereira and Gatlin, 2017).

Eight week trial to determine the effect of dietary active charcoal supplementation on growth performance, biochemical and antioxidants responses, body composition and resistance on Nile tilapia to environmental heavy metal exposure results that active charcoal supplementation reduces in fish feed with 10-20 g active charcoal per kg diet, which suggested that active charcoal supplementation can improve fish performance, enhance antioxidant activities and reduce heavy metal bioaccumulation in fish body (Tawwab and Shady, 2017).

A feeding trial to assess the effects of processed meal from knife fish *Chitala ornata* (KFM) as fish meal replacement in Nile tilapia juveniles showed KFM can partially or totally replace dietary protein from fish meal, mainly with 75% KFM. It results in increase average weight gain, specific growth rate and feed intake (Abarra and Ragaza, 2017).

Inosine monophosphate (IMP) enhances cellular muscles growth and up regulate growth-related gene expression in juveNile tilapia having potential feed additive and supplementation of ≥0.2% IMP for better growth enhancement. It showed IMP supplementation enhances muscle growth by promoting hyperplasia and hypertrophy, it up regulate the growth related gene expression in Nile tilapia juvenile (Asaduzzaman and Kader, 2017).

Dietary spray-dried plasma supplementation mainly at the level of 51.83% g/kg enhances growth performance, intestinal health, hematological profile and cold induced stress resistance in Nile tilapia. It affects leukocytes, lymphocytes and neutrophil counts (Araujo and Barros, 2017).

3. MATERIALS AND METHODS

3.1 Study area

Present experiment was conducted at Fishery Research Unit of Regional Agricultural Research Station (RARS), Parwanipur, Bara is a government agricultural research station which has been contributing the agricultural sector by its continuous researches on various sectors of agriculture such as poultry, animal feed, horticulture, fisheries etc. It is situated at 2 km east from Parwanipur Chowk which lies in highway to Birganj.

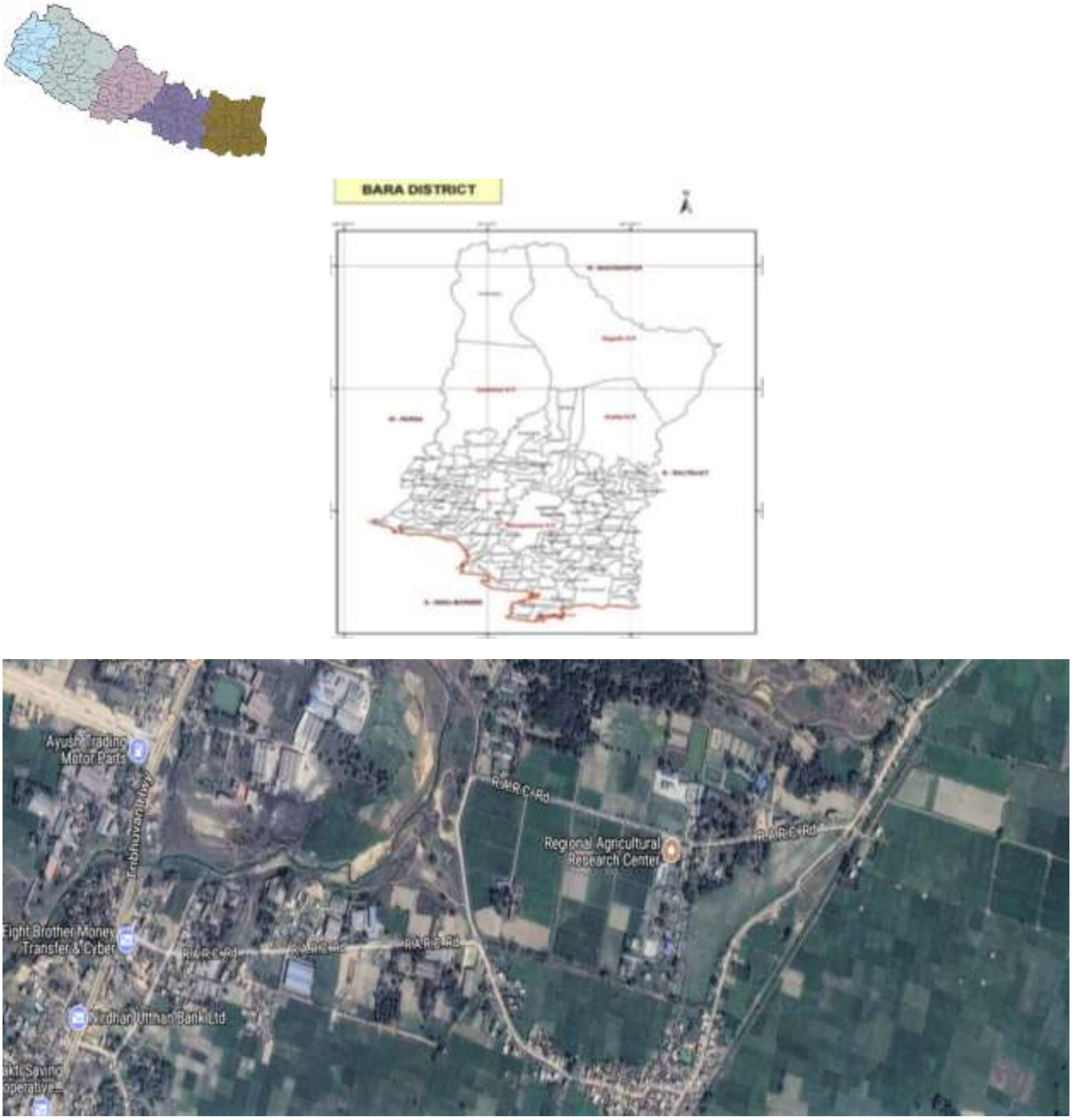

Figure 1. Map of experimental site.

3.2 Experimental set up

Nine cemented tanks of 2m x 3m x 0.8m was used in three replications for experimental purpose. The experimental setup was in Graeco Latin Square Design (GLSD) for 3 feeds containing 45% protein concentration along with other feed materials. Treatments are treatment 1 (i.e. with soybean protein), treatment 2 (soybean and *A. fulica* protein) and treatment 3 (*A. fulica* protein) as shown in Table 1.

Table 1. Experimental tanks setup of different treatments

Cluster 1	Cluster 2	Cluster 3
T1	T2	T3
1	2	3
2	3	1
3	1	2

The tanks were supplied with continuous bored tube well water in sprinkle to maintain water level of 0.8m and continual water exchange for good dissolved oxygen and maintain suitable water condition. Each tank was provided with plastic feeding trays for feeding purpose

3.3 Collection of fingerlings

Mixed sex almost uniform sized fingerlings of *O. niloticus* was collected from RARS own production ponds using net. They were immediately transferred to plastic buckets filled with pond water for further sorting. After sorting each fish were measured and weighed individually. Each tank was stocked with 30 fingerlings of average size 9±677 cm and weight 24±066 g on 24th October, 1 days prior providing the treatment in order to acclimate it. Thus altogether 270 fingerlings were stocked.

3.4 Feed preparation

3.4.1 Collection of *A. fulica*

About 60 kg of *A. fulica* was collected from moist areas of Birgunj (Parsa), Rajghata and Mahindranagar (Bara) for formulation of fish feeds.

3.4.2 Cleaning, Drying and Grinding

A. fulica was cleaned thoroughly with clean water and crushed with hammer to make it shell free. Later it was sun dried and grinded in mortar and pestle to make it fine powdered form.

3.4.3 Fresh feed formation

The properly dried fine *A. fulica* powder was sent to Department of Food Technology and Quality Control (DFTQC), Babarmahal, Kathmandu for its crude protein concentration test. After carrying out its protein test it was mixed with other feed ingredients like rice bran, wheat flour, mustard oil cake and soybean to prepare fish feed having 45% crude protein concentration by using Pearson Square Method. Vitamin and Mineral premix was also added at the rate of 1%. The fish feed was made fresh while feeding them as shown in Table 3.

Table 2. Crude protein contents of different feed ingredients used in feed preparation.

S.N	Ingredients	Crude Protein Content in 100 g
1	Rice bran	15.00%
2	Mustard oil cake	28.80%
3	Wheat flour	13.70%
4	Soybean	37.00%
5	Dried *A. fulica*	46.60%

Table 3. Various fish feed that was formulated.

Controlled	Treatments	
T1 (Soybean 100% as protein source)	T2	T3
	Soybean and *A. fulica* (50:50%) as protein source	*A. fulica* (100%) as protein source

3.5 Feeding

Feeding of fingerlings i.e. 5 % of its bodyweight was given from next morning of stocking in experimental tanks for continuous 2 months. Feed quantities was adjusted every 15 days after sampling in accordance with the average fish weight. Fish were fed twice a day, in morning around 8.00 am and in evening around 4.00 pm.

3.6 Growth check

All the fish were scooped out using scoop net for growth check. Growth check was done in interval of every 15 days during which weight and length of individual fish was recorded by digital balance and measuring scale. It was done in morning around 6.00 to 7.00 am to avoid stress to fish. Any mortality during these period was also recorded.

3.7 Water quality parameters

Water quality parameters like temperature, dissolved oxygen and pH were recorded every 15 days just before sampling on morning around 6.00 am. Water quality parameters were studied using various equipment as shown in Table 4.

Table 4. Instruments used to measure water quality parameters.

S.N	Parameters	Unit	Instruments used
1	Temperature	°C	DO meter ($A_{1.39110}$, Lutron)
2	Dissolved oxygen	mg/l	DO meter (A_{139110}, Lutron)
3	pH		pH meter (HANNA, USA)

3.8 Data analysis

The analysis of experimental data was done using following formulae

Analysis of data was be done using the following formulae

Daily weight gain (g/fish/day) = $\frac{Mean\ final\ weight(g) - Mean\ initial\ weight(g)}{Culture\ period(days)}$

Apparent feed conversion ratio=$\frac{Quantity\ of\ feed\ intake\ (g)}{Net\ weight\ gain(g)}$

Net fish yield (kg/m^2/yr) =$\frac{total\ weight\ gain\ (kg)\ x\ 365}{culture\ area\ x\ culture\ period}$

Gross fish yield (kg/m^2/yr) =$\frac{total\ final\ weight(kg)\ x\ 365}{culture\ area\ x\ culture\ period}$

Survival (%) =$\frac{Total\ harvested\ number}{Total\ stocked\ number}$ x 100

3.9 Statistical analysis

The experiment was carried out in GLSD. One way ANOVA was used to test the significance difference between the treatments at 5% confidence level.

4. RESULTS

4.1 Growth pattern

There was significant difference in growth pattern of fish in different treatments (Figure 2). The growth performance of fish in different treatments is in descending order of T2, T3 and T1 (Appendix I).

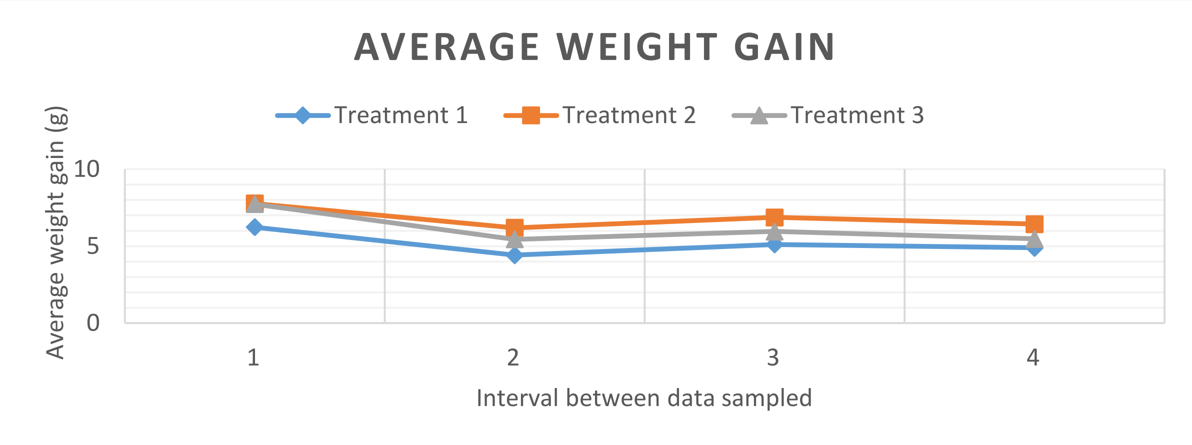

Figure 2. Average weight of fish in different treatments during sampling.

4.2 Apparent feed conversion ratio

Figure 3 shows the apparent feed conversion ratio (AFCR) of fish in different treatment during different sampling periods. The AFCR at 2nd November for T1, T2 and T3 were 3.090, 2.173 and 2.324 respectively. AFCR of all the treatments increases but in T1 and T3 it increases more i.e. from 3.090 to 5.427 in T1 and from 2.324 to 4.366 in T3 during first 15 days. AFCR later increases almost in similar way during next 30 days till 8th December, then during last 15 days AFCR increases drastically in all treatments mainly in T3.

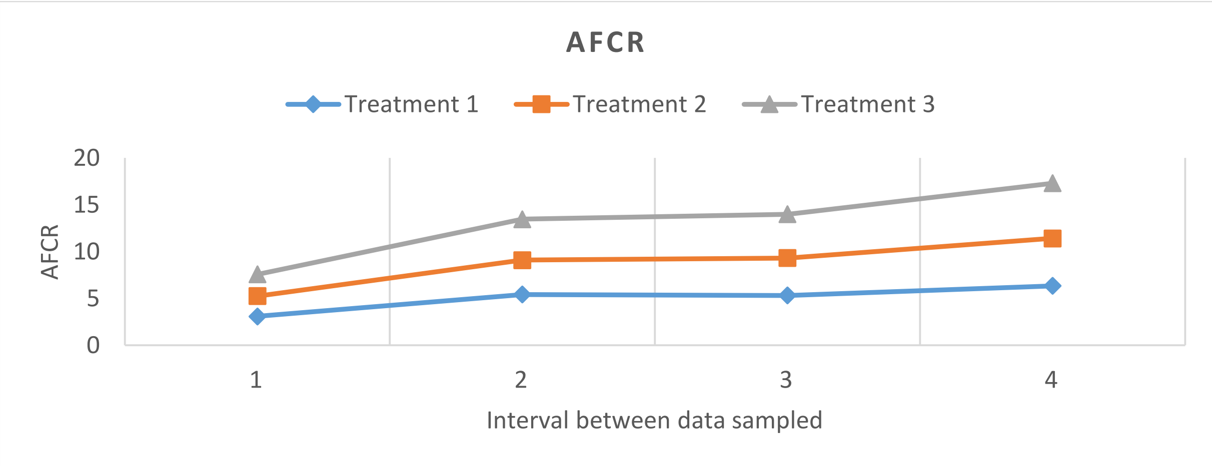

Figure 3. Apparent feed conversion ratio of different treatments.

4.3 Daily weight gain

Figure 4 shows average daily weight gain (DWG) of fish in different treatments during experimental sampling. There was increase in DWG of fish in first 15 days which suddenly decrease in next 15 days and alternate increase and decrease in DWG was observed in next half monthly sampling. Thus, there was gradual and continuous fluctuation in daily weight gain of fish throughout the experiment.

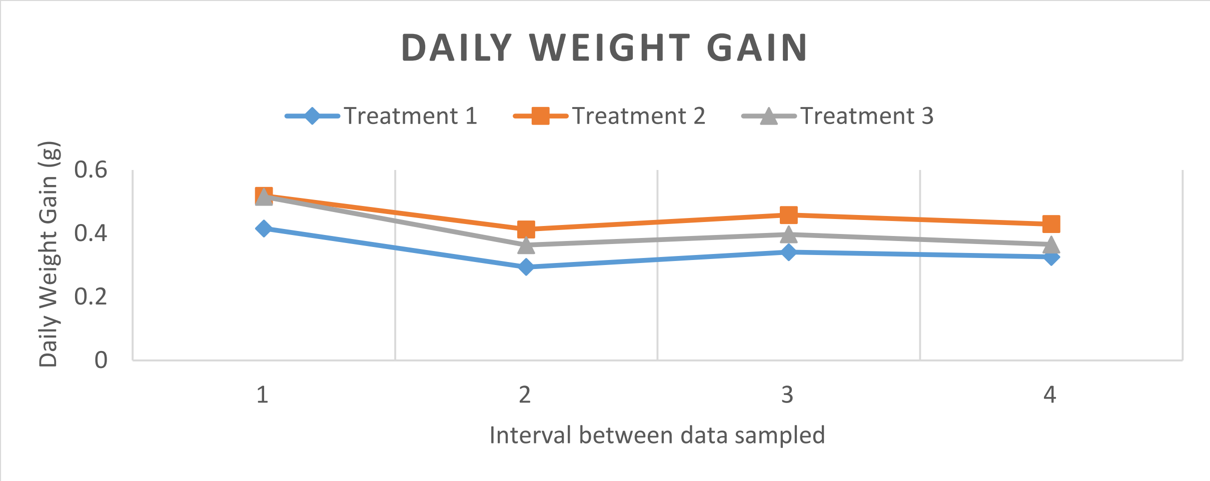

Figure 4. Daily weight gain of fish in different treatments.

4.4 Growth and Production

The growth and production of fish was calculated by the previous mentioned formulae.

Table 5. Growth and Production parameter in different treatment

Parameters	T1	T2	T3
Mean stocking weight (g/fish)	25.688	22.544	23.966
Total stocking weight (g/m^3)	160.555	140.902	149.791
Mean harvesting weight (g/fish)	46.333	49.8111	48.577
Total harvesting weight (g/m^3)	868.75	933.958	910.833
Gross fish yield (kg/m^2/yr)	1.7616	1.8938	1.8469
Net fish yield (kg/m^2/yr)	0.7849	1.0367	0.9357
Average DWG (g/day/fish)	0.344	0.454	0.410
Survival (%)	100	100	100
Feed conversion ratio	5.052	3.723	4.318

There was no significance difference ($p>0.05$) in average weight gain, AFCR, daily weight gain, gross fish yield and net fish yield. The average initial weight of individual fish was 24±066 g during stocking. Mean stocking weight was in order of T1, T3 and T2 (25.688, 22.544 and 23.966 g in T1, T3 and T2 respectively). Though its total harvesting weight was in its inverse order of mean stocking weight i.e. T2 (933.958 g), T3 (910.833 g) and T1 (868.75 g). T1 was provided with high total stocking rate of 160.555 g while T2 with lower total stocking rate of 140.902 g, but there was reverse result in mean harvesting weight i.e. higher in T2 (49.811 g) and lower in T1 (46.333 g). Gross fish yield was higher in T2 (1.8938 $kg/m^2/yr$) and lower in T1 (1.7616 $kg/m^2/yr$). Net fish yield was in 0.7849 g, 1.0367 g and 0.9357 g in T1, T2 and T3 respectively which was slightly higher in T2 compared to T1 and T3. Average daily weight gain was o.344 g, 0.454 g and 0.410 g in T1, T2 and T3 respectively. The feed conversion ratio was higher in T1 (5.052) and lower in T2 (3.723). There was no mortality in any of the treatments or replicates, thus 100 % survival rate was found throughout the trial.

4.5 Temperature

Figure 5 shows average temperature in different sampled dates. The average temperature during the study period was 23.04°c. It shows decreasing trends of temperature throughout the experimental period with highest temperature of 26.9°c recorded on 24th October and lowest of 20.16°c recorded on 23th November as shown in Appendix II.

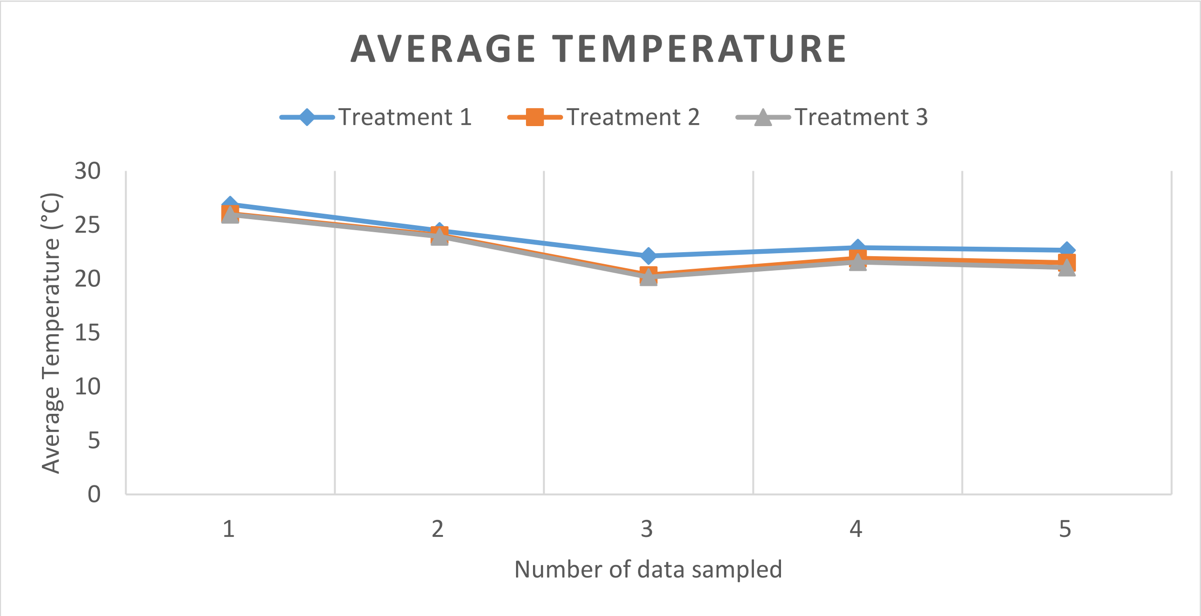

Figure 5. Average temperature of fish experimental tanks in each sample.

4.6 Dissolved Oxygen

Figure 6 shows the average Dissolved Oxygen of different treatments on different sampled dates throughout the experimental period. The average DO during the study period was 7.70 mg/l. It shows almost steady DO with slight fluctuation. The highest DO was 8.13 mg/l recorded on 22[th] December and lowest on 6.76 mg/l on 24[th] October.

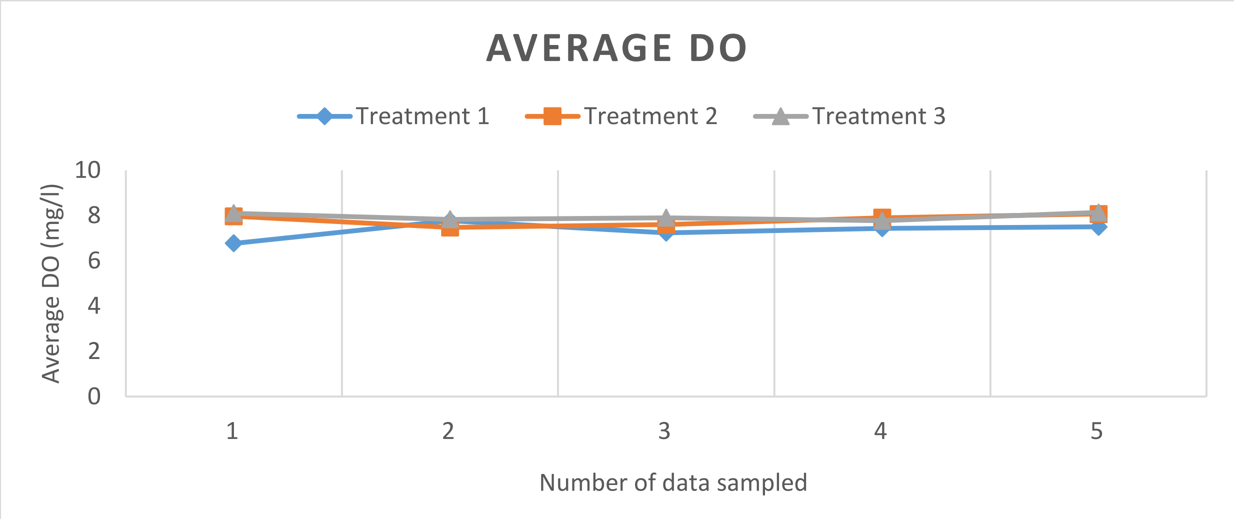

Figure 6. Average Dissolved Oxygen of fish experimental tanks in each sample.

4.7 pH

Figure 7 shows the graphical representation of average pH of different treatments during various sampling dates. pH of all treatments follows same fashion of fluctuation. The average value of pH ranged from 7 to 7.3 throughout the trial.

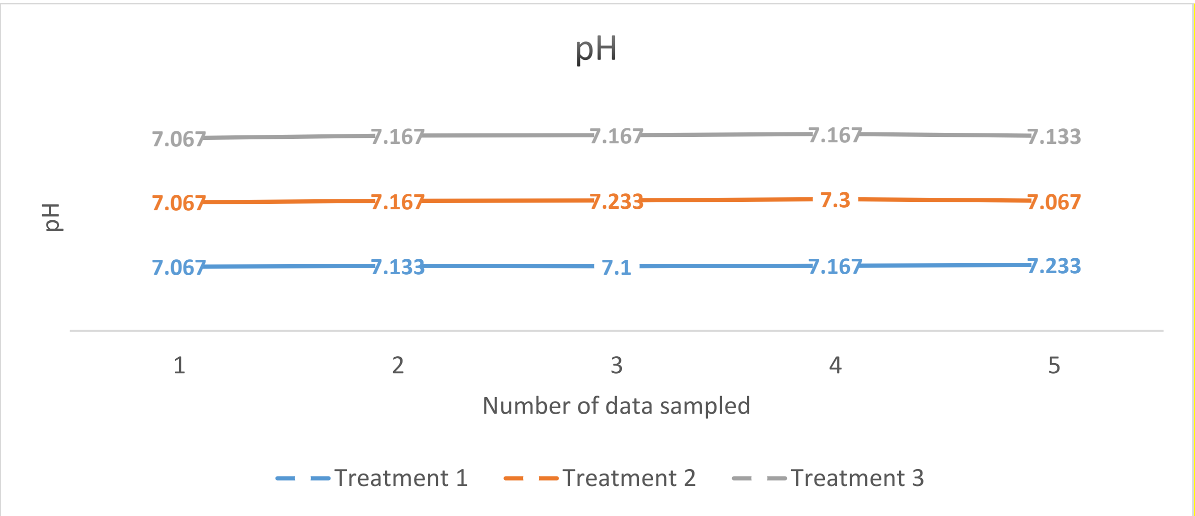

Figure 7. Average pH of fish experimental tanks in each sample.

5. DISSCUSION

The present study was carried out to evaluate GAS as alternative dietary protein source for Nile tilapia. Three diets were prepared with 45% alternative protein concentration, one with 100 % soybean protein (T1) considered as controlled diet and others with 50-50 % soybean and GAS protein (T2) and 100 % GAS protein (T3) as experimental treatment diets. Study showed no significance difference in average weight gain, AFCR, daily weight gain, gross fish yield and net fish yield in Nile tilapia fed with GAS as their alternative dietary protein source. It supports that soybean protein can be partially or totally replaced by GAS protein in Nile tilapia. The present result is supported by Abarra and Ragaza (2017) who replaced fish meal with processed Knife fish *Chitala ornata* (KFM) with high caloric contents in Nile tilapia juvenile and found partial or total replacement mainly with 75% KFM increase average weight gain, specific growth rate and feed intake. Similarly the results are comparable with earlier studies on positive response of mirror carp fed tropical earthworm meal (Rawling et al., 2012) due to high protein content i.e. 50-70%, good mineral and amino acid contents (Feedipedia, 2018). Other protein sources such as full fat and defatted maggot with meal having crude protein content of 40-60%, lipid content ranging 9-25% and high lipid content (Feedipedia, 2018) were also found to demonstrate better growth performance in *C. gariepinus* (Fasakin et al., 2003) which favors the present result. Further Marques et al. (2016) suggests all life stages of *Aurelia* sps are accepted as food for gill head sea bream *Sparus aurata* which supports the use of high protein animals as fish food. Studied on juvenile Nile tilapia suggests that dietary protein based menhaden fish meal to soybean based meal ratio of 1.22 to 1.35 gave high weight gain, lower average daily feed intake, lowest feed conversion ratio and greater specific growth rate (Koch and Hyde, 2016). Similarly the use of 30% inclusion of copra and karnel meal in Nile tilapia gave good growth response without any adverse effect on feed intake or pellet nutrient losses prior to ingestion (Obirikorang and Skov, 2015).

Poultry byproduct meal with substitution of total protein content when compared with fish meal based control diet 100% fish diet, indicated replacement up to 40% of protein content in practical feed is significant (Abdel-warith et al., 2001) due to high protein content ranging 75-90% with relatively low content of ash (less than 10%) and fat (less than 15%) (Feedipedia, 2018). Though others poultry byproducts of low quality have protein content in the 55-75% range and higher amounts of ash (up to 15%) and fat (more than 15% and up to 30%) (Feedipedia, 2018). Similarly various trophic level fishes like Bighead carp (*Hypophthamichthys nobilis*), Grass carp (*Ctenopharyngodon idella*) and mud carp (*Cirrhinus molitorella*) of omnivorous chain produced high quality fish fed with different food waste and meat meal (Cheng et al., 2015). Locally available plant and animal ingredients contributed in good quality striped catfish fingerlings without compromising growth performance, feed utilization and carcass traits (Chau et al., 2012) and appropriate proportion of different types of food wastes like kitchen, restaurants, sloth house satisfied basic nutritional requirement of lower trophic level fishes like grass carp and Tilapia (Wong et al., 2016) contributing economic sustainable waste management.

In contrast trout fed with fish meal containing freeze-dried worm as an alternative dietary protein source showed negative growth performances (Hilton, 1983).

The average weight gain was quite high i.e. 6.23, 7.778 and 7.733 at first sampling in T1, T2 and T3 respectively compared to further sampling. Similar result was obtained in case of DWG and NWG, which might be due to optimum growth temperature. The value of average weight gain, DWG and NWG later decline because of low water temperature, atmospheric temperature, lack of live foods etc. AFCR in all the three treatments was almost same which might be resulted due to maximum feeding and same environmental condition in all the tanks.

The growth performance of Nile tilapia fed with GAS protein did not find significantly higher, compared to soybean, it might be due to its low level of fat (less than 3%), phosphorous, potassium and magnesium (Abouo, 1990).

The optimal temperature for growth is 28-36°c and growth decline with temperature decrease (Teichert-Coddington et al., 1997; FAO, 2014). Water temperature during first sampling was optimum which later drops throughout the experimental period due to decreasing atmospheric temperature and cemented tanks (Bandyopadhyay et al., 1996; Naderi et al. 2002) because of its porous nature. The average temperature in the present study was 23.04°c which was quite low for Nile tilapia growth.

DO concentration in water is dependent on water temperature and also partial atmospheric pressure above the water level (Patil et al., 2012). Nile tilapia cannot survive DO below 0.7 mg/l. Tilapia can survive acute low DO concentration for several hours, though its ponds should be managed to maintain DO concentration above 1 mg/l (Popma, 2005). Its correlations with water body gives direct and indirect information on bacterial activity, photosynthesis, stratification and availability of nutrients (Premlata, 2009).The average DO during the study period was 7.70 mg/l which was good for Nile tilapia growth. The DO level was good throughout the study period due to continuous water supply in the form of sprinkles. DO fluctuated between 6.1 to 9.3 mg/l during the study period.

pH is the measure of acidity and basicity of water and is measured in pH scale which express the concentration of hydrogen ions indirectly. pH in water is governed by the amount of free Co_2, Carbonates and Bicarbonates. Change in pH value change the other aquatic chemical parameters, thus its value plays major importance in good fish culture. Nile tilapia can survive pH ranging from 5-10 but do best in PH ranges from 6-9 (Popma, 2005). The average pH during the study was 7.149 which lies in between its optimal value. There was only slight difference in the pH value in different treatments which might be due to continuous water exchange.

The height of experimental tank was 1 m which was filled with water level of 0.8 m. Thus, transparency of tanks was very clear, fish feeding on the diets can be seen clearly due to low feed waste and continuous water exchange during whole experimental period.

6. CONCLUSION

The present feeding trial on Nile tilapia replacing soybean protein with GAS protein in order to evaluate GAS as an alternative dietary protein source for Nile tilapia showed no significance difference among growth parameters like average weight gain, Apparent feed conversion ratio, daily weight gain, gross fish yield and net fish yield. Nile tilapia fed with mixed soybean and GAS as protein source gave slightly higher growth than soybean alone or GAS alone as protein source. The physic-chemical parameters like temperature, dissolved oxygen, pH, transparency etc. also favors the Nile tilapia growth positively with no mortality when fed with Giant African Snail as protein source.

Hence, GAS can be used successfully in Nile tilapia culture either as partial or total replacement of soybean as protein source.

7. RECOMMENDATIONS

From the above carried study, following recommendations can be drawn:

- Extended study on monoculture and polyculture of Nile tilapia in natural conditions using GAS as food source should be carried out.
- Feeding trials on others carnivorous fishes like *Clarias batracus, C. gariepinus, Heterropneustis fossalis* etc. should be carried out.

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Appendices

Appendix-1: Weight records of different treatments at different sampling dates in gram.

Treatments	Date					
	Replicates	24th Oct.	8th Nov.	23rd Nov.	8th Dec.	22nd Dec.
Treatment 1	T1R1	805	985	1176	1356	1506
	T1R2	740	932	1074	1224	1404
	T1R3	767	956	1020	1150	1260
Treatment 2	T2R1	635	855	1063	1261	1434
	T2R2	666	916	1092	1295	1482
	T2R3	728	958	1131	1348	1567
Treatment 3	T3R1	700	946	1119	1307	1474
	T3R2	671	908	1071	1247	1416
	T3R3	786	999	1153	1325	1482

Appendix-2: Water quality parameters in different treatments

Treatments		Date														
		24th Oct.			8th Nov.			23rd Nov.			8th Dec.			22nd Dec.		
		Temp.	DO	PH	Temp.	DO	PH	Temp.	DO	PH	Temp.	DO	PH	Temp.	DO	PH
Treatment 1	T1R1	27.3	6.1	7.0	24.3	8.2	7.1	23.4	7.1	7.0	24.1	9	7.1	23.2	8.1	7.3
	T1R2	27.4	7	7.0	24.5	7.5	7.2	21.2	7.4	7.2	24.4	6.3	7.1	23.7	7.0	7.2
	T1R3	26	7.2	7.2	24.6	7.6	7.1	21.8	7.2	7.1	20.2	7	7.3	21.1	7.4	7.2
Treatment 2	T2R1	25.7	7.8	7.0	24.1	7.7	7.1	19.9	7.0	7.3	21.5	6.6	7.2	20.7	7.1	7.1
	T2R2	26.2	9.3	7.1	24.1	7.6	7.2	20.6	8.2	7.0	22	8.7	7.3	21.8	8.4	7.0
	T2R3	26.2	6.8	7.1	24	7.1	7.2	20.6	7.6	7.4	22.2	8.4	7.4	22	8.7	7.1
Treatment 3	T3R1	26.1	7.6	7.0	24	8.1	7.0	20.7	7.2	7.3	22.1	8.3	7.3	21.9	8.7	7.2
	T3R2	26	8.4	7.2	24	7.9	7.3	20.3	7.5	7.1	21.3	8.5	7.1	20.7	8.2	7.0
	T3R3	25.8	8.3	7.0	23.8	7.5	7.2	19.5	9	7.1	21.3	6.5	7.1	20.6	7.5	7.2