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Effect of Protein Level on Growth Performance of Nile Tilapia (*Oreochromis niloticus*)

By

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Dedication

To my parents with love

And to my brothers and sisters

Nariman

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Above all my deep thanks and gratitude to Almighty Allah for giving me health and patience to complete this study.

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ABSTRACT

This study was conducted at Sudan University of Science and Technology, Department of Fisheries and Wildlife Science, to investigate the feed efficiency of three formulated diets from local ingredients with different dietary protein levels (25, 30, and 35%) on growth and performance of Nile Tilapia fingerlings (*Oreochromis niloticus*).

The experimental diets were obtained from El-Fath processing fish diet, 10 kilometers south of Khartoum (Soba). Fish were fed at 5% biomass per day during the study period.

The results revealed that the best growth rate and food conversion ratio (FCR) was obtained with 25% dietary protein level followed by 35% and 30% respectively, while the poorest growth rate and (FCR) was recorded in the fish on the diet of 30% protein level.

The protein efficiency ratio (PER) and apparent net protein utilization (NPU) were directly proportional with protein level. Thereafter, further increase in the dietary protein level to some extend led to decline in growth rate.

بسم ا الر ن الرحيم

خلاصة الأطروحة

أجريت هذه الدراسة في مزرعة الأسماك بكلية الطب البيطري والانتاج الحيواني قسم علوم الأسماك والحياة البرية لتحديد الكفاءة الغذائية لثلاثة أنواع من علائق الأسماك المصنعة من المكونات التجارية المحلية (مركز الفتح) والتي تحتوي على ثلاثة مستويات من نسبة البروتين (25، 30 و 35%) على نمو وأداء أصبعيات أسماك البلطي النيلي.

قدمت هذه العلائق لأصبعيات البلطي النيلي والتي يتراوح وزنها الكلي بين 12- 20جرام لمدة شهرين بمعدل 5% من وزنها حيث أوضحت نتائج التجربة أن أعلى معدل نمو وكفاءة تحويلية (FCR) تم الحصول عليها من العليقة ذات مستوى بروتيني 25% ثم تليها العليقة 35% وأقل معدل نمو وكفاءة تحويلية تمت ملاحظتها في الاسماك التي غذيت بالعليقة التي تحتوي على مستوى بروتيني 30%.

عندما حللت النتائج إحصائياً وجدت أن كفاءة البروتين التحليلية (PER) وكفاءة البروتين المستخدم في العليقة (NPU) تتناسب مع مستوى البروتين. كما وجدت أيضاً أنه كلما زاد مستوى البروتين في العليقة عند حد معين ينخفض مستوى النمو في السمكة.

أما بالنسبة للكفاءة التحويلية وبروتين العليقة المستخدم توجد علاقة واضحة بينها وبين متسوى البروتين في العليقة كلما زاد مستوي البروتين إنخفضت نسبة البروتين المستخدم والكفاءة التحويلية.

CHAPTER ONE
INTRODUCTION

Pond culture is not a traditional farming in most parts of Africa and it was introduced after the 2nd World War. There was an initial spectacular aquaculture development with 300.000 ponds being operated, mainly rearing *Tilapia spp*, in about 20 African countries by the end of fifties (Meschkat, 1967).

The importance of aquaculture for the supply of animal protein to humans is constantly increasingly being particularly valuable in regions, in spite of which other types of husbandry are available.

In contrast to intensive and intensive farming systems where the cultured species derive all or a substantial part of their nutrient needs from naturally available pond food organism, fish maintained under intensive clear-water culture conditions are totally dependent on the external provision of a nutritionally "complete" diet throughout their culture cycle (Tacon, 1985).

Studies on finfish nutrition have been described mostly towards developing low-cost, especially by substitution of the animal protein ingredients with plant protein substitutes (Crus and Landencia, 1977; Jackson *et al*., 1982; Vila and Arieli, 1982). In aquaculture the cost is often the highest recurring cost. Apart from providing a suitable diet and reducing the ingredients cost, proper husbandry practices also could provide a considerable saving (DeSilva, 1985).

The development of artificial diets (i.e. complete pelleted feeds) for use within aquaculture production systems has been based largely on the nutritional and manufacturing techniques pioneered and development by the intensive poultry industry (Tacon, 1987).

The successful intensification of culture methods for *Oreochromis niloticus* may be achieved by accurate diets satisfying all the nutritional requirements as formulated. The satisfactory diet formulation depends mainly on quantitative, qualitative and source of protein (plant and animal protein) in consideration of profitability and economic visibility.

Recently many studies have been attempted to determine exact dietary protein requirements of *Oreochromis niloticus* from readily available local materials to maximize growth.

As a result of recent development and improvements in the techniques of fish culture particularly those concerned with the production of eggs, fry and juvenile fish aquaculture production has been growing at the rate of 10% per annum up to the present time more than 300 different species of finfish have been cultivated all with different feed and nutritional requirements.

The main objective of this study is to evaluate the feeding efficiency of three different fish diets on growth performance of Nile Tilapia (*Oreochromis niloticus*).

CHAPTER TWO

LITERATURE REVIEW

2.1 General:

As a general view fish culture in Sudan has been practiced many years ago, while the progress is poor, in spite of availability of the basic of fish culture (fertile land, natural water, good selected culture species and the research skill).

This last is considered as important factor for developed culture so for maximize output and achieve the highest possible efficiency of resource.

Referred to culture species in Sudan, the selected species *O. niloticus* showed high growth rate even with a low protein diet exploiting both the natural productivity of the rearing environment and the distribution of supplementary feed. They tolerate a wide range of environmental conditions and show good resistance to disease, they mature quickly and produced frequently in captivity (seed production does not constitute a major problem) and in specific case of Sudan they are traditionally familiar and accepted by the consumer.

Researchable many studies were carried out to increase production and maximize growth of *O. niloticus* such studies were on water quality, reproductive biology, food and feeding ecology in spite of these studies the field of food and feeding is still needs much more research specially in supplementary feed and adjusting dietary protein requirements.

2.2 Food, feeding ecology and growth of *Oreochromis niloticus*:

Naturally *O. niloticus* can be considered a plant to feeder as starter feed which beginning with phytoplankton and omnivorous and about to be herbivorous at the end moves to ingested zooplankton after a while it become omnivorous and about to be herbivorous at the end (Bishai, 1963). This change in feeding behavior have led to setting of experiment aiming to provision of food suits all stages of lifecycle and attaining level of growth at minimal cost. In a wide application the traditional rearing of *O. niloticus* depended mostly on extensive methods where the fish obtained all their nutrition from aquatic environment in which they were cultured. Nutrient input into the culture system was limited per unit area, high production level depend on good nutrition as well as proper culture management, and the use of feeds in *O. niloticus* culture systems has increased production and profits considerably.

2.3 Artificial and supplementary feeds:

Feed represents the largest expenditure item in fish culture and protein is the most expensive nutrient in the diet in *O. niloticus* farming the natural food contribute significant amounts of nutrient with compounded feeds formulated for increased yield. Coactal (1977); Veverica *et al*., (1999) evaluate alternative cheap feed supplements for *Oreochromis niloticus* farming from local ingredient rice bran, wheat bran, cassava meal and corn meal in consideration of availability and cost of compound other preliminary studies conducted by Lili *et al.* (2001) have shown that the performance of different bran in promoting *Oreochromis niloticus* growth. Maize bran for example gives highest fish yield followed by wheat bran, while rice bran yielded the least. Beside various proportional studies by Alim (1996), for the supplementary feeds (wheat bran, groundnut cakes, sesame cakes and fish meal) mixtures resulting in the best economic formula that would yield the best conversion efficiency. Seeking ways for increasing *Oreochromis niloticus* production Lili *et al.* (2001) found that to compare locally available rice bran with two compounded laboratory formulated diets resulted that the compound diets has similar nutritional value and promoted better fish growth than rice bran.

Michael and Imre (1993) reported that to local abundance of certain raw materials is incurred for formulated diets for aquaculture production. So in Bangladesh mainly use wastes of slaughter house fish silage pulse bran while in Thailand is used trash fish, soy bean curd, leaf meal and sweet potatoes. This formula apparently high protein with cheaper cost compared with commercial available feed. Thus greater effort should be done to find local substitute ingredient for successfully been used as *Oreochromis niloticus* specially for fry and finger lings.

2.4 Source of protein in diet formulation diets:

The qualitative and quantitative of protein in *O. niloticus* diets mainly depends on source of protein.

2.4.1 Animal protein:

Is quite important for formulation diets, the main source of it is found in fish meal which still constitute substantial part of the feed formulation for *Oreochromis niloticus* and many other species however the rising cost and uncontained availability of fish meal have forced nutritionist and feed manufacturers to use less expensive, readily available plant protein to substitute for fish meal (Lim and Dominy, 1989).

2.4.2 Plant protein

Because of the limited supplies and high price of animal protein (fish meal) other alternative sources of protein must be considered. Plant proteins are generally cheaper per unit of nutrient protein nutrient than animal protein (Alceste, 2000). Although a large amount of protein feed-stuffs has been used in domestic animal feeds, commercial aquaculture feeds for grow out contain 25-45% (Crude protein). Thus only high protein content plant ingredient such as oilseed residues, are used in fish feed. The most commonly utilized by feed manufactures are soybean meal, peanut meal, cottonseed meal, sun flower seed meal. Olivera (2002) evaluated the effect of substituting animal protein with mixture of plant feedstuffs, soybean meal, alfalfa leaf concentrate in diets for *Oreochromis niloticus* frying in different percentage protein level (25, 30, 35, 40 and 45%) resulting that fish meal fed with 30 diet showed the best growth performance. The best incidence cost was obtained with 25% and high profit index with 30% the results suggest that it is possible to replace is 65% of animal protein with mixture of plant protein. Also Hossain, Foken and Becker (2002) evaluated the nutrition value of sesbania seed meal as a possible fish meal substitute in diet of *Oreochromis niloticus* by formulated iso-nitrogenous and iso-energetic diets to contain 32% crude protein and 18.4 kg gross energy resulting that diets with sesbania meal were similar and significantly ($P<0.05$) better than the other dietary ingredients. The extent of plant protein utilization depends on availability and acceptability by *O. niloticus*. Siddiqui and

Adam (1989) used date palm leaf silage as alternative fish feed, they found that the growth performance and feed utilization was less efficient in smaller weight fish (9.1 g) than the larger fish (125 g) and considerable a result of fiber accumulated in the high levels of plant protein in fish diets in some case resulted in reduced growth and poor feed efficiency. Probably proper balance of essential nutrients or decease of palatability and pellet water stability value (Lim and Dominy, 1989).

2.5 Dietary protein requirement:

Dietary protein is always considered to be primary importance in fish feeds as the protein requirements of fish are higher than those of domestic animals and protein sources account for more than half of the total feeds cost. Alceste (2000) protein is the basic building nutrient of cultural fish and usually accounts for 68.85% of the dry matter of fish carcasses.

Marty (2003) reported that the level of dietary protein producing maximum growth of *O. nitolicus* depends on the emergency diet, the physiological state of the fish such as (age and environmental factors) the level of dietary protein producing maximum growth of *Oreochromis niloticus* decreases with increasing fish age) and quality beside the level of food intake. Johnny (2002) added range from 32-36% in fingerling feed and 28-32% in feed for larger than 40 grams body weight the amount of energy provided from fats is generally restricted to 4-8% of the diets, the higher content is fed to small fish and gradually decreased with increasing size. Some studies have been created to determine the exact dietary protein requirements of *O. niloticus*. Hughes (1977) gives the recommended optimum protein with weight dependence in the fry 0.5 g about 50%, P 0.5 – 10 g (40%) P and 10–30 g) about 30-35% protein level. Siddigui and Adam (1987) compared four artificial diets with dietary protein levels of 20, 30, 40 and 50%. They found tat for fry the best growth was obtained with 40% dietary protein and about 30% protein is optimum for maximum growth for young tilapia and the feed conversion efficiency (FCE) increased from 20 to 30% dietary protein and there decreased for diets containing 40 and 50% protein.

Other preliminary feeding trial was conduced by Corazon (2002) on finger lings fed with high protein diet 25% and low protein diet 18% is found that high growth in case of high protein and this is refer to lower percentage into amount of protein.

In the present result Ogunji (2001) compared four different formulated diets 35, 40, 45 and 50% crude protein in ringer lings of *Heterobranchus longifilis* and found that specific growth rate (SCR) and food conversion ratio (FCR) and weight gain increased as the dietary protein level increase and the treatments fed with 35 and 40% crude protein diets gave low performance and the best growth was obtained in treatment fed with 45% crude protein.

Other researcher, Johnny (2002) reported that the dietary requirement depended on essential amino acid which content in protein, requirement, this follows the concept that protein requirement and ensure maximum growth.

2.6 Energy requirement:

The dietary protein is energy ratio required for maximum growth decreases with increasing size of Tilapia. Sinfree and Stickeny

(1981) found that small *O. aureus* (2.59) grew best when the diet contained 35% protein with a digestible energy, protein (DE/P) ratio of 8.2 kcal/g of protein. Larger fish (7.5 g) grew maximally when fed a diet containing 9.4 kcal DE/g protein.

Nubaryk (1980) reported that small *O. niloticus* (1.77 g) grew maximally when the DE ratio was 8.3 kcal/g for a 36% protein diet. He also found that as DE content of the diet increased, food consumption decreased, but that amount of protein in the diet did not affect consumption rate.

2.7 Essential fatty acid requirement:

Tilapias appear to have a dietary requirement for fatty acids of the linoleic (n-6) supplementation of tilapia diets with vegetable oils (soybean or corn oils) rich in 18.2 n-6 has given better performance than those containing fish oil high in 20.5 n-3 fatty acids (Takouchi, Satoh and Watanabe, 1983a). The optimum dietary levels of n-b fatty acids have been estimated to be about 1% for *T. zillii* (Takeuchs, 1983[a]) and 0.5% for *O. niloticus*.

2.8 Vitamins requirement:

Relatively little information is available on vitamin requirements of tilapias. One reason for this is that most tilapia culture is in ponds where the fish consume large quantities of natural foods, which probably satisfies their vitamin needs, vitamin supplements are often detected from practical feeds for tilapia cultured under extensive conditions in ponds. Culture system where limited of no natural food organisms are present, supplemental vitamin must be added to commercial feeds. Due to the lack of information on vitamin requirements for tilapias, allowances established for other warm water species are used.

2.9 Mineral requirements:

Minerals are inorganic elements required by animals in maintaining many of their metabolic function, these functions can be summarized as follows (Jauncey, 1992).

Structure of hard skeletons such as bones and teeth. Structure of soft tissues. Nerve impulse and transmission and muscle contraction. Minerals serve as components of many enzymes, vitamin, hormones and respiratory pigments. Little information is available on the mineral requirements of tilapia and therefore their response to dietary minerals is not fully understood. The requirements of only eight minerals namely: calcium, phosphorus, magnesium, zinc, manganese, potassium, iron and chromium. The mineral requirements of tilapia depend on fish size and mineral contents of both culture water and fish feeds.

2.10 Water quality:

The most crucial factor affecting the success on failure of a fish-farming venture is the water supply. The quantity of water available determines the quantity of fish, which can be produced, but if the water quality is unsuitable, then clearly the fish farm is bound to fail anyway. Each species has preferred ranges for the various parameters of water quality, such as temperature dissolved oxygen and salinity and ideally the fish farm should operate at the optimum levels of each parameter to achieve fast growth and efficient performance.

2.11 Feed storage:

Fish feeds usually contain relatively high amounts of fish meal and fish oil. They are very susceptible to rancidity. In addition, ascorbic acid is highly volatile, but critical to normal growth and development of most species of fish. For these reasons, fish feeds should be purchased frequently. Feeds should be stored in a cool, dry place and should never be kept on hand for more than three months. Refrigeration of dry feeds is not recommended because of the high moisture content of that environment. Freezing is an acceptable way of extending the shelf life.

2.12 Fish uses and food value:

Fish plays a vital role in feeding the world's population. Recent global statistics revealed wide variations in fish consumption, but people in the developing countries are generally much more dependent on fish as a part of their daily diets than people living in the developed world. It may be used for human consumption with excellent nutrient content when prepared from small fish (Patti, 1991).

Fish is an important and highly desirable food for people suffering from protein energy mal-nutrition, which is a leading cause of infant mortality in the developing world. It can be available force against endemic goiter caused by lack of dietary iron and iodine. Each 100 grams of lean or white fish contains less than 1% of fat about 18% of protein and energy value range of 50-80 kcal.

Only fish contains 8-15% of fat and so have higher energy value (80100 hcal/100g) when processed, preserved and cooked properly. Fish retains most of its high nutrients content. However this can be lost with poor handling and storage. Fish protein has a high biological value similar to the protein of land animal but the contents of protein are some-what less than meat and there is often a large waste in the scales and bones.

Marine fish are rich sources of iodine in the diet and a good source of fluoride. Small fish may be a useful source of calcium when eaten has a whole together with the bones (Passmore and Eastwood, 1986).

2.13 Fish production in Sudan:

The natural fisheries of Sudan are divided into two main sectors, the inland fisheries (fresh water fisheries) and the marine fisheries of the Red Sea. The land fisheries are composed of the main Nile and its tributaries which are 6500 km long, and specially the reservoirs formed by the dams on the rivers. Jebel Aulia reservoir on the White Nile, Rosaries and Sennar reservoirs on the Blue Nile, Khashm-Algerba reservoirs on Atbara river and Nubia Lake, which is the Sudanese portion on Nasir reservoir. It lies in the northern part of Sudan and it was formed by the construction of the Egyptian high dam south of Aswan. It is the richest source of fish in the main Nile inside the Sudan, in addition to the Sud region at upper White Nile. On the other hand the marine fisheries are at the Sudanese coast line on the Red Sea which extends to 720 km, and a continental shelf of about 9800 km^2 which is unsuitable for trawling due to its irregular coral beds (Souness, 1978). This area is endowed with fine fisher, shell fishes, crap and sustainable fish stock of Sudan is about 110.000 tons (Fishery Department Statistics, 1995).

About 68% of this is found in the Southern States and specially in the Sudd region due to the existence of favourable natural conditions for fish production and growth, water covers about 2 million hectares.

The total area of Sudan is bout 20 million hectares. Fishermen are considered in most of the developing countries as one of the most neglected and poorest groups within society, having traditional inherited fishing methods, which are not adapted to modern fishing methods.

2.14 Fish farming (Aquaculture):

Capture fisheries will continue to make the major contribution to world fish supplies, but aquaculture (farming of fish aquatic plants) is expected to fill the gap between the demand and supply and according to (FAO, Fishery Department, fact file, 1998), the aquaculture sector's contribution to total food fish supplies is about 29%.

Sudan is qualified to expand in aquaculture as the essential inputs such as land, water and agricultural byproducts, which are the main ingredients of fish feed are available. Also the irrigation channels, natural pools and (Hafeers) can be used as fish farms.

The first fishery farm has been established in 1952 and since then the sector witnessed horizontal increase in number and area of farms, reaching 147 registered in Khartoum, Gezira, Kassala and White Nile States, 138 farmers are in Khartoum State and 9 in the other states, yet only four of these were operated. The area of these farms ranged between half and 30 feddans. The operated farms were not used under economic basis, but just for recreation and sells consumption (Abdel Alwahab, 1997).

Actually, most of the fish farmers were established in order to complete the farm decoration, i.e. vegetable fruits, diary, poultry and fisheries (Abdel Alwahab, 1997). Most of the farms are constructed of earth basins with some trials in the natural pools (e.g. Rahad in Western Sudan) and in cages (Jebel Aulia Dam site) beside oyster farms in the Red Sea. The average annual production per feddan is expected to be 4 tons of tilapia (Buliti), (Osman, 1993).

The main reasons of the failure of this sector in Sudan are not institutionally treated leaving the problem unsolved in addition to lack of equipments and skillful trained staff; moreover the problem of power can be included (Abdel Wahab, 1997).

CHAPTER THREE

MATERIALS AND METHODS

3.Study area:

The study was conducted at the Department of Fisheries and Wildlife, College of Veterinary Medicine and Animal Production, Sudan University of Science and Technology, Khartoum North, Sudan photo (1).

Photo (1): Fisheries experimental farm.

3.2 Experimental size:

Seventy two fish individuals of Nile Tilapia (*Oreochromis niloticus*) were collected from fish farm of the Fisheries Department by gill nets for this study (Photo 2). The positive aquaculture characteristics of Nile Tilapia are their tolerance to poor water quality, fed on available and cheap feed and it is a hardy fish, which can withstand handling during experimental study (Mahdi, 1973).

Photo (2): Weighing of experimental fishes during the study period.

3.3 Experimental diets:

Three different experimental diets in protein level 25% (diet A), 30% (diet B), and 35% (diet C) the diets were obtained from El Fath processing fish diet plant, 10 kilometers south of Khartoum for this study. Chemical composition of the experimental diets is shown in Table 1.

Table (1): Chemical composition(%) of the experimental diets.

Diets	DM	CP	CF	Ash	Fat
A	95.37	35	21.95	8.5	5.0
B	95.65	30	20.44	10.1	5.3
C	94.5	25	19.95	9.9	4.3

Where:

A=25 level of protein

B=30 level of protein

C=35 level of protein

DM= dry matter

CP=crude protein

CF= crude fiber

3.4 Experimental trials:

The experiment was conducted in six mosquito nets act as hapa (190 x 100 x 90 cm, Photo 3) fitted in the fish farm pond (20 x 15 m^2).

Experimental fish average weight was 20 g), standard length 7cm and total length 9.5 cm. These species were stocked at 12 fingerlings for each mosquito net (hapa).

Fish were fed with three different levels of protein diet (25, 30 and 35%), at 5% biomass per day, for the period Feb-April 2007.

Photo (3): Shows mosquito nets of fish rearing (hapas).

The feeding rate was adjusted every 10 days after sampling the population (photo 4). Feeding was done twice a day at 9:0 am and 3:0 pm with daily ration divided into two parts.

Photo (4): Shows sampling and feeding trial.

Temperature:

The temperature of the water fluctuated between 30and33^{o}C during the period of experiment using the ordinary centigrade thermometer.

pH:

pH was measured by a digital pH meter fluctuated between 7 and 8.2.

Proximate analysis:

Three randomly selected sacrificed fish from each group were ground homogenously for proximate analysis to determine moisture, protein, fat and ash according to AOAC (1980).

Moisture content:

Determination of moisture was based on weight loss of 5 g of fish meat. Fish samples (5 g each) were put in an oven at 100^{o}C overnight cooled in desiccators and reweighed. The moisture content was calculated as follows:

$$\text{Moisture \%} = \frac{\text{Initial weight} - \text{oven dried weight}}{\text{Initial weight}} \times 100$$

Crude protein:

Kjeldahl method was used to determine fish nitrogen content. Crude protein was obtained by multiplying the amount of nitrogen by 6.25.

Fat content:

Fat content (ether extract) of each sample was determined according to Soxhlet method, using 23 gm of fish samples. The extraction continued for 5 hours at 60^{o}C. Fat percentage was calculated as follows:

$$\text{Fat \%} = \frac{\text{Extracted fat weight}}{\text{Initial weight}} \times 100$$

Ash content:

Two grams of ground fish samples were placed into a dried crucible of known weight. The crucible was placed inside a muffle at 150^{o}C.

And increased gradually till it reached 600^{o}C and the sample was incinerated that temperature for 3 hours. Then crucible was taken out cooled into desiccators and weighed. The ash percentage was calculated as:

$$\text{Ash \%} = \frac{\text{Crucible wt containing incinerated fish – crucible wt empty}}{\text{Fish sample wt. before incineration}} \times 100$$

Statistical analysis:

The finding data of this experiment were analyzed by completely randomized design by Comez and Comez 1980.

CHAPTER FOUR

RESULTS

The results in tables (2 and 3) during study period show that the best growth rate and food conversion ratio (FCR) was obtained with 25% dietary protein level, followed by 35% and 30% respectively, while the poorest growth rate and (FCR) was recorded in the fish on the diet of 30% protein level.

The protein efficiency ratio (PER) and apparent net protein utilization (NPU) were directly proportional with protein level.

Table (2): Summary of the effect of protein level on performance of Nile Tilapia (*Oreochromis niloticus*) during the study period

Parameter	Diet A	Diet B	Diet C
SGR	0.89	0.66	0.69
FCR	3.4	4.9	4.4
FCE	29.9	23.1	22.5
PER	0.85	0.62	0.55
NPU	32.4	29.8	29.5
Weight gain	21.32	18.8	20.7

- Where:
- SGR= Specific growth rate
- FCR= Food conversion ratio
- FCE= Food conversion efficiency.
- PER= protein efficiency ratio.
- NPU= Apparent protein utilization

Table (3): Effect of different diets and period on the body weight of studied Nile Tilapia

Diets Period	Diet (A)	Diet (B)	Diet (C)	Overall	Sig
P1	24.76 ± 7.6	30.36± 5.7	29.58 ± 4.3	28.23±6.5[c]	**
P2	27.55 ±8.8	29.74 ± 10.5	32.52 ± 4.9	29.94±8.5[bc]	
P3	29.47±8.4	32.25±11.5	31.50±10.9	31.12±10.3[bc]	
P4	29.07±12.3	44.78± 5.8	29.02±14.4	34.29±15.19[b]	
P5	33.05±13.7	40.17±14.6	33.47±16.6	35.56±15.1[b]	
P6	41.96±17.2	46.05±17.2	37.68±18.4	41.90±17.5[a]	
Overall	31.0 ± 12.9[b]	37.22± 26.9[a]	32.29 ± 12.9[b]		
Sig.	**				

Where:
a,b means within the same column followed by different superscripts are significantly ($P< 0.05$) different.
** Highly significant ($P< 0.01$).

Table (4): The effect of diet and time on the standard length of studied sp.

Diets Period	Diet (A)	Diet (B)	Diet (C)	Overall	Sig
P1	8.15± 1.2	8.95± 0.8	8.80±0.65	8.63 ±0.9	N.S
P2	8.69 ± 1.2	8.63± 2.7	9.35±0.71	8.89 ± 1.7	
P3	8.86 ± 1.1	8.75± 2.7	8.68±2.70	8.76 ±2.3	
P4	8.52 ± 2.7	8.81±3.4	8.13±3.70	8.49 ±3.3	
P5	8.87±2.8	9.58±3.0	8.52±3.90	8.99±3.3	
P6	8.86±2.6	9.94±3.1	8.72 ± 4.00	11.18 ±5.7	
Overall	9.6 ± 1.2	9.11 ± 2.7	8.70 ± 2.90		
Sig.	N.S				

Where:
N. S : Not significant

Table (5): The effect of diet on the total length of studied sp.

Diets Period	Diet (A)	Diet (B)	Diet (C)	Overall	Sig
P1	10.02 ±1.2	11.03 ±0.7	10.76 ±0.7	10.60 ±1.0	N.S
P2	10.53 ± 1.4	10.44 ± 3.3	11.39 ± 0.7	10.78 ± 2.1	
P3	10.84 ± 1.3	10.58 ± 3.3	10.58 ± 3.3	10.66 ± 2.8	
P4	10.44 ± 3.4	10.79 ± 4.2	9.94 ± 4.5	10.39 ± 4.5	
P5	11.01 ± 3.5	11.18 ± 3.7	10.31 ± 4.7	11.05 ± 4.0	
P6	11.55 ± 3.7	12.18 ± 3.8	10.68 ± 4.9	11.47 ± 4.1	
Overall	10.73 ± 2.7	11.14 ± 3.4	10.61 ± 3.6		
Sig.	N.S				

Where:
N. S : Not significant

CHAPTER FIVE

DISCUSSION

The study was carried out to investigate the feed efficiency of three formulated diets from local ingredients with three dietary protein levels (25, 30 and 35%) on growth performance of Nile Tilapia fingerlings (*Oreochromis niloticus*).

The results revealed, that the best growth rate and food conversion ratio (FCR) were obtained with 25% dietary protein level followed by 35% and 30%, respectively, while the poorest growth rate and FCR was recorded in the fish on the diet of 30% protein level.

The protein efficiency ratio (PER), apparent net protein utilization (NPU) and food conversion efficiency (FEC) were directly proportional with protein level. Also, when analyzed statistically were found significant and progressively decreased with increasing of protein level, suggesting that the proportion of dietary protein used for catabolic processes (energy production) increased with the level of protein. These findings were in agreement with Turker *et al*. (2005). Thereafter, further increase in the dietary protein level led to decline in growth rate.

Feed conversion ratio (FCR) and protein efficiency rates (PER) in the present study fell within the rate reported by Day and Plascencia (2000) and Fournier *et al*. (2003, 2004) for Atlantic trout fed diets where fish meal was substituted by soybean protein concentrate, other feed ingredients or a mixture of plant proteins. The findings of this study were higher than those reported by Person-Le Ruyet *et al*. (2002). For other species reared in different O_2 concentration for 30 days (FCR=0.69-0.70, PER= 3.2-3.3, SGR=1.75-2.02). The results of this study might have been better than those obtained by other authors due to fish size and suitable water temperature. However, the values from the fishes fed diet 25% protein level significantly different ($P>0.05$) on the standard length and total length of the studied fishes. But there was no significant variation in the intervals of the feeding time (Tables 4 and 5). The poor growth rate increment in the diets 30 and 35% protein level diets could be the reason for the worse handling and management during the period of the study. The mean value for FCR of 25% protein level diet is 3.4 and 30% protein level diet is 4.4 and 35% protein level diet obtained in this study is greater than the value of 2.45 reported by Fox *et al*. (1994) in the same species fed by formic acid ensiled shrimp head meal based diet. Nevertheless, the values of SGR, FCR and PER (Table 1) obtained in this study are poorer than the mean values of 2.58, 1.61 and 1.52 for SGR, FCR and PER, respectively reported by Fagbenro *et al*. (2004).

Nengas *et al*. (1999) stated that, low performance of some of the formulated fish meals tested in their studies could be due to an insufficient essential amino acid or fatty acid contents. In this study, the high protein levels did not show higher growth rate among the studied fish species suggesting that the poorer growth of fish fed on 35% protein level could be attributed to a lack of some certain amino acids, but rather to processing condition quality of the ingredients, poor digestibility, or a combination of these factors.

This discrepancy points to the fact that 25% protein level in the fish diet is better digested and utilized by fishes than the high protein level as in the diets with 35% protein level and 30% protein level.

Generally, the trend of the results suggests that 25% protein level can be suitable and the best level protein for fish feed in the tropical region (Sudan).

CHAPTER SIX

CONCLUSIONAND RECOMMENDATIONS

1. The study is conducted in order to find the best and cheaper diets, using local ingredients in addition to satisfying the nutritional requirements of *Oreochromis niloticus* fingerlings, therefore, the agricultural by products and fish meal are best alternative materials to supply adequate fish feed.
2. The period of experiment was not enough.
3. The experiment in fish nutrition should be done for enough time so as to get the better findings there is a need for further studies in this field

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APPENDICES

App. (1) Body measurement during study period

Treat	Period	Rep	Wt	T_ L	S_L
1	1	1	26.0	10.0	7.5
1	1	1	28.5	11.0	9.0
1	1	1	28.0	11.0	9.0
1	1	1	29.0	12.0	10.0
1	1	1	33.0	11.5	9.5
1	1	1	33.0	11.5	9.5
1	1	1	33.0	11.2	9.5
1	1	1	36.0	11.5	10.0
1	1	1	32.0	11.0	9.0
1	1	1	32.0	11.5	9.3
1	1	1	34.5	11.0	9.5
1	1	1	33.5	11.0	9.0
1	1	2	10.2	8.4	6.7
1	1	2	19.0	9.0	7.0
1	1	2	14.5	8.0	6.0
1	1	2	21.5	9.0	7.0
1	1	2	22.0	9.0	7.5
1	1	2	18.0	9.5	7.5
1	1	2	17.8	8.5	7.0
1	1	2	20.0	9.0	7.5
1	1	2	22.6	10.0	8.0
1	1	2	14.0	8.0	6.5
1	1	2	17.3	9.0	7.0
1	1	2	18.9	9.0	7.2
2	1	1	24.0	10.5	7.5
2	1	1	25.0	10.0	8.0
2	1	1	24.0	10.0	8.0
2	1	1	24.0	10.5	8.5
2	1	1	26.0	11.5	8.5
2	1	1	27.0	11.0	9.0
2	1	1	24.0	10.0	8.0
2	1	1	24.0	10.5	8.5
2	1	1	27.4	10.5	8.5
2	1	1	24.5	10.0	8.0

2	1	1	25.4	10.5	8.5
2	1	1	25.5	10.0	8.0
2	1	2	38.0	12.0	10.0
2	1	2	38.0	12.0	10.0
2	1	2	36.0	11.5	9.5
2	1	2	35.0	11.5	9.5
2	1	2	34.0	11.5	9.5
2	1	2	36.3	12.5	10.5
2	1	2	38.5	12.4	10.0
2	1	2	35.9	11.5	9.5
2	1	2	33.5	11.0	9.0
2	1	2	38.3	11.5	9.8
2	1	2	34.5	11.5	9.5
2	1	2	30.0	11.0	9.0
3	1	1	33.0	11.5	9.5
3	1	1	34.4	11.2	9.5
3	1	1	35.0	11.7	9.5
3	1	1	32.5	11.5	9.5
3	1	1	35.0	11.5	9.5
3	1	1	30.0	10.5	8.7
3	1	1	34.0	11.5	9.5
3	1	1	35.4	11.5	9.5
3	1	1	32.8	11.0	9.0
3	1	1	33.0	11.2	9.0
3	1	1	35.0	11.5	9.5
3	1	1	33.7	11.5	9.5
3	1	2	24.7	10.0	8.0
3	1	2	24.7	10.0	8.0
3	1	2	22.0	9.8	8.0
3	1	2	21.0	9.5	8.5
3	1	2	26.2	11.0	9.0
3	1	2	28.5	10.0	8.0
3	1	2	26.5	10.0	8.0
3	1	2	26.3	10.0	8.0
3	1	2	27.0	10.5	8.5
3	1	2	25.3	10.0	8.0
3	1	2	29.0	11.0	9.0
3	1	2	25.0	10.5	8.0
1	2	1	37.5	12.5	10.5
1	2	1	33.9	11.9	9.8

1	2	1	35.7	11.9	9.5
1	2	1	27.0	11.5	9.5
1	2	1	32.0	11.0	9.0
1	2	1	41.5	12.5	10.5
1	2	1	33.0	11.5	9.5
1	2	1	39.0	12.5	10.0
1	2	1	36.0	12.0	10.0
1	2	1	37.0	12.0	10.0
1	2	1	34.5	11.5	10.0
1	2	1	38.5	12.0	10.0
1	2	2	13.0	8.5	7.0
1	2	2	19.0	9.0	7.3
1	2	2	19.5	9.0	7.5
1	2	2	22.0	10.0	8.0
1	2	2	20.0	9.0	7.5
1	2	2	20.0	9.5	7.9
1	2	2	18.2	8.5	7.3
1	2	2	25.4	9.5	7.5
1	2	2	21.0	9.5	7.9
1	2	2	23.7	10.0	8.0
1	2	2	14.5	8.5	7.0
1	2	2	19.5	9.0	7.5
2	2	1	36.0	11.5	10.0
2	2	1	26.5	10.1	8.2
2	2	1	38.0	12.0	10.0
2	2	1	27.1	11.0	9.0
2	2	1	39.0	13.0	10.5
2	2	1	29.0	11.0	9.5
2	2	1	31.8	11.5	9.5
2	2	1	35.0	12.0	10.0
2	2	1	27.5	10.5	9.0
2	2	1	28.5	10.5	8.5
2	2	1	28.0	10.5	8.5
2	2	1	26.0	10.5	8.5
2	2	2	42.0	12.5	10.0
2	2	2	40.0	12.0	10.5
2	2	2	38.0	12.0	10.0
2	2	2	28.0	11.0	9.0
2	2	2	30.0	10.5	8.5
2	2	2	32.5	11.5	9.5

2	2	2	35.5	11.5	9.5
2	2	2	30.0	12.0	10.0
2	2	2	41.0	13.0	10.5
2	2	2	24.5	10.5	8.5
2	2	2	0.0	0.0	0.0
2	2	2	0.0	0.0	0.0
3	2	1	38.0	12.0	10.0
3	2	1	33.5	12.0	10.0
3	2	1	40.0	12.0	10.5
3	2	1	42.4	12.5	10.0
3	2	1	36.2	12.0	10.0
3	2	1	30.0	11.5	9.5
3	2	1	36.0	12.0	10.0
3	2	1	37.5	12.0	10.0
3	2	1	37.5	12.0	10.0
3	2	1	37.0	12.2	10.0
3	2	1	32.5	11.9	9.9
3	2	1	36.5	12.0	10.0
3	2	1	29.0	11.5	9.5
3	2	2	26.8	10.5	8.5
3	2	2	26.5	10.5	8.5
3	2	2	27.0	10.8	8.6
3	2	2	25.0	10.4	8.5
3	2	2	30.0	10.9	8.8
3	2	2	33.9	11.0	9.0
3	2	2	26.0	10.2	8.3
3	2	2	32.0	11.0	9.0
3	2	2	30.0	11.5	9.0
3	2	2	25.8	10.0	8.0
3	2	2	31.5	11.0	9.0
1	3	1	35.0	12.0	10.0
1	3	1	38.5	12.4	10.2
1	3	1	37.4	11.9	9.8
1	3	1	35.0	11.5	9.5
1	3	1	37.5	12.0	10.0
1	3	1	39.0	12.6	10.0
1	3	1	41.8	12.7	10.3
1	3	1	36.0	11.5	9.5
1	3	1	28.4	11.5	9.0
1	3	1	40.0	12.5	10.4

1	3	1	40.0	12.0	9.7
1	3	2	23.0	9.7	8.0
1	3	2	25.5	10.5	8.2
1	3	2	21.6	9.7	7.7
1	3	2	23.6	10.5	8.2
1	3	2	20.0	9.0	7.5
1	3	2	24.0	10.0	8.0
1	3	2	21.5	9.0	7.5
1	3	2	23.2	10.2	8.4
1	3	2	15.0	8.4	6.6
1	3	2	21.8	9.6	8.8
1	3	2	20.6	9.4	7.8
2	3	1	43.0	12.6	10.8
2	3	1	31.1	11.5	9.7
2	3	1	30.0	10.9	8.7
2	3	1	33.5	11.4	9.3
2	3	1	28.0	10.5	8.3
2	3	1	27.7	10.6	9.0
2	3	1	30.5	10.6	8.7
2	3	1	30.9	11.0	9.4
2	3	1	38.4	12.0	10.0
2	3	1	40.0	12.5	10.5
2	3	1	38.5	11.5	9.7
2	3	1	27.5	10.6	8.5
2	3	2	40.6	12.0	10.0
2	3	2	40.0	12.2	10.1
2	3	2	42.2	12.2	10.0
2	3	2	45.1	12.6	10.2
2	3	2	30.9	10.8	8.8
2	3	2	44.6	12.7	10.5
2	3	2	33.0	11.5	9.5
2	3	2	41.8	12.9	10.6
2	3	2	25.6	10.5	8.8
2	3	2	31.1	11.0	9.0
2	3	2	0.0	0.0	0.0
2	3	2	0.0	0.0	0.0
3	3	1	39.2	12.0	10.0
3	3	1	39.0	12.0	10.0
3	3	1	36.9	12.0	10.0
3	3	1	34.0	12.0	9.9

3	3	1	38.8	12.0	10.0
3	3	1	44.5	12.5	10.5
3	3	1	37.0	12.3	10.0
3	3	1	41.0	12.0	10.0
3	3	1	33.0	11.9	9.5
3	3	1	40.0	12.3	10.0
3	3	1	40.0	12.5	10.3
3	3	1	0.0	0.0	0.0
3	3	2	28.5	11.0	9.0
3	3	2	29.5	11.0	9.0
3	3	2	34.0	11.4	9.4
3	3	2	26.8	10.5	8.5
3	3	2	35.2	11.6	9.5
3	3	2	28.5	10.7	8.5
3	3	2	27.2	10.5	8.5
3	3	2	28.0	10.5	8.5
3	3	2	29.0	11.5	9.5
3	3	2	31.8	10.7	8.7
3	3	2	34.1	11.2	9.2
3	3	2	0.0	0.0	0.0
1	4	1	37.5	12.0	10.0
1	4	1	42.0	12.5	10.5
1	4	1	41.2	12.8	10.5
1	4	1	43.8	13.3	10.7
1	4	1	37.0	12.2	10.2
1	4	1	39.0	12.1	10.0
1	4	1	42.6	13.0	10.5
1	4	1	36.4	12.0	9.8
1	4	1	28.2	11.5	9.2
1	4	1	44.0	12.9	10.2
1	4	1	41.3	13.0	10.4
1	4	1	0.0	0.0	0.0
1	4	2	22.0	10.3	8.2
1	4	2	32.0	11.0	9.0
1	4	2	24.3	10.2	8.5
1	4	2	16.3	9.0	7.4
1	4	2	25.5	11.0	9.0
1	4	2	25.4	10.5	8.5
1	4	2	23.4	9.8	8.3
1	4	2	21.1	9.8	7.9

1	4	2	27.0	11.0	9.0
1	4	2	22.2	10.0	8.0
1	4	2	25.5	10.7	8.8
1	4	2	0.0	0.0	0.0
2	4	1	46.6	14.0	11.4
2	4	1	31.8	11.5	9.5
2	4	1	40.7	12.5	10.4
2	4	1	34.0	12.0	9.5
2	4	1	33.0	12.5	10.4
2	4	1	40.0	12.6	10.5
2	4	1	312.0	12.0	9.5
2	4	1	45.0	13.0	11.0
2	4	1	27.2	10.9	8.5
2	4	1	41.3	12.7	10.5
2	4	1	29.5	11.2	9.0
2	4	1	0.0	0.0	0.0
2	4	2	46.6	13.2	10.7
2	4	2	46.5	13.6	11.0
2	4	2	44.4	12.9	10.5
2	4	2	46.4	13.6	11.0
2	4	2	40.0	12.4	10.0
2	4	2	32.0	11.5	9.5
2	4	2	40.5	12.0	10.0
2	4	2	37.4	12.4	10.2
2	4	2	26.6	11.4	9.4
2	4	2	33.3	11.2	9.0
2	4	2	0.0	0.0	0.0
2	4	2	0.0	0.0	0.0
3	4	1	33.6	12.2	9.8
3	4	1	46.7	13.0	10.5
3	4	1	35.5	11.9	10.0
3	4	1	40.2	12.7	10.4
3	4	1	42.4	13.0	11.0
3	4	1	42.1	12.5	10.5
3	4	1	44.5	13.0	10.8
3	4	1	30.7	11.0	9.0
3	4	1	42.3	12.5	10.0
3	4	1	33.5	11.7	9.7
3	4	1	37.5	12.5	10.4
3	4	1	0.0	0.0	0.0

3	4	2	35.8	11.6	9.5
3	4	2	26.7	11.0	8.7
3	4	2	30.8	11.4	9.4
3	4	2	28.4	11.2	9.2
3	4	2	26.0	11.0	9.0
3	4	2	29.5	12.0	9.5
3	4	2	28.7	11.0	9.0
3	4	2	29.0	11.5	9.3
3	4	2	32.6	12.0	9.5
3	4	2	0.0	0.0	0.0
3	4	2	0.0	0.0	0.0
3	4	2	0.0	0.0	0.0
1	5	1	28.3	11.5	9.2
1	5	1	45.5	13.0	10.0
1	5	1	44.0	13.0	10.5
1	5	1	41.2	13.0	10.5
1	5	1	48.0	13.5	10.7
1	5	1	46.4	14.0	11.5
1	5	1	47.5	13.3	10.7
1	5	1	42.2	12.5	10.2
1	5	1	53.5	13.9	10.4
1	5	1	38.0	12.5	10.0
1	5	1	42.3	13.0	10.5
1	5	1	0.0	0.0	0.0
1	5	2	30.5	11.5	9.4
1	5	2	32.0	11.3	9.2
1	5	2	25.0	10.8	8.5
1	5	2	31.8	11.0	9.0
1	5	2	33.5	12.0	10.0
1	5	2	29.0	10.8	9.0
1	5	2	31.0	12.0	9.7
1	5	2	24.0	10.5	8.5
1	5	2	39.0	11.5	9.6
1	5	2	22.5	10.5	8.5
1	5	2	18.0	9.3	7.5
1	5	2	0.0	0.0	0.0
2	5	1	50.0	13.5	11.0
2	5	1	51.5	14.5	11.5
2	5	1	45.5	13.0	10.6
2	5	1	41.0	12.5	10.0

2	5	1	49.0	13.5	11.0
2	5	1	45.0	13.4	10.8
2	5	1	34.3	12.0	10.0
2	5	1	29.4	11.5	9.3
2	5	1	35.0	12.5	10.0
2	5	1	40.0	12.7	10.4
2	5	1	34.4	12.0	9.7
2	5	1	35.6	12.0	9.8
2	5	2	52.5	13.9	11.0
2	5	2	51.0	13.2	10.2
2	5	2	50.0	13.5	10.9
2	5	2	40.0	12.5	10.0
2	5	2	55.5	13.0	11.4
2	5	2	31.0	11.5	9.0
2	5	2	50.0	13.7	11.3
2	5	2	47.5	13.2	10.5
2	5	2	38.6	12.0	10.0
2	5	2	57.5	14.3	11.6
2	5	2	0.0	0.0	0.0
2	5	2	0.0	0.0	0.0
3	5	1	53.6	13.5	10.8
3	5	1	35.2	12.1	9.9
3	5	1	46.7	13.0	10.5
3	5	1	36.2	12.6	10.4
3	5	1	45.5	13.2	11.0
3	5	1	48.8	13.4	11.8
3	5	1	47.8	13.6	11.0
3	5	1	41.1	12.4	10.2
3	5	1	51.7	13.6	11.0
3	5	1	46.6	11.2	10.2
3	5	1	42.1	13.0	10.4
3	5	1	0.0	0.0	0.0
3	5	2	34.0	12.5	10.4
3	5	2	35.0	11.5	9.3
3	5	2	39.2	12.1	10.0
3	5	2	30.3	11.5	9.4
3	5	2	30.9	11.8	9.8
3	5	2	34.0	12.0	9.8
3	5	2	33.8	11.5	9.7
3	5	2	35.9	11.6	9.6

3	5	2	34.9	11.5	9.5
3	5	2	0.0	0.0	0.0
3	5	2	0.0	0.0	0.0
3	5	2	0.0	0.0	0.0
1	6	1	59.0	14.0	11.5
1	6	1	54.6	13.5	11.2
1	6	1	52.0	13.0	10.7
1	6	1	67.2	15.0	12.0
1	6	1	58.0	13.5	11.0
1	6	1	52.0	13.6	11.0
1	6	1	45.5	13.0	10.9
1	6	1	50.0	13.0	10.6
1	6	1	56.0	13.5	11.5
1	6	1	55.0	13.8	11.4
1	6	1	34.0	11.9	9.5
1	6	1	0.0	0.0	0.0
1	6	2	59.2	12.4	10.0
1	6	2	46.7	11.5	9.7
1	6	2	31.0	11.0	9.2
1	6	2	26.0	12.5	8.5
1	6	2	40.0	12.5	10.5
1	6	2	44.0	13.0	10.5
1	6	2	46.0	12.0	14.0
1	6	2	38.0	11.5	9.7
1	6	2	30.0	11.3	9.2
1	6	2	22.0	9.7	8.0
1	6	2	41.0	12.2	10.2
1	6	2	0.0	0.0	0.0
2	6	1	64.5	14.5	12.0
2	6	1	49.0	13.5	11.0
2	6	1	37.1	12.5	10.2
2	6	1	40.0	12.7	10.2
2	6	1	62.0	14.2	11.5
2	6	1	48.0	13.3	10.6
2	6	1	44.5	12.2	10.5
2	6	1	40.0	12.3	10.0
2	6	1	47.0	12.6	10.5
2	6	1	33.0	12.0	10.0
2	6	1	53.7	13.5	11.0
2	6	1	58.0	14.0	11.7

2	6	2	62.0	14.5	11.5
2	6	2	61.5	14.3	11.5
2	6	2	60.0	14.0	11.3
2	6	2	53.0	13.7	11.0
2	6	2	57.0	14.5	11.8
2	6	2	38.0	12.5	10.2
2	6	2	64.0	14.2	11.7
2	6	2	48.0	13.0	10.5
2	6	2	53.0	13.5	11.0
2	6	2	32.0	11.0	9.0
2	6	2	0.0	0.0	0.0
2	6	2	0.0	0.0	0.0
3	6	1	57.0	14.0	11.5
3	6	1	55.0	14.2	11.5
3	6	1	48.0	13.5	11.0
3	6	1	46.0	13.0	10.5
3	6	1	47.0	13.5	11.0
3	6	1	46.0	13.0	10.5
3	6	1	55.0	13.0	10.5
3	6	1	58.5	13.5	11.0
3	6	1	35.5	12.5	10.0
3	6	1	47.0	13.0	10.7
3	6	1	42.0	12.5	10.2
3	6	1	0.0	0.0	0.0
3	6	2	43.0	12.8	10.5
3	6	2	32.5	11.5	9.4
3	6	2	34.0	11.7	9.5
3	6	2	43.0	13.0	10.5
3	6	2	44.0	12.5	10.3
3	6	2	46.0	12.0	10.5
3	6	2	45.0	12.7	10.4
3	6	2	44.0	12.2	10.0
3	6	2	36.0	12.4	10.0
3	6	2	0.0	0.0	0.0
3	6	2	0.0	0.0	0.0
3	6	2	0.0	0.0	0.0