INSTITUTE OF ANIMAL PHYSIOLOGY AND ANIMAL NUTRITION

Study to determine maximum growth capacity and amino acid requirements of Tilapia genotypes

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LIST OF ABBREVIATIONS

BW	body weight
^{o}C	centigrade
CMC	carboxyl methyl cellulose
CP	crude protein
DM	dry matter
d	day
E	energy
E.A.A	essential amino acid
FCR	feed conversion ratio
Fig.	figure
g	gram
GE	gross energy
HCL	hydrochloric acid
I.U.	international unit
kg	kilogram
kJ	kilo joule
l	liter
m	meter
m^3	cubic meter
ME	metabolizable energy
mg	milligram
MJ	mega joule
ml	millimeter
P:E	protein energy ratio
NFE	nitrogen free extract
NPU	net protein utilization
O.	Oreochromis
PER	protein efficiency ratio
PPV	productive protein value

SGR	specific growth rate
Thr	threonine
W	weight

1. INTRODUCTION

The present shortage of animal proteins in Egypt is attributed to the fact that the population is continuously increasing while the production of animal proteins cannot cover the necessary requirement. Therefore, fish farming is becoming more important. In the developing countries like Egypt, where the problem is drastic, it is believed that Tilapia culture can offer one of the solutions, specially in view of ever depletion of existing fisheries.

It has been found that increasing fish production in Egypt is feasible through the development of fish farming. Due to the suitable climate, availability of cultured fish seed and water availability, the potential of fish farming is very high. That is why the industrial aquaculture is expanding very rapidly in Egypt . About 44,500 hectare of fresh and brackish water are currently used for aquaculture with semi-intensive culture being the most common. Aquaculture production has jumped from 15,000 tons in 1984 to 76,000 tons in 1996, representing 17,6% of total fish production in Egypt (El-SAYED,1998).

Egypt has vast areas of fishery resources of about 6.15 million hectare are open water's .The total annual production of fish from this area is in the order to 450,000 tons in 1998 (ICLARM,1999).

Today, Tilapia is one of the most popular fish in Egypt. Success in the culture of Tilapia is attributed to its ability to resist poor water quality and disease, to tolerate wide ranges of temperature and salinity, low oxygen levels and to convert many low quality organic, animal, agricultural and domestic waste materials into high quality protein. Tilapia breeds easily in captivity . Tilapia pure species male *Oreochromis niloticus* and Red hybrid Tilapia (*O. mosambicus* x *O. hornorum*), are growing faster than other Tilapia breeds and desirable for the consumer. The success of intensive Tilapia culture depends on a large extent of supplemental feeding. Because protein is the most expensive nutrient in the feed, it is necessary to know the exact protein level and the amino acid requirements for the optimum utilisation of protein and intensive growth. This will of course help in formulating a well-

balanced, mixed diet for economic feeding of Tilapia. In this context, basic research is essential for a more detailed knowledge about growth process in Tilapia of different genotype corresponding to the supply of amino acids and energy. Furthermore, there is practical no comparative information about the growth capacity of Tilapia genotypes based on nutrient deposition studies and the possibility to use a growth model for calculations of maximum protein deposition resp. amino acid requirements in terms of different percentage of growth capacity. The present study was undertaken, to overcome these limitations and to come to conclusions, mainly corresponding to

- Growth capacity of different Tilapia genotypes and age periods
- Optimal protein : energy supply for Tilapia of different genotypes/age periods
- Conditions for optimising growth and feed efficiency of Tilapia.
- Threonine requirement and model calculation.

2. REVIEW OF LITERATURE

Basic information about nutrient requirements and feeding behavior of fish are important for the calculation of experimental diets and further conditions of the experimental work for estimation of maximal growth capacity. The aim of the following review is to give a general idea about this aspects, mainly for Tilapia genotypes.

2.1. Feeding behavior

The feed and feeding habits of Tilapia genera *Sarotherodon* and *Oreochromis* have been studied by several authors (LOWE, 1958; MORIARTY, 1973; BOWEN, 1982).They reported that the characteristic diet of adult Tilapia is of plant matter or detritus of plant origin. Blue-green and green algae, macrophytes, periphyton, amorphous and detritus are found to be a primary natural feed for Tilapia. They also found that juveniles of Tilapia can be fed on phytoplankton, zooplankton and on other small invertebrates. MESKE (1985) found that majority of Tilapia are herbivorous. Some species, such as *T. zillii* prefer higher plants. Other species such as *T. sparmanni* and *T. esculenta* are omnivores. Some authors confirmed that Tilapia are omnivorous.

According to their conclusions, it is evident that there are different views on the main feed of Tilapia in the fry, young and adult stages. These differences may be due to the fact that this species could change its feeding habits (filter - feeding or surface - grazing) among different life stages and environments (JAUNCEY, 1998).

2.2. Dietary requirements

Generally, fishes need the same nutrients and energy which are essential for terrestrial animals for maintenance and growth . Fishes take their nutrients from the natural aquatic organism or from the artificial feeds. When the natural feed is not sufficient, artificial diets have to be added, containing the necessary nutrients, energy and other additional components

such as minerals and vitamins. Deficiency of those substances may cause a reduction in the growth rate of fish and may lead to susceptibility against diseases.
As stated by LOVELL (1998), some nutritional physiological differences between fishes and farm animals can be found:

a. Energy requirements are lower in fish than in warm-blooded animals, thus giving fish a higher protein to energy ratio.
b. Fish require the same lipids as warm-blooded animals do, such as omega-3 (n-3) series fatty acids for some species
c. The ability of fish to absorb soluble minerals from the water minimizes the dietary need for some minerals.

Most species of fish have comparable nutritional requirements. Some differences were found in the essential fatty acid and the ability to assimilate carbohydrates (HALVER, 1989).

2.2.1. Dietary protein requirements

Protein is the basic building nutrient of any growing animal and muscles which are anatomically by far the major components of the fish body. According to JAUNCEY (1998) the body protein usually counts for 65 – 85 % of the dry matter content of fish. Generally, the ability of fish and terrestrial animals to synthesize protein at a rate which is required to promote growth from carbon skeleton are limited. Therefore, fish diets must contain the highest amount of amino acids required. According to JAUNCEY and ROSS (1982) the amount of dietary protein required to produce maximum growth of fish is influenced by the following factors:

a. The energy concentration of the diet.
b. The amino acid composition of the dietary protein and amino acid availability.
c. The physiological state of the animal (age, weight and maturity) and feeding habits.
d. The level of feeding.
e. The environmental conditions.

These factors must be considered when determining the optimum protein levels for various species to obtain reliable values which could be applied in Tilapia fish nutrition.

A lot of experiments have been carried out to determine the optimal protein level for many species of fish. It was found to be 20 to 60% for fish (HASTING,1979). Most species of warm-water fish have protein needs similar to channel catfish, thus protein levels of 30 to 60% will probably be adequate for most fish diets (LOVELL, 1980). Maximum growth and optimum utilization were achieved when carp were fed on diets containing 35 – 45% crude protein (OGINO and SAITO, 1970; OGINO et al., 1976). CRUZE and LAUDENICA (1978) reported that the best growth rate of Nile Tilapia was on a diet with 36% crude protein containing fish meal as source of protein. DAVIS and STICKNY (1978) found that the optimum protein requirement for Tilapia aurea was 36%. Male Tilapia hybrids fed on diets with protein level of 20 ,25 , 30 and 35% showed no significant difference in growth rate between diets. JAUNCEY (1982) found that the maximum level of dietary protein producing a maximum growth for Tilapia mossambicus was 40% with dietary protein : energy ratio of 116.6 mg protein per kcal metabolizable energy. WANG et al. (1985) fed groups of *Tilapia niloticus* on diets with protein levels ranging from 13 to 40% and found maximum growth in fish fed on 30% crude protein. SIDDIQUI et al. (1988) fed Tilapia on diets containing 20, 30, 40 and 50% crude protein and they found that the best growth rate was obtained when dietary protein was 40% and 30% for fry and young Tilapia, respectively. EL - SAYED and TESHIMA (1992) fed fry *Oreochromis niloticus* on diets containing white fish meal as sole source of protein and they found that 45% protein content resulted in maximum growth. Also ABDELGHANY (2000) fed Tilapia *Oreochromis niloticus* of 35 g initial weight on diets containing casein and gelatin as protein sources . The protein content of the diets varied from 15 to 50% in 5% increments and he indicated that the maximum weight gain, protein deposition and energy deposition were obtained at a protein level of 40%.The large variations in the optimum protein level or the protein requirements among tilapia species may be due to differences in the measurement methodology, fish age, fish size, feed allowance, quality of the protein, energy content and environmental conditions (HALVER, 1989). The most criteria used for protein evaluation was generally growth rate and the protein level which gives

optimum growth. The optimum growth was generally taken whether from a peak growth or from the broken line or break point method and both were based on weight gain. This could also mean that protein requirement is the minimum amount to meet requirements for amino acids and to achieve maximum growth .The results of these experiments are summarized in table 1.

Several investigators have studied endogenous excretion losses and nitrogen retention in a variety of fish species, very few investigators have determined the maintenance requirement for protein using either purified or semipurified diets. OGINO and CHEN (1973) obtained a maintenance requirement of 0.95 g protein / kg body weight / day for carp fed on casein as the sole protein source. 1.6 g protein / kg body weight / day was found for rainbow trout fed on fishmeal as sole source of protein (KAUSHIK *et al.*,1981) and 1.3 g protein / kg body weight / day for channel catfish fed on casein – gelatin mixture (GALTIN *et al* .,1986). 2 g / kg body weight / day were observed for Tilapia fed on fish meal - soybean meal (KAUSHIK *et al.*,1994). The maintenance requirement in summary was found to be about 1 g protein /kg BW / day based on data from the above studies (HALVER ,1988).

Table 1: Optimum protein level for maximum growth of Tilapia species

Species	Optimum level (%)	Size (g)	Protein source	FCR	Reference
O. mossambicus	40	0.5-1.0	Fish meal	1.5	JAUNCEY (1982)
O. mossambicus	30-35	6.0-30	Fish meal	1.6-1.8	ROSS (1982)*
O. niloticus	35	0,013-26	Fish meal	1.8	SANTIAGO *et al*. (1982)
O. niloticus	45	0,012	Fish meal (white)	1.1	El-SAYED & TESHIMA (1992)
O. niloticus	30	3.5-10.0	Casein	0.85	WANG *et al*. (1985)
O. niloticus	25	9-17	Casein	0.80	WANG *et al*. (1985)
O. niloticus	30-40	0.8-40.0	Fish meal	1.72-1.89	SIDDIQUI *et al*. (1988)
O. niloticus	28-30	Fry	Fish meal	n.d.	DE-SILVA & PERERA (1985)
O. aureus	36	0.3-0.5	Soybean meal or fish meal	n.d.	DAVIS & STICKNEY (1978)
O. aureus	56-34	Fry-7.5	Casein-albumin	2.5-2.8	WINFREE &STICKNEY (1981)
T.zillii	35	1.3-1.5	Casein	n.d.	MAZID et al. (1978)
Hybrid (*O. niloticus x O.aureus*)	24	3-8	Fish meal (white)	1.42	SHIAU & HAUNG (1989)
Hybrid (*O. niloticus x O.aureus*)	30-35	106-156	Fish meal + Soybean meal	n.d.	VIOLA & ZOHAR (1984)
Hybrid (*O. niloticus x O.mossambicus*)	25	7.5-9.1	Fish meal	n.d.	OBERST et al. (1983)
O. niloticus	35-40	35	Casein + Gelatin	1.11	ABDELGHANY (2000)

n. d.: not determined

*quoted from JAUNCEY and ROSS (1982).

2. 2. 2. Amino acid requirements

Proteins are complex structures composed of amino acids as basic units. These amino acids can be divided into two groups, essential and nonessential. The essential amino acids can not be synthesized and have to be supplied by feeding. The nonessential amino acids can be synthesized by the animal in a quantity to support maximum growth. The protein quality depends in a large extent on concentration of essential amino acids in the protein sources and their amino acid availability. A high quality protein must contain an adequate amount of each amino acid to meet the requirements of the fish.

Most monogastric animals, including fish, require the same indispensable amino acids, namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. The essentiality of amino acids can be determined in fish by feeding a purified diet containing isolated crystalline amino acids as a control diet and feeding of test diets similar to control, except that one amino acid under study. Test diets that produce no growth or markedly less than the control, represent amino acids that are essential for fish.

Earlier studies were carried out to determine the amino acid requirements for fish species. (HALVER, 1957) formulated the first successful purified diet and established the essential amino acid requirements of Chinook salmon. Other studies have been attempted to adapt such a diet to other species with varying success. NOSE *et al*. (1974) demonstrated that fish diets deficient in each of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine and valine failed support growth until the deleted amino acid was supplemented. It would thus seen reasonable to assume that carp require the same ten amino acids reported to be essential for the other species. However, MAZID *et al*. (1978) reported that *Tilapia zillii* require the same ten essential amino acids such as the other fish species do. Table 2 shows the species of fish requiring the same ten essential amino acids.

Table 2: Fish species requiring the ten essential amino acids

Species	Reference
Chinook salmon *Oncorhynchus tschawytscha*	HALVER (1957)
Channel catfish *Ictalurus punctatus*	DUPREE & HALVER (1970)
Common carp *Cyprinus carpio*	NOSE *et al.* (1974)
Red belly tilapia *Tilapia zilli*	MAZID *et al.* (1978)
European eel *Anguilla anguilla*	ARAI *et al.* (1972)

The quantitative amino acid requirements are determined for several species of fish by feeding graded levels of one amino acid limiting in a test diet containing crystalline amino acids or a combination of purified protein and crystalline amino acids. The test diet is formulated in the way that the amino acid profile is identical to chicken whole egg protein or fish muscle, except for the amino acid being tested. This experimental design has been successfully used to determine the amino acid requirements for several fish species, such as salmon (KLEIN and HALVER 1970), channel catfish (WILSON *et al.,* 1978) and Japanese eel (NOSE, 1979).

OGINO (1980) reported a new simple method to measure the deposition rates of essential amino acid in the carcasses of growing carp and rainbow trout fish under near optimal conditions and then related these data back to feed intake and dietary protein level. The final method based on the assumption that there is a direct correlation between the relative proportions of EAA in tissue and the dietary requirements (TACON and COWEY 1985). This analysis of the tissue EAA pattern (each EAA expressed as a proportion of total EAA) and determination of the requirements for a single EAA (to fix the level of the proportion of the dietary protein) will permit extrapolation of the rest of the EAA. This work is supported by terrestrial animal experiments and re - evaluation of EAA data of fish. More studies were conducted by KYU-KIM et al, (1991) to determine the requirement of sulfur containing amino acids and utilization of DL - methionine by rainbow trout. They found that the methionine requirement of fingerling rainbow trout is 0.52 (1.49)% of diet (% of dietary protein), when a diet contains excess cystine .The cystine replacement value (on an equal molar sulfur basis) of L - cystine for L - methionine is approximately 42%. The total

requirement of trout for sulfur containing amino acids is about 0.8 (2.3)% of diet (% of dietary protein). Also KYU-KIM et al., (1992) reported that the lysine and arginine requirements of young trout are 1.30 (3.71)% and 1.41 (4.03)% of diet (% of dietary protein). They compared these works with the work from OGINO (1980) and found a large difference in the results may be due to the feed intake or feeding system. KYU-KIM (1993) showed that the total aromatic amino acid requirement is 1.5% of dry diet or 4.3 % of dietary protein. CHITHRA, N. and DELBERT, M. (1993) indicated that the total sulfur amino acid requirement for juvenile hybrid striped bass was 2.9% of dietary protein when fed on semipurified diets containing 35% crude protein. Fish muscle and crystalline amino acid were supplemented with graded levels of methionine. TIBALDI et al. (1994) reported that the arginine requirement of Eropean sea bass *Dicentrarchus labrax* was 1.81 ± 0.005% of the diet. Also TIBALDI et al. (1999) reported that the threonine requirement of Eropean sea bass *Dicentrarchus labrax* was 1.12-1.26% of the diet. TONI RUCHIMAT et al. (1997) reported that the quantitative lysine requirement of juvenile yellow tail (*Seriola quinqueradiata*) by using broken line analysis was determined to be 1.78% of the dry diet or 4.13% of dietary protein. They also reported that, if lysine availability of protein sources used are considered, the calculated requirement was 1.66% of the dry diet or 3.85% of the dietary protein.

ABDELGHANY (2000) suggested that the amino acid cystine could supply 42% of Nile Tilapia requirement for sulfur containing amino acid on molar sulfur basis to attain maximum performance in terms of growth rate and efficiency of utilized dietary protein (PER). The results also showed that the other growth parameters, feed conversion and productive protein value were as high when 65% of the total sulfur containing amino acids came from cystine, as when almost (about 97%) came from methionine. Table 3 shows the amino acid requirements of some fish species.

Table 3: The essential amino acid requirements of some fish species (% of CP)

EAA	Chinook salmon [a)]	Japanese eel [b)]	Common Carp [c)]	Channel catfish [d)]
Arginine	6.0	4.5	3.8	4.3
Histidine	1.8	2.1	1.4	1.5
Isoleucine	2.2	4.0	2.3	2.6
Leucine	3.9	5.3	4.1	3.5
Lysine	5.0	5.3	5.3	5.1
Methionine + Cystine	4.0	3.2	1.6	2.3
Phenyalanine + Tyrosine	5.1	5.8	2.9	5.0
Threonine	2.2	4.0	3.3	2.0
Tryptophan	0.5	1.1	0.6	0.5
Valine	3.2	4.0	2.9	3.0

a) NRC (1993)
b) NOSE (1979)
c) OGINO (1980)
d) WILSON (1991)

More investigations were conducted to determine the quantitative amino acid requirements for Tilapia by JACKSON and CAPPER (1982). They fed *S. mossambicus* on diets with 40% crude protein (50% of the protein from fish meal, soybean meal and groundnut meal; 50% a mixture of crystalline amino acids). They concluded that methionine, lysine and arginine requirements were respectively 1.33, 4.1 and 4 percent of the crude protein. Further requirements are presented in table 4. Also JAUNCEY et al.(1983) determined amino acid requirements for *O. mossambicus*. SANTIAGO (1985) defined the quantitative EAA requirements for Tilapia *O. niloticus* using a basal casein / gelatin diet containing 28% crude protein, supplemented with crystalline amino acids. Muscle and egg EAA profiles were compared with these requirement data and it was suggested the amino acid requirements as a percentage of dietary protein. ODUM and EJIKE (1991) reported that the requirements of arginine and lysine were 1.48% and 1.76% respectively for *O .niloticus* as a percentage of the diet. Also GABER (1994) fed Tilapia mossambicus with an average body weight of 0.9 g on two different feed mixtures made with two different crude protein levels (diet A = 40% CP

diet B = 23% CP). The results indicated that *Tilapia mossambicus* had an average body weight of 17.1 and 8.6 g for diet A and B, respectively. The increase of crude protein in the feed up to 40% caused a significant increase of the content of amino acids in the body (as % of carcass fresh weight). The amino acid requirements are given as percent of a 40 % protein diet. Some data concerning these requirements are summarized in table 4.

Table 4: Essential amino acid requirements for Tilapia genotypes (% of CP)

EAA	*S. mossambicus* [a)]	*O. mossambicus* [b)]	*O.niloticus* [c)]	*O. mossambicus* [d)]
Arginine	4.0	2.8	4.2	2.2
Histidine		1.1	1.7	2.2
Isoleucine		2.0	3.1	2.4
Leucine		3.0	3.4	2.8
Lysine	4.10	3.8	5.1	2.9
Methionine + Cystine	3.2	1.0	2.7	1.5
Phenyalanine + Tyrosine		2.5	3.8	1.7
Threonine		2.9	3.8	1.8
Tryptophan		0.4	1.0	1.3
Valine		2.2	2.8	1.9

a) NRC (1993)
b) JAUNCEY et al. (1983)
c) SANTIAGO (1985)
d) GABER (1994)

According to the observations which are presented in table 4, it can be concluded that there is a large variation in amino acid requirements due to different studies. These differences can be explained by the different methods used for determining amino acid requirements or by the other experimental conditions such as age of fish, size and weight. More investigations are required to reach more detailed information about amino acid requirements that can be applied in the nutrition of Tilapia.

2. 2. 2. 1. Essential amino acid supplementation

The initial aim of fish culture is to transform dietary protein into tissue protein efficiently. Alternative protein in diets for Tilapia should replace natural proteins not only in quantity but also in quality in order to provide optimum growth and feed conversion. The protein sources commonly used in fish diets can be divided into two parts. The first part are plant protein sources and the second part are animal protein sources. Some protein sources are deficient in some essential amino acids, while others have excess to these amino acids. For instance, casein has nearly adequate amounts of all amino acids, except arginine, while gelatin has excess of arginine and is deficient in the other amino acids. Combination of protein sources and/or supplementation of crystalline amino acids to the diet are useful methods for correcting the deficiency of single amino acids. Essential amino acid supplementation of fish feeds could be used to raise levels to those optimal for the target species.

Many authors have used these methods to improve the quality of protein sources in fish nutrition. TANAKA et al. (1977) added free amino acids such as L-tryptophan, L-alaninie, L-methionine and L-proline to increase the performance of casein diets for carp. RUMSEY and KETOLA (1975), VIOLA et al. (1982, 1983), MURIA (1986) and SHIAU et al. (1987) observed that the growth rate of some fish species was improved when soybean diets were supplemented with amino acids such as methionine and lysine. TESHIMA et al. (1986) found that *Tilapia niloticus* fed on gelatin with casein at ratio 1 : 3 improved their growth rate. This may be due to the high levels of arginine in gelatin which can correct the deficiency of arginine in casein. VIOLA et al.(1992) found with the common carp that 0.5% dietary lysine supplement was nutritionally equivalent and economically superior to 5% crude protein in the diet. ADEPARUSI and OLUTE (2000) studied the effect of replacement of 20, 40, 60 and 80 % of menhaden fish meal in a control diet containing 30% crude protein with toasted lima beans supplemented with 1.4% methionine on growth of *Oreochromis niloticus*. This study shows that the lima bean, when toasted and supplemented with methionine, can qualitatively replace 40 - 80% fish meal protein in the diet of *Oreochromis niloticus*. On the other hand SOLIMANA (2000) showed that there was no significant effect on growth

parameter, feed conversion ratio and protein utilization when *Oreochromis niloticus* was fed on diets with 36% crude protein containing fish meal replacement with 10 and 20% black seed meal and supplemented with lysine and methionine. EL- SAYED (1990) obtained the same results when Nile Tilapia were fed with either decorticated cottonseed meal or corticated cottonseed meal, supplemented or not supplemented with 0.5% lysine showed no significant differences in body weight gain.

Evidence of deficiency in dietary amino acids in fish generally reduced growth rate, appetite and showed poor feed conversion. A few amino acid deficiencies lead to anatomical abnormalities. For instance, deficiency of methionine causes lake trout to develop bilateral lens cataracts and suffer poor growth and survival rates (POSTON et al., 1977; PAGE, 1978). Tryptophan deficiency causes scoliosis (dorsa- ventral curvature of the spine) and lordosis in Sockeye salmon (HALVER and SHANKS, 1960) and rainbow trout (SHANKS et al., 1962; KLOPPEL and POST, 1975), but not in the channel catfish (WILSON et al., 1978). Further effects of tryptophan deficiency in rainbow trout including abnormal calcium deposits in the kidney and bony plates are reported by KLOPPLE and POST (1975). Lysine deficiency causes caudal fin rot, i. e. loss of much of the fin in rainbow trout (KETOLA, 1983). This is the pathology symptoms caused as result of lysine deficiency for rainbow trout may not be due to the specific amino acid deficiency, but rather to either a nutritional stress or a sensitivity of the specific genetic line of rainbow trout used in that work since this particular pathology has not been reported by any other investigator.

2. 2. 2. 2. Amino acid availability

Amino acid availability for various proteins provide information about the availability of each of the essential amino acids for the specific protein sources evaluated. It is necessary not only to know amino acid profiles of proteins fed, but also to determine the availability of these amino acids. Certain amino acids may not be available to the fish because of incomplete protein digestion or because of some nonprotein compounds bound to the amino acid. In particular, two of essential amino acids, lysine and methionine, readily undergo changes during processing of foodstuff that may render them unavailable to the fish.

Lysine is a basic amino acid and in addition to the α- amino group normally found in a peptide linkage, lysine contains a second amino group which is free and reactive. Although chemically measurable, it can not be biologically available in fish diets (COWEY and SARGENT 1972, COWEY 1978). For example, when some proteins are over heated in the presence of reducing sugar, the reducing sugar may react with the ε - amino group of lysine, by Maillard reaction, making lysine biologically unavailable. Lysine also interacts with gossypol, reducing the availability of cottonseed meal.

Methionine is difficult to be measured chemically in foodstuffs. It is relatively easily oxidized, especially during processing, to form sulphoxide or sulphone. Five protease inhibitors have been reported in soybean. Protease inhibitors basically act by binding with chymotrypsin or trypsin, rendering them inactive. They are also known to display an effect on metabolism of certain amino acids such as cystine. Soybean is often rich in lysine and poor in methionine. Almost all protein inhibitors are heat labile and will be broken down when cooked to produce soy oil and soybean meal. Therefore, the biological values of soybean will be better when supplemented with amino acid methionine to meet the amino acids requirements for fish. The apparent and true availability of amino acids from several protein sources for channel catfish are reported by WILSON et al. (1981).

2. 2. 3. Energy requirement

Generally, energy is not a nutrient, it is released during the different metabolic oxidation processes of fat, carbohydrates and proteins (amino acids). Fish obtains the energy required from feed or, in periods when deprived of feed, from the body stores. According to NRC (1993) fish has lower energy requirements than warm-blooded animals and is considered to have more energy efficiency than mammals and birds (table 5) for the following reasons:

a. Fish excretes about 85% from the nitrogenous waste as ammonia while most of the N-components are excreted as urea in mammals and as uric acid in birds. Therefore, fish require lower energy for nitrogen excretion than other animals (GOLDSTEIN and FORSTER, 1970).
b. Heat increment could be 30% or more in mammals, in fish it was found to be 3 – 5% of ME for rainbow trout (SMITH et al., 1978) and about 22% of ME for *Tilapia niloticus* (MEYER - BURGDORFF et al., 1989).
c. Fish requires less energy to maintain position in the water and does not need to regulate body temperature. The maintenance energy requirements are therefore lower for fish than for other animals.

Table 5: Comparison of efficiency of utilization of energy by fish, chicken and cattle

Animal	Protein %	ME: protein ratio (kJ /g)	Efficiency		
			Weight gain/(g) of feed consumed (g)	Protein gain/(g) of protein consumed (g)	Protein gain /MJ ME consumed(g)
Channel catfish	32	35.6	0.84	0.36	11.2
Broiler chicken	18	67.0	0.48	0.33	5.5
Beef cattle	11	100.5	0.13	0.15	1.4

Source: LOVELL (1979)

2. 2. 3. 1. Lipids as source of energy

The use of lipid as source of energy for animal diets has been recognized for many years. Traditionally, lipids, namely fats and oils are thought of being primarily sources of energy due to their high digestibility and they ability to release the highest energy per gram as compared to protein and carbohydrate. However, their digestibility is known to be related to the length of the carbon chains and the degree of unsaturation or melting point. There are two major requirements for dietary lipids: first, as source of metabolic energy and second, to maintain the structure and integrity of cellular membranes in the form of phospholipids (JAUNCEY 1982; STEFFENS, 1985). Dietary lipids also provide a vehicle for absorption of

fat soluble vitamins and provide other compounds such as sterols, that play a vital role in the structure of biological membranes at both cellular and subcellular level (HALVER, 1980, STEFFENS, 1985).

2. 2. 3. 2. Carbohydrate as source of energy

The nutritional value of carbohydrates varies among the fish species. Warm-water fish can use much more amounts of dietary carbohydrate than cold-water and marine fish. The ability of fish to utilize carbohydrates as a source of energy depends on its enzymatic capacity to degrade carbohydrate. The α- amylase activity is the highest in herbivorous fish, followed by omnivorous and carnivorous fish, respectively. Therefore, herbivorous and omnivorous fish can utilize carbohydrate as a source of energy more than carnivorous fish (BRETT and GROVES, 1979; CHOW and HALVER, 1980).

In general, it is very important to provide the exact amount of energy in diets for fish. A dietary excess or deficiency of useful energy can reduce growth rate, because energy is needed for maintenance and voluntary activity. The diet containing excess energy can restrict food consumption and thus prevent the intake of necessary amounts of protein and other nutrients for maximum growth (ANDREWS, 1979). Excessively high energy/nutrient ratios can also lead to a deposition of large amounts of body fat (fatty fish). On the other hand, dietary protein will be used for energy when the diet is deficient in energy in relation to protein (LOVELL, 1998).

2. 2. 3. 3. Sparing effect of protein by lipids and carbohydrates

The nutrition of fish points out that proteins are not only important as amino acid sources, providing the enzymatic and structural components of cells but also as a source of energy. Because protein is the most expensive source of energy, the aim in fish feeding is to maximize the utilization of protein for growth by supplying adequate amounts of alternative dietary energy sources such as lipids and carbohydrates. Lipids are a potential source of energy. This was indicated by several studies with some fish species such as trout.

TACKEUCHI (1978) found that the protein level in trout diets could be reduced from 48 to 35 percent with no loss in weight gain, given dietary lipid levels of 15 - 20 %. Also TAKEUCHI et al. (1979) found that increasing the lipid content from 5 to 15 % in diets containing 22, 32 and 41 % protein (from casein) had little or no effect on growth rate, feed conversion, energy retention or protein utilization of carp. Some experiments were conducted by JAUNCEY (1982) using diets containing 21, 29, 37 and 45 % protein with levels of dietary lipids of 6, 12 and 18 % at each protein level. The protein sparing effect of dietary lipids for mirror carp at 20^{o} C was evident from the increased specific growth rate (SGR), protein efficiency ratio (PER) and apparent net protein utilization (NPU) at each protein level with increasing levels of dietary lipids. It is possible to reduce the protein content of diets containing 18 % lipid from 45 to 29 % with no losses of weight gain and with improved utilization of dietary protein. Similar results were found by DUPREE et al. (1979), weight gain and feed efficiency of channel catfish increased as the level of bleached menhaden oil was increased to 15 % of the diet. However, the growth was decreased when the oil level was increased up to 20 %. WINFREE and STICKNEY (1981) fed S. aureus on diets with a lipid level from 2 to 8.6% and found that the optimum protein and lipid levels were 5.3% and protein content 56% for Tilapia up to 2.5 g body weight and decreased to 4.4 % lipid with protein content 34% for fish up to 7.5 g body weight. The results from WANG et al. (1985a, b) indicate that the maximum growth of *Tilapia niloticus* up to 9 g was obtained with diets containing 5.4 – 8.1 % lipid and 30 % protein. VIOLA and ARIELI (1983) fed Tilapia hybrid (S. aureus x S. niloticus) on diets with 25 % protein and lipid levels of 4 – 8 % from varied sources as poultry oil, soybean oil, acidulated soybean oil, fish oil or acidulated cottonseed oil. They found that varying the sources and levels of oil in the diet did not improve growth rate or feed conversion of fish reared in both cages and pond systems. JAUNCEY and ROSS (1982) reported that Tilapia do not appear to utilize high amounts of lipids as efficient as salmons or carp and suggested that 6 – 10 % lipid is to be included into the diet of tilapia for maximum utilization of protein and also reported that lipids more than 12 % resulted into depressed growth. In contrast to the previous studies, DE- SILVA et al. (1991) fed red Tilapia hybrid on lipid ranges from 6 – 24 % and recommend that 18 % lipids spared dietary protein up to 30 %.

Finally, these studies describe the beneficial effects, both economically and nutritionally of increasing the level of dietary lipids as compared to conventional (low fat) fish feeds.

The use of carbohydrates as a protein sparing energy source has less attention than the use of lipids for some purpose and there is still some dissension about the level of dietary carbohydrates that should be included in commercial fish rations, specially Tilapia. BUHLER and HALVER (1961) showed that increasing the level of dextrin in diets containing 38 % protein from 0 to 48 % increased protein efficiency ratio (PER) from 1.65 to 2.37, thus demonstrating the protein - sparing action of dietary carbohydrates in Chinook salmon. They also added that the dietary level of dextrin has raised the liver size and glycogen content without apparent pathological effects. DUPREE and SNEED (1966) found that with channel catfish, increasing the level of dextrin in the diet from 2.5 to 10 % increased weight gains, but further increase to level of 15 to 20 % depressed growth.

An experiment conducted by CHIOU and OGINO (1975) has shown that in contrast to rainbow trout, carp was capable to digest 85 % of the ingested starch at dietary levels from 19 to 48 %. This would seem to suggest better utilization by carp of higher levels of dietary carbohydrate. Also ANDERSON et al. (1984) studied the utilization of diets containing carbohydrates of different molecular complexity and their effect on growth, feed conversion and carcass quality of Tilapia. They found that *O. niloticus* can utilize both simple and complex carbohydrates. The growth was improved as the level of glucose, sucrose, dextrin and starch was increased from 0 to 40 %. OSMAN (1991) found that Tilapia hybrid fingerlings fed on four diets with protein level of 20 to 35 % to study the effect of partial replacement of protein by carbohydrates. He found that body weight gain, SGR, FCR and NPU were higher in groups fed on 25 % protein with 62 % total carbohydrates than in the other groups. LIM (1989) reported that Tilapia can digest carbohydrates in feedstuffs relatively well, much better than salmons. SHIAU and PENG (1993) fed Tilapia hybrids on three protein levels, three carbohydrate levels and three carbohydrate sources. This study seems merely to confirm those of others showing that carbohydrates do spare protein in

Tilapias and that glucose is inferior to starch and dextrin as a carbohydrate source, and that even the highest carbohydrate level of 41 % tested was well utilized.

Finally, the energy level and P:E ratio that produce the highest growth by Tilapia species are found in table 6. From this table and after converting all energy forms to ME it can be concluded that the optimum P : E ratio for Tilapia fry and small fish up to 2.5 g is ranging from 28 to 35.5 mg protein/kJ ME, while the adult fish have a P:E ratio ranging from 21.5 to 30 mg protein/kJ ME. Table 6 shows the optimum protein : energy ratio producing maximum growth for Tilapia species.

Table 6: Optimum Protein : Energy ratio for maximum growth of different Tilapia species

Species	Size range (g)	Protein level (%)	Energy scale	P:E ratio g protein/MJ	Authors
T.zillii	1.4 – 1.8	35	GE	22.8	MAZID et al. (1979)
S.aureus	2.5 – 7.5	34	DE	25.8	WINFREE & STICKNEY (1981)
S.mossambicus	0.5 – 1	40	ME	27.9	JAUNCEY (1982)
T. niloticus	6 - 9	29	GE	15.4	WANG et al. (1985)
T.niloticus	12	41	ME	26.7 – 29.5	MAGOUZ (1990)
O. niloticus	14	30	GE	17.9	YONG et al. (1989)
O. niloticus	0.12	45	GE	16.7	EL-SAYED & TESHIMA (1992)
T. hybrid	1.24	30- 35	GE	20.6	SHIAU et al. (1987)

2. 2. 4. Mineral Requirements

All inorganic elements found in an animal body are essential in the diet. However, dietary need for minerals has been demonstrated in one or more animal species. Those required in large quantities are termed major and those required in trace quantities are called trace minerals. Fish probably requires the same minerals like warm blooded animals for tissue formation, various metabolic processes and for optimum growth. Ca and P are the most important minerals that fish needs. LOVELL (1980) found that fish uses inorganic elements to maintain osmotic balance between fluids in the fish body and water. According to

ROBINSON et al. (1984) group of fingerlings *Tilapia aurea* were fed on diets containing graded level of calcium ranging from 0.17 to 3.2 % and reported that the range between 0.17 to 0.65 dietary Ca was adequate for optimum growth. Also ROBINSON et al. (1987) reported a requirement in *O. aureus* of 0.5 % for normal bone mineralisation and no pathological signs of phosphorus deficiency were recorded. The next important mineral is magnesium about 70 % of the magnesium in a fish body is in the hard tissue . Other functions of magnesium are as an enzyme activator in carbohydrate metabolism and in protein synthesis, also copper and zinc are very important components of a number of metabolic processes DE SILVA and ANDERSON (1995). VIOLA et al. (1986) observed that a total of 0.7 % P in a diet for Tilapia hybrids was sufficient for normal growth of large fish, small fish with a higher growth rate required a higher level of approximately 1 % P. Zinc is an essential component of more than 80 metalloenzymes. Zinc also an enzyme cofactor playing a role in the metabolism of protein, lipid and carbohydrate. EID and GHONIEM (1994) Suggested a requirement of 30 mg / kg in diet of *O. niloticus.* More investigations about mineral requirements for fish are reported by BEVERIDGE and ANDREW (2000).

2. 2. 5. Vitamin requirements

Vitamins are organic compounds required in the diet in relative small quantities for growth, health and physiological functions in the animal. A vitamin that is essential for some fish species may not to be essential for other species such as Tilapia has been shown that ß-carotene can be biotrasformed from the natural feed in the liver of Tilapia into vitamin A. Some organisms are enable to synthesize some vitamins. The natural feeds are often limited in the vitamin content, so vitamins must be supplied in the diet to achieve normal fish growth (NRC, 1993). One of the most important vitamin for Tilapia feed is vitamin C (ascorbic acid). The reduction of vitamin C causes reduced growth, exopthalmia, poor wound repair and soft opercular bones (AL-AMOUDI et al., 1992; SOLIMAN et al.1986, 1986a). Also vitamin E (tocopherol) is very important in the feed for Tilapia species. It causes reduce of growth, poor feed efficiency, skin and fin hemorrhage and anorexia (SATOH et al., 1987; ROEM et al., 1990a). SOLIMAN and WILSON (1992) reported that the dietary riboflavin requirement for blue Tilapia was determined to be 6 mg riboflavin/kg of the diet. They also reported that the

dietary pantothenic acid requirement for blue Tilapia was determined to be 10 mg calcium d-pantothenate/kg of diet. On the other hand some vitamins are not required for Tilapia such as cyanocobalamin, inositol and choline (LIMSUWAN and LOVELL, 1981; ROEM et al., 1990). The suggested premix for Tilapia feeds (mg or IU per kg of feed) is presented in table 7. More details about vitamin requirements of Tilapia are reported by JAUNCEY (1998).

Table 7: The suggested premix for Tilapia feeds
(mg or IU per kg^{-1} of feed).

Vitamin	Complete Premix [1)]	General Premix [2)]
Thiamine (B1)	2.5mg	0 –1mg
Riboflavin (B2)	6mg	3 –5mg
Pyridoxine (B6)	3mg	0 –1mg
Pantothenic acid	10mg	3 –5mg
Nicotinic Acid (Niacin)	14mg	6-10mg
Biotin	1mg	0-0.5mg
Folic Acid	1mg	0-0.5mg
Cyanocobalamin (B12)	0.01 (NR)*	0
Inositol	300 (NR) *	0
Choline	400 (NR) *	0
Ascorbic Acid	75mg	50mg
Retinol (A)	1000 IU	500 IU
Cholecalciferol (D3)	1000 IU	200 IU
Tocopherol (E)	50mg	10mg
Menadione (K)	1mg	0

*) No requirement determined as shown by studies on Tilapia.

1) A complete premix used for hatchery/ broodstock/experimental feeds where natural feeding is insignificant

2) A more conservative premix for general, fairly intensive, ongrowing.

Data suggested from JAUNCEY (1998)

3. MATERIAL AND METHODS

The present study was carried out in three experiments. All of three experiments were conducted to determine the growth capacity and maximum N-retention with different genotype of Tilapia, family Chialiade , also to find out the physiological response of different Tilapia genotypes to different protein supply in combination with different energy density. For this purpose 10 semi purified diets were formulated for a N-rise experiment combining 5 crude protein levels ranging from 16% - 48% crude protein. The effect of dietary treatments should be evaluated based on growth rate, body composition, nutrient deposition, feed and nutrient utilization. Furthermore, the results of protein deposition were used for estimations of protein deposition capacity.

3.1. Experimental systems

3.1.1. Experimental facility

The experiments were carried out at the Institute of Animal Physiology and Animal Nutrition , Georg-August-University Goettingen, in a recirculating system facilitated with 38 circular fiber glass tanks. Each tank had 0.9 m diameter and 350 l water volume (Figure 1). In this system 90 – 95 % of the water was recycled and freshwater (Goettingen tap water) was added to the system at a daily rate of about 5 – 10 % in order to replace water losses through evaporation and sludge drained. Each culture tank was supplied continuously by mixture of biologically filtered water and freshwater. From the culture tank the excessive water flew out through standpipe and was collected in a reservoir tank (1.4 x1.1 m and 0.75 cubic meter water volume) under the culture tank. From here the water is pumped upwards to the biological trickling filter where nitrification process takes place.

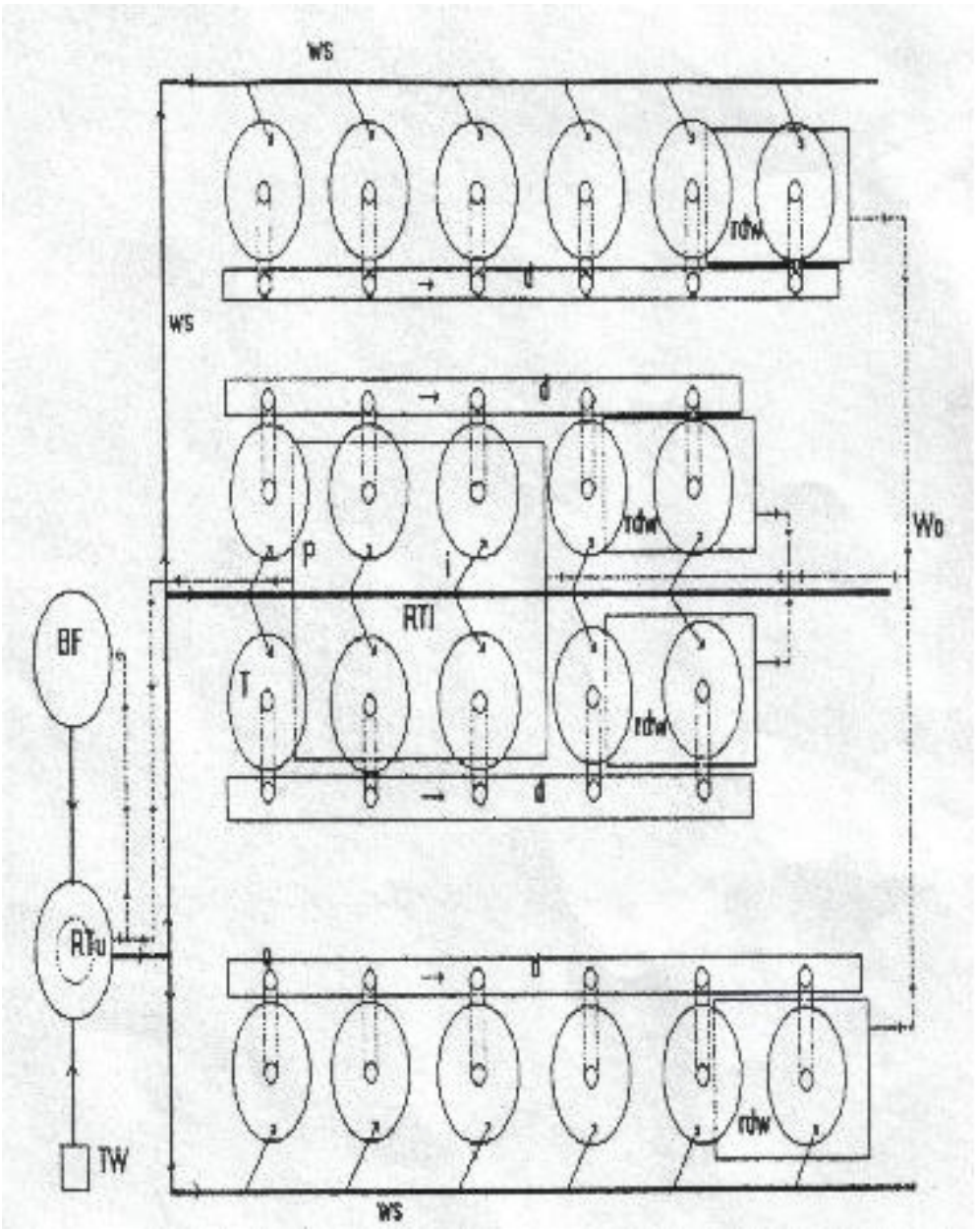

Figure 1: Diagram of the closed water recirculation system unit.

(TW= tap water supply, Rtu = upper reserve tank, BF= biological filter, ws = water supply (mixture of biological filtered and fresh water), d= drainage, Wo = water outflow, rdw = reception of drained water, Rtl = lower reserve tank for collection and pumping the drained water, P = pump).

3.1.2. Experimental fish

Three different genotypes of fingerling Tilapia were used for the present experiment. The first all male pure *Oreochromis niloticus* was produced in the work of Aquaculture and Fresh waters ecology of the Institute of Animal Breeding and Animal Genetics of Goettingen University. The second genotype, Red Tilapia (*O. mossambicus* x *O. hornorum*), used in the second experiment was imported from Marriott fish farming, Alexandria, Egypt. The third genotype, (*Oreochromis niloticus* x *O. mossambicus* x *O. hornorum*) used in the third experiment, was obtained by hybridization under commercial conditions of a fish farm in Germany.

All of the fish used in each experiment were raised in fiber glass tanks by recirculating tank system at a temperature of 28^{o}C and fed with a standard diet (MAGUZ, 1990) ad libitum by automatic feeders up to an average body weight of 2 – 3 g. The standard diet composition is documented in the appendix (table A.1). After this period the fish were fed at a level of 10 % of the body weight for 4 times daily by hand until the expected weight was reached (12 g per fish). During that time the fish were fed also with the standard diet “Goettingen feed”.

3.1.3. Experimental procedure

The fish of each experiment were hand graded and distributed on the recirculating tank system one week prior to starting of the trial. The fish were starved for one day prior to weighing. 25 fish weighing approximately 12 g per fish were stocked to each tank. At the beginning of the experiment 15 fish from each experiment were randomly selected, killed by tranquilizing solution (2-Ethoxy-ethanol) and frozen for initial analysis of body composition. During the experiment fish were fed by hand three times per day. Water temperature of culture tank was daily checked and the culture tank water was sampled biweekly for monitoring water quality parameters. The water quality is documented in the appendix (table A2). After 4 weeks, 8 weeks and at the end of each experiment (10 weeks), 9 fish per treatment were selected and weighed for chemical analysis of body composition.

3.1.4. Feeding system

Initially the fish were fed at a rate of 7% of the body weight. After the first period (two weeks), because of inactive feed consumption, the feeding level was reduced to 6% of the body weight in the second period. During the third period 5% and finally 4% from the body weight of fish were supplied. The fish were fed 3 times daily by hand at 8.00 h, 12.00 h and 16.00 h. The fish groups were weighed weekly and the diets were also adjusted weekly according to the growth of the fish**.**

More Details of experimental design are summarized in table 8.

Table 8: Experimental design of feeding experiments

Experiment	1	2	3
Diets per experiment	10	10	10
Number of replication	3	3	3
Number of fish per tank	25	25	25
Number of fish samples/treatment *)	9	9	9
Initial body weight (g/fish)	12.3 ± 0.1	12.4 ± 0.1	12.3 ± 0.1
Feeding frequency	3 (by hand)	3 (by hand)	3 (by hand)
Experimental period (day)	70	70	70

*) for determination of body composition

3.2. Experimental diets

3.2.1. Diet formulation

The semi purified diets of each experiment were formulated from practical ingredients. The dietary protein sources were derived from fish meal and wheat gluten in constant ratio (3 : 1). The diets were formulated to contain 5 crude protein levels ranging from 16 - 48 % crude protein in 8 % increments. The diets from 1 to 5 were isoenergetic with energy level of 15.6 MJ ME/kg. The diets 6-10 were adapted in energy with levels ranging from 13.6 MJ ME/kg to 17.6 MJ ME/kg. The protein : energy ratio for diets 1-5 was ranging from 10.3 to 30.7 g protein / MJ ME and from 11.7 to 27.3 g protein / MJ ME for diets 6-10. Threonine

was calculated to be the first limiting amino acid except diet 8 with threonine supplementation. Tryptophan was added to each experimental diet to cover the amino acid requirement for Tilapia according to NRC (1993). Wheat starch and oil (soybean oil : fish oil = 1 : 1) were used as additional source of energy to adjust the energy content of the diets. Cellulose powder was supplemented as an inert bulker. The chemical analysis of each ingredient is presented in table 9. The amino acids composition of experimental diets is presented in table 10, while the approximate analysis of experimental diets composition is presented in table 11. The vitamin mixture used was a commercial mixture of the Vilomix 910109 (Deutsche Vilomix Tiernahrung GmbH) which was added to all experimental diets at a constant level of 1% as recommended by MEYER - BURGDORFF (1985) and the minerals were added as mixture based on the work of SCHÄFER (1995).

Table 9: Proximate analysis of feedstuffs used in the experiments

Proximate analysis (%)	Fish meal	Wheat gluten
Dry matter	92.00	90.68
Crude protein	77.10	87.60
Ether extract	10.54	7.06
Crude ash	11.29	1.15
N.F.E *	1.07	4.19

* Nitrogen free extract

3.2.2. Diets manufacture

The experimental diets of the study were prepared by individually weighing of each component and by thoroughly mixing the minerals, vitamins, L - Threonine , L- Tryptophan, CMC with wheat starch. This mixture was added to other components together with oil. This mixture and the other components were intensively mixed in a cutter (E. Müller und Söhne, Type MTK 20 special). Cold water (up to 30 % of the total amount) was added after the ingredient has been perfectly mixed with continuous turning over until the mixture became suitable for making granules. The wet mixture was passed through granule machine with 2 mm diameter. The produced pellets were dried at room temperature for three days (approximately 10 % moisture was achieved). The dried pellets were stored in a cool room at

2^{o}C. The amino acid composition of the experimental diets calculated from amino acid composition of the components, is presented in table 10.

Table 10: The amino acid composition of the experimental diets (g/kg)

Diets / Amino acids	1	2	3	4	5	6	7	8	9	10	E.A.A req. **
Lysine	8.23	12.35	16.48	20.59	24.72	8.23	12.35	16.48	20.59	24.72	14.30
Arginine	8.40	13.60	16.75	20.94	25.13	8.40	13.60	16.75	20.94	25.13	11.80
Histidine	3.09	4.58	6.15	7.65	9.17	3.09	4.58	6.15	7.65	9.17	4.80
Isoleucine	6.15	9.23	12.30	15.37	18.44	6.15	9.23	12.30	15.37	18.44	8.70
Leucine	11.29	16.93	22.57	28.22	33.86	11.29	16.93	22.57	28.22	33.86	9.50
Phenylalanine + Tyrosine	10.73	16.16	21.47	26.83	32.21	10.73	16.16	21.47	26.83	32.21	10.50
Methionine + Cystine	6.39	8.68	11.58	14.47	17.37	6.39	8.68	11.58	14.47	17.37	7.50
Valine	7.20	10.80	14.39	17.98	21.59	7.20	10.80	14.39	17.98	21.59	7.80
Threonine	5.67	8.52	11.35	14.18	17.02	5.67	8.52	13.20*	14.18	17.02	10.50
Tryptophan	1.65	2.47	3.30	4.12	4.94	1.65	2.47	3.30	4.12	4.94	2.80

* L-Thr supplemented

** Essential amino acid requirement for Tilapia according to NRC (1993).

Table 11: Composition and proximate analysis of the experimental diets

Diets	1	2	3	4	5	6	7	8	9	10
Ingredient (g/kg										
Fish meal	158	237	316	395	474	158	237	316	395	474
Wheat gluten	46	69	92	115	138	46	69	92	115	138
Wheat starch	580	495.6	414	328.8	247.6	674.6	546.6	414	280	149
Soy oil + Fish oil	119.6	102	81.6	64.6	44	25	51	81.6	113.6	142.6
Vit-Mix [a]	10	10	10	10	10	10	10	10	10	10
Min-Mix [b]	36.4	36.4	36.4	36.4	36.4	36.4	36.4	36.4	36.4	36.4
L-Tryptophan	0.25	0.40	0.50	0.60	0.70	0.25	0.40	0.50	0.60	0.70
L-Threonine	--	--	--	--	--	--	--	0.75	--	--
Cellulose	29.75	29.60	29.50	29.40	29.30	29.75	29.60	28.75	29.40	29.30
C.M.C [c]	20	20	20	20	20	20	20	20	20	20
Composition (%)										
Dry Matter	88.16	88.56	89.02	88.88	88.76	88.15	88.81	88.62	89.79	90.55
Crude protein	17.20	24.20	32.10	40.30	48.50	17.30	24.50	32.80	40.40	48.40
Ether extract	15.01	14.04	12.85	12.88	10.63	5.47	8.45	12.60	17.05	20.58
Crude ash	6.63	7.97	9.32	10.54	11.94	6.46	7.78	8.95	10.42	11.25
N.F.E [d]	61.16	53.79	45.73	36.28	28.93	70.77	59.27	45.65	32.13	19.37
ME MJ/kg [e]	15.60	15.60	15.60	15.60	15.60	13.60	14.60	15.60	16.60	17.60
CP : E Ratio`*)	10.30	15.40	20.50	25.60	30.70	11.70	16.50	20.50	24.10	27.30
CP : E Ratio *[1]	10.30	14.50	19.30	23.90	29.10	11.60	15.70	19.60	22.80	25.90

a)Vitamin mixture (per kg feed): 10000 I.U. Vit. A, 1000 I.U. Vit. D_3, 50 mg Vit. E, 100 mg Vit. B_1, 100 mg Vit. B_2, 100 mgVit. B_6, 100 mg Vit. B_{12}, 20 mg Vit. K_3, 200 mg Vit. C, 500 mg Nicotinic acid, 500 mg Inositol, 200 mg Ca-Pantothenate, 20 mg Folic acid, 5000 mcg Biotin and 2000 mg Cholinchloride.

b)Mineral mixture (per kg feed) : 16.40 g Monocalciumphosphate, 5.50 mg $MgSO_4$. $7H_2O$, 7.53 g NaC1, 4.50 g K_2SO_4, 2.0 g Fe-Gluconat, 0.40 g $ZnSO_4.7H_2O$, 50 mg $MnSO_4$. H_2O, 15 mg $CuSO_4$, 4.75 mg KJ and 0.25 mg $CoCl_2.6H_2O$.

c)Carboxyl methyl cellulose.

d) Nitrogen free extract.

e) ME metabolic energy calculated by using 18.8, 33.5 and 13.8 kJ/g for protein, lipid and carbohydrate respectively according to BRETT and GROVES (1979).

* Calculated CP:E ratio.

*1 determined CP:E ratio

3.3. Sample preparation for chemical analysis

At the beginning, after 4 weeks, 8 weeks and at the end of each experiment, 9 fish from each treatment were selected for chemical analysis of body composition. Sampled fish were killed and frozen soon afterward and kept under cold storage conditions at a temperature of –20^{o}C. To obtain a homogenous material, fish were thawed and autoclaved by using a steam sterilizer at 2.5 kg/cm^{2} and 139^{o}C for 4 hours. Afterwards the sample of each group was homogenized with a mixer and stored under freezing conditions until the chemical analysis took place.

Table12: Summarized data of fish sampling for measurements of body composition

Experiments	1	2	3
Diets	10	10	10
Number of replications per diet	3	3	3
Number of fish per sample	3	3	3
Total fish samples for chemical analysis	90	90	90

3.4. Analytical methods

The chemical analysis of diets and fish carcasses were carried out according to the methods described by NAUMANN and BASSLER (1976 – 1997). The nitrogen free extract for diets was calculated by subtraction the total percentage of crude protein, crude fat and crude ash from 100%. The fat content in the fish carcass was calculated by subtraction the total percentage of crude protein and crude ash from 100%. The crude protein content of diets and whole body of fish was determined by using LECO FP 2000 system according to Dumas method for determination of nitrogen. The multiplication with the factor of 6.25 was the base, to calculate the crude protein content. The amino acid composition of feed components was determined by using amino acid analyzer LC 3000. The feed samples (0.25g) were firstly hydrolyzed by using 10 ml 6 N HCl in an air drier at 110°C. To insure total hydrolysis, the process took place under nitrogen gas for 24 hours. The sample was transfered to a volumetric

flask and made up to volume with NaOH (7.5 mol/l) and NaOH (2 mol/l) buffer to reach a pH of 2. After adding the internal standard the sampel was centrifuged for 10 minutes at 14.000 rounds per minute. Norleucine, a synthetic amino acid was added as an internal standard. The hydrolysate samples were stored under cold conditions at 4^0 C until used. The sulfur containing amino acid group (Methionine and Cystine) are destroyed by the acid hydrolysis described above, therefore, a pretreatment (oxidation) with performic acid was essential for protection. The free amino acids were determined by ion exchange chromatography using an automatic amino acid analyzer (LC 3000, Biotronik, München). For determination of gross energy content in the diets an adiabatic bomb calorimeter LECO AC-350 system was used .

3.5. Experimental parameters

The evaluation of experimental diets is performed by using a number of criteria. Growth, feed conversion ratio, protein efficiency ratio and net protein utilization and protein retention were the most important criteria. Each of these criteria involves the measurement of various parameters. These parameters were used to evaluate the growth performance ,quality of diets, especially protein quality and amino acid balance.

1.Growth.

a) Percentage weight gain = (Mean final weight – Mean initial weight)/ Mean initial weight x 100

b) Specific growth Rate = [ln final weight(g) – ln initial weight (g)]/ time (d) x 100

2. Feed conversion ratio = feed intake (g) / weight gain (g)

3.Protein utilization

a)Protein efficiency ratio = weight gain (g) / protein intake (g)

b)Productive protein value = [protein gain (g) / protein intake (g)] x 100

c)Net Protein Utilization =

[(protein gain (g) + protein maintenance requirement* (g)) / protein intake (g)] x 100

* protein maintenance requirement 1g/kg/BW/d, as stated from HALVER (1988)

4. Calculation of maximum nitrogen deposition capacity

Each growing animal, also fish, has a genetically determined capacity for maximum N-deposition. The sum of this capacity (C) plus the corresponding N-maintenance requirement (NMR) can be defined as the maximum capacity for N-retention (A). The interrelationship between N-intake and N-retention can be described by N-utilization model from GEBHARDT (1966).

$$y = \mathbf{A\,(1 - e^{-bx})} \qquad \text{where}$$

y = Daily N-deposition + Daily N- maintenance requirement per $BW_{kg}^{0.67}$ (mg)

A = calculated daily maximum theoretical capacity for daily N-retention y (dependent on genotype and age) (mg)

x = Daily N- intake per $BW_{kg}^{0.67}$ (mg)

b = Slope of N-utilization curve (dependent on protein quality, independent on N-intake)

e = Basic number of natural logarithm.

The data for estimation of N-retention capacity can be generated by classical N-rise experiments with subsequent analysis of body composition and determination of N-deposition or by N-balance studies. The recent experiments were based on determination of body composition only.

All N-deposition data were adapted to the model by statistical program MATHEMATICA 3.0 for Linux (WOLFRAM, 1997). The aim of this adaptation is to minimize the sum of deviation squares X^2 following the function $X^2 = \varepsilon; /Fi - fi/^2$, where Fi represents the experimental value and fi the corresponding estimating value.

Generally the estimation of N-retention capacity gives results which are higher than real N-retention under in vivo conditions. So we prefer the term "theoretically maximum of N-retention capacity", indicating that this value is really an upper limit for this parameter.

In spite of this fact, these A-values or corresponding C-values are generally useful as the "genotype-factor" within our physiological based N-utilization model (GEBHARDT, 1966). These values can be identified and used for further calculations within the model, mainly for evaluation of amino acid efficiency and amino acid requirements.

3.6. Statistical analysis

All of the results data were subjected to one–way analysis ($P<0.05$) of variance (SPSS 8/PC program). The treatment means were compared according to the method of Duncan new multiple range test (Duncan1955).

$Y_{ij} = \mu + D_i + e_{ij}$

Where :

Y_{ij} = The observation of the j th individual from D th Diet.

μ = The overall mean.

D_i = The Fixed effect of the D th Diet.

e_{ij} = The random error associated with the individual j.

4. RESULTS

Three experiments were conducted to determine the growth capacity of different genotypes of Tilapia, and to find out the physiological response of different protein supply on growth of Tilapia. The experiments were carried out in the fish recirculating system of the Institute of Animal Physiology and Animal Nutrition, University of Goettingen.

4. 1. Experiment 1

The first experiment was conducted with male *O. niloticus* (genotype 1) with survival rate about 80 to 100%. The different survival rate was a result of high aggressiveness of male animals and not effected by dietary treatments.

4.1.1. Feed intake and growth performance

The growth data and feed intake are presented in table 13.The average body weight (12.3±0.1g) between groups of male *O.niloticus* at the start of the experiment were not significantly (P≤0.05) different, indicating that groups were randomly divided and homogenous. After 28 days of the experimental start, the average body weights were significantly (p≤0.05) affected by the increasing dietary protein levels in the diet. The body weight and final weight gain of fish fed on different protein level fell into three clusters. The groups of fish fed on diets 4,5,9 and10, which were containing 40% and 48% crude protein with different energy levels, had a significantly (p≤0.05) higher body weight than the other treatment. Groups of fish fed on diets 2,3,7 and 8 which were containing 24% and 32% crude protein, were intermediate. Diets 1 and 6 which contained 16% crude protein had a significantly lower body weight . After 56 days of the experimental period also the diets 4,5,9 and 10 resulted in a significantly (p≤0.05) higher body weight than the other treatments. At the end of the experiment, the groups of fish fed on diets 4,5,9 and 10 still had higher final body weights than the other groups followed by group 8, which was fed on a diet containing 32% crude protein and supplemented with amino acids (threonine and tryptophan) to cover

the amino acids requirements for Tilapia according to NRC (1993). The groups fed on diets 1, 2, 6 and 7 had a significant (P≤0.05) lower body weight. The data of weight gain as percent of the initial body weight of fish are also presented also in table 13. It is show, the data of weight increasing with increasing of dietary protein level. Data of feed intake had similarly the same pattern as the results of body weight and final weight gain.

Table13: Growth parameters dependent on test diets and age (genotype 1)

Diet	Initial body weight (g)	Body weight (g)			Feed Intake (g)	Weight gain as % of the initial body weight
		28 days	56 days	70 days	70 days	
1	12.2	25.6 ^{e} ± 2.8	46.0 ^{e} ± 5.2	60.0 ^{e} ± 5.1	86.4 ^{d} ± 5.5	390.4 ^{e} ± 40.2
2	12.2	31.5 ^{d} ± 2.1	59.0 ^{cd} ± 4.5	78.0 ^{d} ± 9.0	104.6 ^{c} ± 7.1	539.3 ^{d} ± 73.7
3	12.2	34.2 ^{d} ± 1.1	68.3 ^{c} ± 4.5	92.3 ^{c} ± 11.2	109.7 ^{bc} ± 9.3	660.9 ^{c} ± 92.1
4	12.3	43.5 ^{b} ± 2.5	106.7 ^{a} ± 6.5	139.7 ^{a} ± 7.7	129.1 ^{a} ± 16.6	1035.8 ^{a} ± 69.6
5	12.2	43.8 ^{b} ± 2.5	102.7 ^{a} ± 7.6	136.3 ^{a} ± 12.8	124.5 ^{ab} ± 2.1	1014.6 ^{a} ± 107.3
6	12.2	25.8 ^{e} ± 2.0	45.3 ^{e} ± 5.0	58.3 ^{e} ± 6.6	85.4 ^{d} ± 6.1	376.7 ^{e} ± 52.7
7	12.3	30.5 ^{d} ± 2.1	57.7 ^{d} ± 3.7	73.0 ^{de} ± 4.0	98.1 ^{cd} ± 5.7	490.3 ^{de} ± 30.5
8	12.3	39.0 ^{c} ± 1.7	87.3 ^{b} ± 4.9	116.7 ^{b} ± 9.1	123.9 ^{ab} ± 13.1	848.9 ^{b} ± 79.7
9	12.4	44.9 ^{ab} ± 04	99.0 ^{a} ± 4.0	132.7 ^{a} ± 4.4	129.1 ^{a} ± 13.3	964.2 ^{ab} ±36.7
10	12.2	48.3 ^{a} ± 2.3	108.3 ^{a} ± 7.3	145.3 ^{a} ± 8.0	139.5 ^{a} ± 10.8	1088.0 ^{a} ± 65.2

1* Mean values in the same column with the same superscript are not significantly different (p ≤ 0.05).

The differences in final body weight of fish were reflected by differences in specific growth rate (SGR) which are presented in table 14. After 28 days, the average SGR was significantly (p≤0.05) higher in the groups fed on diets 4, 5, 9 and 10 which were containing 40% and 48% of crude protein with different energy levels. The intermediate groups were fed on diets 3 and 8 (+Thr) containing 32% crude protein, followed by groups 2 and 7 fed on diets containing 24% crude protein. The lowest (p≤0.05) SGR was expressed after diets 1 and 6 with a crude protein level of 16%. After 56 days, the groups 4 ,5 ,9 and 10 also had a significant (p≤0.05) higher SGR than the other treatment. However, group 1 and 6 had a significant (p≤0.05) lower SGR than the other groups. At the end of the experimental period (70 days) the data of SGR showed the same tendency as the results of SGR from the second period (56 days).

There were no significant effects between the groups with diets 4, 5, 9 and 10. The SGR was found to be higher than in the other fish groups. The lowest results of SGR were observed after diets 1 and 6 with a crude protein content of 16% . In general, SGRs were increased with increasing crude protein in the diets.

Table 14: Specific growth rate (SGR) dependent on test diet and age (genotype1)

Diet	SGR (g/d)		
	28 days	56 days	70 days
1	2.63 ^{e} ± 0.36	2.35 ^{e} ± 0.19	2.26 ^{e} ±0.12
2	3.39 ^{cd} ± 0.24	2.80 ^{d} ± 0.14	2.64 ^{d} ±0.16
3	3.72 ^{bc} ±0.00	3.09 ^{c} ±0.10	2.90 ^{c} ±0.16
4	4.53 ^{a} ±0.21	3.85 ^{a} ±0.13	3.47 ^{a} ±0.00
5	4.56 ^{a} ± 0.21	3.80 ^{a} ±0.13	3.43 ^{ab} ±0.11
6	2.67 ^{e} ± 0.27	2.33 ^{e} ±0.19	2.22 ^{e} ±0.17
7	3.22 ^{d} ±0.25	2.73 ^{d} ±0.11	2.54 ^{d} ±0.00
8	4.11 ^{b} ± 0.18	3.50 ^{b} ±0.11	3.21 ^{b} ±0.11
9	4.55 ^{a} ± 0.00	3.69 ^{ab} ±0.00	3.36 ^{ab} ±0.00
10	4.91^{a} ± 0.23	3.89 ^{a} ±0.11	3.54 ^{a} ±0.00

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4. 1. 2. Feed Conversion Ratio (FCR)

As given in table 15 the average FCR after 28 days, indicate a low level with a low protein diets and improved feed conversion when the protein level was 32% and more. The group of fish fed on diets 10, 9, 5, 4 and 8 had a significant ($p \leq 0.05$) better FCR with levels of FCR less than 1 : 1 (0.86, 0.93, 0.95 and 0.97) followed by diet 8 with a level of FCR 1.07 which was only numerical different. The groups of fish fed on diets 1 and 6 had the most worse ($p \leq 0.05$) FCR. After 56 days of the experimental start the average data of FCR were 0.96, 0.98, 1.03 and 1.08 after diets 10, 5, 4 and 9. Statistical analysis revealed that the groups of fish fed on diets 10, 5, 4 and 9 had a significant ($p \leq 0.05$) better FCR than the other groups

of fish, while the group fed on 32% CP level without supplementation of threonine resulted in intermediate feed conversion (1.35). The significant decreased ($p \leq 0.05$) feed conversion in the group of fish fed on diets 1 and 6 with a crude protein level of 16% at two different levels of energy indicates the lack of protein in the diets. At the end of the experimental, the groups fed on diets 5, 4, 10, 9 and 8 expressed a significant ($p \leq 0.05$) better feed conversion than the other groups. The highest feed conversion data were 1.81 and 1.86 for fish fed on diets 1 and 6 indicating the lowest level of feed efficiency. In general, the FCR data followed the same pattern like weight gain.

Table 15: Feed conversion ratio (FCR) dependent on test diets and age (genotype1)

Diet	FCR (g/g)		
	28 days	56 days	70 days
1	1.88 ^{d} ± 0.31	1.84 ^{e} ± 0.13	1.81^{d} ± 0.00
2	1.40 ^{c} ± 0.11	1.55 ^{d} ± 0.00	1.60 ^{c} ± 0.11
3	1.23 ^{bc} ± 0.00	1.35 ^{c} ±0.00	1.37 ^{b} ± 0.12
4	0.97 ^{a} ± 0.00	1.03 ^{ab} ± 0.00	1.01^{a} ± 0.12
5	0.95 ^{a} ± 0.00	0.98 ^{ab} ± 0.00	1.00 ^{a} ± 0.00
6	1.80 ^{d} ± 0.19	1.85 ^{e} ± 0.16	1.86 ^{d} ± 0.14
7	1.47 ^{c} ± 0.12	1.59 ^{d} ± 0.00	1.61^{c} ± 0.00
8	1.07 ^{ab} ± 0.00	1.12 ^{b} ± 0.00	1.18 ^{a} ± 0.00
9	0.93 ^{a} ± 0.00	1.08 ^{ab} ± 0.00	1.07 ^{a} ±0.11
10	0.86 ^{a} ± 0.00	0.96 ^{a} ± 0.00	1.04 ^{a} ±0.00

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4. 1. 3. Protein Efficiency Ratio (PER)

Protein efficiency ratios were calculated for each group and are presented in table 16. After 28, the protein efficiency ratio were 2.42 and 2.64 for groups 5, 10 fed on a diet containing 48 % crude protein with different level of energy. Statistical analysis revealed that fish fed on diet 5 had a significant ($p \leq 0.05$) lower PER than groups 2, 1 and 6, with diets

containing 24 % and 16 % crude protein . After 56 days, the groups of fish fed on diets 1 and 6 showed a significant ($p \leq 0.05$) higher PER than the other groups of fish. The groups fed on diets 5, 10, 9 and 3 had a significant ($p \leq 0.05$) lower PER than the other groups. The lower PER value was observed after diet 5 (2.33), indicating the surplus in the diet.

At the end of the total experimental period (70 days) the PER data can be divided in three clusters. The first in the groups of fish fed on diets 1 and 6 with a crude protein content of 16% and different levels of energy. The second in groups of diets 3, 9, 4, 7, 8 and 2. The third in groups 10 and 5. The statistical analysis revealed that the diets 1 and 6 had a significant ($p \leq 0.05$) higher PER, while the groups fed on diets 3, 9, 4, 7, 8 and 2 with a crude protein level from 24% to 40% show intermediate result. Group 10 and 5 had significant ($p \leq 0.05$) lower PERs than the rest groups, indicating the high protein content of these diets.

In general, the PER values decreased steadily with increasing levels of the dietary protein.

Table 16: Protein efficiency ratio (PER) dependent on test diets and age (genotype1)

Diet	PER (g/g)		
	28 days	56 days	70 days
1	$3.54^{a} \pm 0.63$	$3.57^{a} \pm 0.26$	$3.62^{a} \pm 0.15$
2	$3.33^{ab} \pm 0.27$	$2.99^{bc} \pm 0.14$	$2.92^{b} \pm 0.20$
3	$2.82^{bcd} \pm 0.10$	$2.57^{cde} \pm 0.00$	$2.54^{bc} \pm 0.23$
4	$2.87^{bcd} \pm 0.19$	$2.68^{cd} \pm 0.14$	$2.77^{b} \pm 0.38$
5	$2.42^{d} \pm 0.10$	$2.33^{e} \pm 0.00$	$2.29^{c} \pm 0.19$
6	$3.64^{a} \pm 0.40$	$3.54^{a} \pm 0.30$	$3.51^{a} \pm 0.26$
7	$3.11^{abc} \pm 0.25$	$2.87^{bc} \pm 0.15$	$2.82^{b} \pm 0.11$
8	$3.16^{abc} \pm 0.16$	$3.02^{b} \pm 0.00$	$2.87^{b} \pm 0.00$
9	$2.92^{bcd} \pm 0.00$	$2.53^{de} \pm 0.12$	$2.57^{bc} \pm 0.28$
10	$2.64^{d} \pm 0.14$	$2.38^{de} \pm 0.16$	$2.19^{c} \pm 0.18$

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4. 1. 4. Productive Protein Value (PPV)

The effect of dietary protein levels appeared clearly when dietary protein utilization was measured in terms of productive protein value (PPV). PPV is a better measure of feed quality than PER because PPV does not include the deposition of fat as it is done with PER. Therefore, PER is less sensitive to measure dietary protein utilization on fatty fish than PPV. PPV also decreased with increasing of dietary protein levels as it is shown in table 17. The highest PPV after 28 days, was found in fish fed on diet 1 containing 16% crude protein and the CP:E ratio of 10.30. The lowest value of PPV was found in fish fed on diet 5 containing 48% crude protein and CP:E ratio of 30.70. PPV seemed to be affected by dietary fat content. The statistical analyses revealed that the group 1 had a significant ($p \leq 0.05$) higher PPV than the other groups, except diets 8, 2 and 9. Group 5 had a significant ($p \leq 0.05$) lower PPV than groups 2, 8 and 1. After 56 days of the experiment, also groups 6 and 1 were containing 16% crude protein and CP:E ratio 11.70 and 10.30, had a significant ($p \leq 0.05$) higher PPV than the other groups of fish. After diets 5 and 10, containing 48% crude protein and a CP:E ratio 30.70 and 27.30 a significant ($p \leq 0.05$) lower PPV was observed except diet 9. At the end of the experiment (70 days) the results showed the same pattern as in the second period of the experiment. The highest PPV was found after diets 1 and 6 and the lowest PPV was found in diets 10 and 5.

Table 17: Productive protein value (PPV) dependent on test diets and age (genotype 1)

Diet	PPV (%)		
	28 days	56 days	70 days
1	51.5 ^{a} ± 4.3	52.5 ^{a} ± 1.1	47.8 ^{ab} ± 6.2
2	46.1 ^{ab} ± 2.5	45.4 ^{b} ± 1.6	44.8 ^{abcd} ± 4.7
3	40.4 ^{bc} ± 4.1	42.4 ^{bc} ± 2.0	39.2 ^{cde} ± 2.3
4	40.7 ^{bc} ± 1.8	44.1 ^{bc} ± 2.0	42.4 ^{bcd} ± 5.3
5	38.9 ^{c} ± 3.5	37.2 ^{d} ± 4.5	37.2 ^{de} ± 4.4
6	42.4 ^{bc} ± 3.1	54.7 ^{a} ± 4.1	52.4 ^{a} ± 4.7
7	42.5 ^{bc} ± 6.5	43.5 ^{bc} ± 2.1	44.1 ^{bcd} ± 1.8
8	47.3 ^{ab} ± 2.8	46.0 ^{b} ± 3.4	46.3 ^{abc} ± 2.1
9	45.6 ^{abc} ± 1.0	41.3 ^{bcd} ± 1.2	41.5 ^{bcde} ± 4.3
10	41.0 ^{bc} ± 2.9	39.8 ^{cd} ± 3.1	33.8 ^{e} ± 5.5

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4. 1. 5. Net Protein Utilization (NPU)

The results of net protein utilization in table 18 indicate that as the content of dietary protein increase, the values of NPU fall dawn. At low dietary protein levels the amino acid composition of the protein is the limiting factor and maximal NPU values are obtained. At higher protein levels, a greater proportion of the protein is utilized as energy. However, high dietary protein levels are necessary for fish to grow reasonably fast. After 28 days, the fish fed on diet 1 containing 16 % crude protein had a significant ($p \leq 0.05$) higher NPU than the other groups of fish, followed by diet 6 and the lowest ($p \leq 0.05$) NPU value is shown after diet 5 containing 48 % crude protein content . At 56 days of the experiment, the fish fed on diets 1 and 6 containing 16 % crude protein with different levels of energy had a significant ($p \leq 0.05$) higher NPU than the other groups, followed by diet 6. The lowest ($p \leq 0.05$) NPU value was

found in the diets 5 and 10 containing 48 % crude protein level with different levels of energy. At the end of the experiment, the results of NPU indicate the same pattern as in the second period of the experiment.

Table 18: Net protein utilization (NPU) dependent on test diets and age (genotype1)

Diet	NPU (%)		
	28 days	56 days	70 days
1	78.1^{a} ± 4.2	63.1^{a} ± 0.7	55.4 ^{ab} ± 5.8
2	63.4 ^{c} ± 2.3	51.8 ^{b} ± 1.3	49.3 ^{bc} ± 4.4
3	53.1^{ef} ± 4.1	47.0 ^{bc} ± 1.8	42.4 ^{cde} ± 2.2
4	49.9 ^{fg} ± 1.7	46.8 ^{bc} ± 2.2	44.6 ^{cd} ± 5.6
5	46.6 ^{g} ± 3.2	39.8 ^{d} ± 4.4	39.0 ^{ed} ± 4.3
6	69.3 ^{b} ± 3.1	65.5 ^{a} ± 3.8	60.1 ^{a} ± 4.2
7	59.7 ^{cd} ± 6.1	49.9 ^{b} ± 2.2	48.8 ^{bc} ± 1.6
8	59.1^{cde} ± 2.7	50.0 ^{b} ± 3.7	49.0 ^{bc} ± 2.1
9	54.6 ^{def} ± 0.9	44.2 ^{cd} ± 1.2	43.6 ^{cd} ± 4.6
10	48.3 ^{fg} ± 2.8	42.3 ^{cd} ± 3.2	35.5 ^{e} ± 5.6

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4.1.6. Body composition

Data concerning the whole body composition at the initial and end of the experiment are presented in table 19. Despite of the fact that there were no significant differences in crude protein content of the final whole body (carcass) among the different test diets ($p \leq 0.05$), except diet 1. The crude protein content of groups 5, 8 and 9 were higher than the crude protein contents of the initial fish carcass. Fat content in final groups were statistically effected by the energy density of the diets. The highest fat content was shown in the group of fish 1 and lowest fat content in group of fish 4. These results were higher than the fat content in the initial fish carcass, effected by the level of energy in the test diets. Crude ash content of final groups were significantly effected by test diets and feed intake. The highest crude ash

was observed in group of fish 6. Dry matter contents were higher than in the initial fish carcass. The data of whole body composition at 28 days and 56 days are documented in Appendix table A3 and A4.

Table 19: Whole body composition of fish at the start and end of experiment (genotype 1)

Diet	Dry matter (%)	Crude protein (%)	Ether extract *[1] (%)	Ash (%)
Initial	23.87	15.35	3.83	4.68
1	28.4^{a} ±1.8	13.7^{b} ±1.3	10.4^{a} ±0.5	4.3^{ab} ±0.2
2	28.8^{a} ±0.3	15.4^{a} ±0.4	9.1^{ab} ±0.4	4.3^{ab} ±0.1
3	26.1^{ab} ±0.4	15.3^{a} ±0.5	6.8^{cd} ±0.7	3.9^{b} ±0.3
4	25.3^{b} ±0.5	15.3^{a} ±0.3	5.7^{d} ±0.6	4.3^{ab} ±0.6
5	26.3^{ab} ±1.0	16.2^{a} ±0.6	5.8^{d} ±0.6	4.3^{ab} ±0.3
6	28.0^{a} ±2.8	15.1^{a} ±0.5	7.7^{bcd} ±2.4	5.2^{a} ±0.2
7	27.4^{ab} ±1.5	15.5^{a} ±0.3	8.0^{bcd} ±1.1	3.8^{b} ±0.5
8	28.2^{a} ±2.2	16.0^{a} ±0.4	8.6^{abc} ±1.5	3.5^{b} ±0.4
9	28.0^{a} ±1.0	16.1^{a} ±0.0	8.1^{bc} ±0.7	3.8^{b} ±0.9
10	27.1^{ab} ±1.6	15.4^{a} ±1.5	7.6^{bcd} ±0.3	4.1^{b} ±0.6

1* Calculated as (fat + NFE)

2* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4.1.7. Nutrient deposition

The initial, after 28 days, 56 days and the final of protein and fat deposition of the experimental fish are presented in table 20 and 21, respectively. The protein deposition after 28 days of the experimental period was significantly ($p \leq 0.05$) higher in the groups fed on diets 10, 5 and 9, containing crude protein levels of 48% and 40%, respectively, followed by the groups 4 and 8 containing crude protein levels of 40% and 32%, supplemented with threonine to cover the amino acid requirement for Tilapia according to NRC (1993). The lowest protein deposition was observed in group 6 and 1. The lowest fat deposition was observed in the group fed on diet 6 and the highest fat deposition was observed in diet 9, no

significant effects were observed between fish of groups 9, 10, 2, 8, 4, 3 and 1. After 56 days, the highest protein deposition was observed in the groups 10, 4, 5 and 9 containing crude protein levels of 48 % and 40 %, respectively. The lowest protein deposition was observed in group 1 and 6 containing 16% crude protein in the diet. The highest fat deposition was observed in groups 10, 4, 9 and 8, respectively and the lowest fat deposition in group 6 with no significant effect between diets 6, 7, 1, 3, 2 and 5. At the end of the experimental period (70 days) the results show a similar pattern with the highest protein deposition in groups of fish 10, 5, 4, 9 and 8 and the lowest protein deposition in group 1, 6 and 7.

In general, the body composition or the crude protein gain and fat gain was effected by dietary protein content, energy density and feed intake. Increasing of energy content in the diet gives the highest level of protein gain and fat gain, an increase of feed intake gives an increase in protein gain and fat gain, too.

Table 20: Protein gain dependent on test diets and age (genotype 1)

Diet	Initial CP Quantity	Protein gain (g)		
		28 days	56 days	70 days
1	1.88	1.94 ^{fe} ± 0.19	4.94 ^{e} ± 0.47	6.36 ^{e} ± 1.17
2	1.87	2.66 ^{cd} ± 0.20	7.09 ^{d} ± 0.65	10.11 ^{cd} ± 1.72
3	1.86	3.16 ^{c} ± 0.30	9.26 ^{c} ± 0.90	12.34 ^{c} ± 1.51
4	1.88	4.42 ^{b} ± 0.26	15.50 ^{a} ± 0.60	19.50 ^{ab} ± 0.75
5	1.87	5.07 ^{a} ± 0.63	14.50 ^{a} ± 2.78	20.11 ^{ab} ± 2.72
6	1.87	1.58 ^{f} ± 0.13	5.10 ^{fe} ± 0.63	6.89 ^{de} ± 1.10
7	1.90	2.47 ^{ed} ± 0.45	6.85 ^{ed} ± 0.30	9.47 ^{de} ± 0.85
8	1.89	3.99 ^{b} ± 0.32	11.39 ^{b} ± 0.24	16.85 ^{b} ± 1.89
9	1.91	5.05 ^{a} ± 0.18	14.12 ^{a} ± 0.44	19.42 ^{ab} ± 0.76
10	1.88	5.59 ^{a} ± 0.45	16.01 ^{a} ± 0.75	20.58 ^{a} ± 3.48

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

Table 21: Fat gain dependent on test diets and age (genotype 1)

Diet	Initial fat quantity	Fat gain (g)		
		28 days	56 days	70days
1	0.47	1.78 ^{abc} ±0.48	4.08 ^{cd} ±1.72	5.81 ^{cd} ±0.80
2	0.47	2.02 ^{ab} ±0.15	4.56 ^{c} ±0.61	6.57 ^{c} ±0.53
3	0.46	1.81 ^{abc} ±0.16	4.18 ^{c} ±0.64	5.85 ^{cd} ±0.73
4	0.47	1.84 ^{abc} ±0.20	7.52 ^{a} ±1.14	7.52 ^{bc} ±1.14
5	0.47	1.60 ^{bc} ±0.00	4.70 ^{bc} ±1.94	7.40 ^{bc} ±1.36
6	0.47	0.90 ^{d} ±0.36	2.29 ^{d} ±0.72	4.03 ^{d} ±1.48
7	0.47	1.43 ^{c} ±0.00	3.54 ^{d} ±0.56	5.45 ^{cd} ±1.18
8	0.47	2.01 ^{ab} ±0.30	6.16 ^{abc} ±1.26	9.68 ^{ab} ±2.67
9	0.48	2.27 ^{a} ±0.00	6.72 ^{ab} ±1.36	10.32 ^{a} ±0.65
10	0.47	2.15 ^{ab} ±0.53	8.06 ^{a} ±1.55	10.68 ^{a} ±1.09

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4.1.8. Calculation of maximum growth capacity

The growth capacity was calculated as the maximum of nitrogen deposition by using a model from (GEBHARDT 1966). The results show that the daily maximum of N-deposition capacity for male *O. niloticus* from 12 to 44 g body weight after 28 days, resulted in 350 mg N/BW $_{kg}$ $^{0.67}$ for groups fed on diets 1- 5 containing 16 % to 48 % crude protein with the same energy level of 15.6 MJ ME/kg and 410 mg N/BW $_{kg}$ $^{0.67}$ for the fish fed on diets 6 – 10 containing 16 % to 48 % crude protein level with energy levels from 13.6 to 17.6 MJ ME/kg, respectively. After 56 days of the experimental period the groups of fish fed on diets 1 - 5 containing 16 % to 48 % crude protein with the same energy level (15.6 MJ ME/kg) resulted in 328 mg N/BW $_{kg}$ $^{0.67}$ and 337 mg N/BW $_{kg}$ $^{0.67}$ for groups fed on diets 6 – 10 containing 16 % to 48 % crude protein with higher energy levels from 13.6 to 17.6 MJ ME/kg, respectively for male *O . niloticus* from 12 to 108g body weight.

At the end of the experiment the groups 1 - 5 fed on 16% to 48% crude protein level with the same energy level (15.6 MJ ME/kg) resulted in 292 mg N/BW $_{kg}^{0.67}$ and 305 mg N/BW $_{kg}^{0.67}$ after diets 6 – 10 containing 16% to 48% crude protein level with energy levels from 13.6 to 17.6 MJ ME/kg, respectively for male *O. niloticus* from 12 to 145g body weight. The maximum N-deposition data are presented in table 22.

In general, the daily N-deposition capacity slightly decreased with the age of fish, also some changes were observed corresponding to energy content in the diets 6-10. The N-deposition curve at the end of experiment (70 days) are presented in figure 2 and figure 3.

Table 22: Calculation of maximum N-deposition capacity dependent on test diets and age (genotype1)

Diet	28 days	56 days	70 days
Isoenergetic diets	350 mg N/BW $_{kg}^{0.67}$	328 mg N/BW $_{kg}^{0.67}$	292 mg N/BW $_{kg}^{0.67}$
Adapted diets	410 mg N/BW $_{kg}^{0.67}$	337 mg N/BW $_{kg}^{0.67}$	305 mg N/BW $_{kg}^{0.67}$

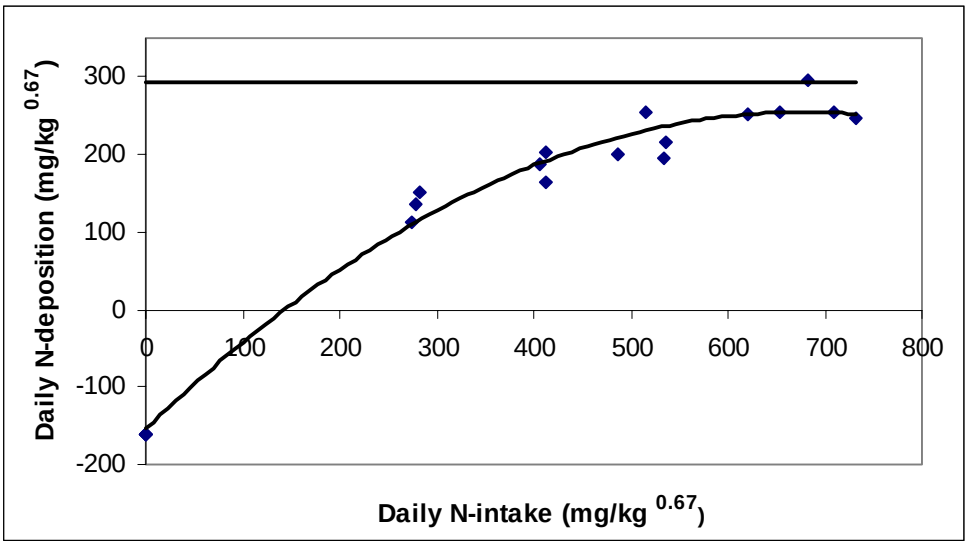

Figure 2: N-deposition curve at the end of the experiment for isoenergtic diets (genotype 1)

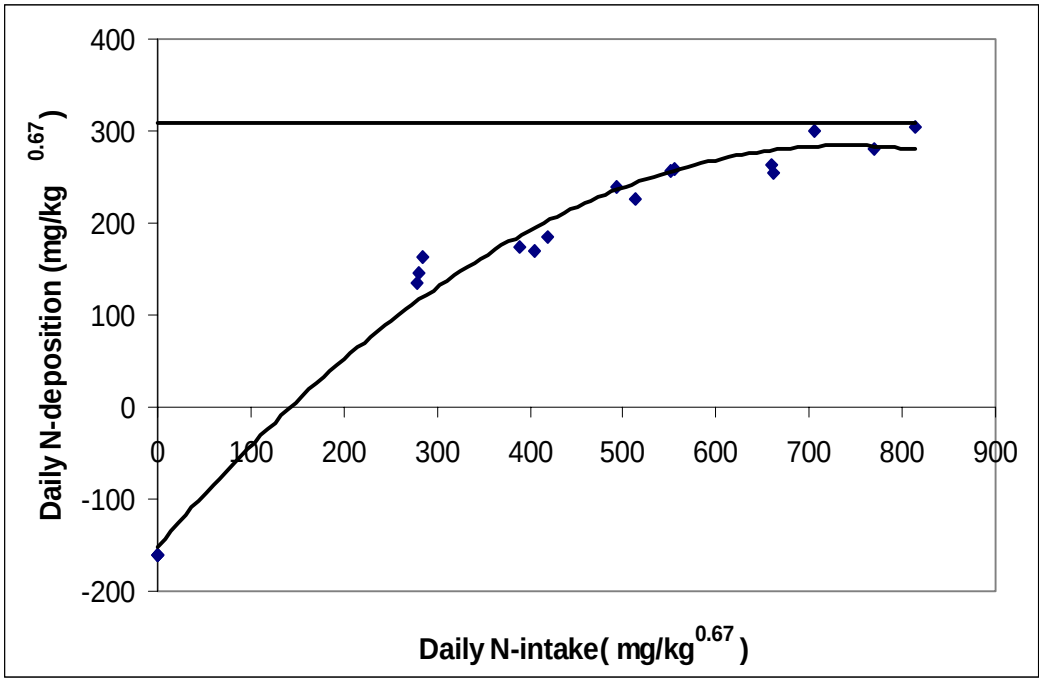

Figure 3: N-deposition curve at the end of the experiment for adapted energy diets (genotype 1)

4.2. Experiment 2

The second experiment was conducted with Red Tilapia (genotype 2) with survival rate about 96 to 100. The different survival rate was a result of male aggressive and not effected be dietary treatment.

4.2.1. Feed intake and growth performance

The growth data and feed intake are presented in table 23. The average body weight (12.4±0.1g) between groups of Red Tilapia at the start of the experiment were not significantly ($p \leq 0.05$) different, indicating that groups were randomly divided and

homogeneous. After 28 days after the experimental start the average body weights were significantly ($p \leq 0.05$) effected by the increasing of dietary protein levels in the diet. The body weight and the final weight gain of fish fed on different protein levels fell into three clusters. The groups fed on diets 10, 5, 4 and 9 containing 40 % and 48 % crude protein levels with different energy levels had a significant ($p \leq 0.05$) higher body weight than the other treatment, while no significant observations were found between fish fed on diets 3 and 8. Diets 2 and 7 were intermediate. Diets 1 and 6 which contained 16% crude protein had a significant lower body weight ($p \leq 0.05$). After 56 days, the diets 10 and 4 had a significant ($p \leq 0.05$) higher body weight than the other treatment. No significant differences were found between diets 10, 4, 5 and 9. The lowest body weight was found in group of fish 6 and 1 fed on diets containing 16 % crude protein. At the end of experiment (70 days) fish fed on diets 10, 9, 5, 4, 8 and 3 had a significant ($p \leq 0.05$) higher final body weight than the other groups, followed by the fish fed on diets 2, 7, 1 and 6 with a lower body weight significant ($p \leq 0.05$). The data of the percentage of weight gain as percent of the initial body weight of fish are presented in table 23. It is show, the data are increasing with increasing of dietary protein level. Data of feed intake had same pattern body weight and final weight gain.

Table 23: Growth parameters dependent on test diets and age (genotype 2)

Diet	Initial body weight (g)	Body weight (g)			Feed intake (g)	Weight gain as % of the initial body weight
		28 days	56 days	70 days	70 days	
1	12.3	24.6 ^{f} ±0.5	36.0 ^{fe} ±1.0	42.6^{b} ±3.2	73.6 ^{d} ±1.7	248.0^{b} ±29.2
2	12.5	32.0 ^{d} ±1.0	52.6 ^{cd} ±6.0	66.0^{b} ±8.5	93.3 ^{bc} ±6.1	425.1^{b} ±67.8
3	12.4	36.6 ^{bc} ±1.1	66.3 ^{ab} ±3.0	89.6^{a} ±2.8	103.9^{ab} ±4.57	625.1^{a} ±26.8
4	12.5	41.0 ^{a} ±1.0	74.6 ^{a} ±5.8	100.0^{a} ±18.7	108^{ab} ±9.2	704.8^{a} ±168.4
5	12.4	41.0 ^{a} ±3.0	72.0 ^{ab} ±12.3	98.0^{a} ±17.3	95.8^{bc} ±14.5	692.0^{a} ±137.1
6	12.2	24.3 ^{f} ±0.5	34.3 ^{f} ±2.1	42.3^{b} ±5.1	70.6^{d} ±4.1	246.5^{b} ±35.3
7	12.3	28.6 ^{e} ±0.5	47.3 ^{ed} ±2.9	63.7^{b} ±4.9	85.3^{cd} ±9.33	417.6^{b} ±40.8
8	12.5	35.6 ^{c} ±1.2	59.6 ^{bc} ±6.1	89.6^{a} ±20.5	94.5^{bc} ±8.7	613.4^{a} ±147.9
9	12.4	39.0 ^{ab} ±1.0	68.3 ^{ab} ±3.2	96.6^{a} ±7.2	95.2^{bc} ±3.2	681.5^{a} ±56.5
10	12.3	41.0 ^{a} ±3.6	78.6 ^{a} ±13.1	106.0^{a} ±20.1	117.5^{a} ±19.9	762.2^{a} ±166.9

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

The differences in final weight in second experiment were reflected also by differences in specific growth rate (SGR) which are presented in table 24. After 28 days, the average SGR was significantly ($p \leq 0.05$) higher in the groups fed on diets 10, 4, 5 and 9 containing 40 % and 48 % crude protein levels, with different energy levels in the diet. In the intermediate groups were fed on diets 3 and 8 (+Thr), containing 32 % crude protein, followed by groups 2 and 7, fed on diets containing 24 % crude protein. The lowest significant ($p \leq 0.05$) difference in SGR showed in group 1 and 6 with a crude protein level of 16 %. After 56 days, groups 10, 4 ,5, 9 and 3 had a significant ($p \leq 0.05$) higher SGR than the other treatment. However, group 1 and 6 had also a significant ($p \leq 0.05$) lower SGR than the other treatment. At the end of the experimental period (70 days). The data of SGR showed the same pattern as the results of SGR from the second period. There were no significant effects observed between groups 10, 4, 5, 9, 3 and 8. The SGR was found to be 2.78 - 3.05. The group of fish fed on diets 6 and 1 containing 16 % crude protein had a significant lower SGR than the other groups with SGR value of 1.77 and 1.78, respectively.

Table 24: Specific growth rate (SGR) dependent on test diets and age (genotype 2)

Diet	SGR (g/d)		
	28 days	56 days	70 days
1	2.49 ^{f} ±0.00	1.91 ^{e}±0.00	1.78 ^{c} ±0.13
2	3.32 ^{d}±0.15	2.54 ^{cd}±0.19	2.36 ^{b} ±0.18
3	3.86 ^{bc}±0.11	2.99 ^{ab}±0.10	2.82 ^{a} ±0.00
4	4.27 ^{a}±0.14	3.19 ^{a}±0.18	2.96 ^{a} ±0.31
5	4.25 ^{a}±0.26	3.13 ^{a}±0.31	2.93 ^{a} ±0.25
6	2.40 ^{f}±0.10	1.83 ^{e}±0.00	1.77 ^{c} ±0.14
7	3.01^{e}±0.00	2.39 ^{d}±0.10	2.34 ^{b} ±0.10
8	3.73 ^{c}±0.00	2.77 ^{c}±0.18	2.78 ^{a} ±0.29
9	4.09 ^{ab}±0.00	3.04 ^{ab}±0.00	2.92 ^{a} ±0.00
10	4.28 ^{a}±0.36	3.29 ^{a}±0.30	3.05 ^{a} ±0.28

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4.2.2. Feed Conversion Ratio (FCR)

As given in table 25, the average of FCR after 28 days of the experimental period indicated that the quality of the diets was improved when the protein level was 32 % and more. The group of fish fed on diets 10, 4, 5 and 9 had a significantly ($p \leq 0.05$) better FCR than the other fish groups with levels of FCR near to 1:1 (1.08, 1.08, 1.09 and 1.18). Fish fed on diets 3, 8 and 2 had also significant different FCRs compared to other groups. Fish fed on diets 6 and 1 had a significant ($p \leq 0.05$) higher FCR than the rest groups. After 56 days, the average FCR was 1.18 and 1.25 for group 5 and 10, respectively . Statistical analyses revealed that fish fed on diets 10 and 5 had a significant ($p \leq 0.05$) lower FCR than the other fish groups. While no significant differences were found between the groups 9, 4, 8 and 7 fed on diets containing 40 % and 32 % CP supplemented with the amino acid threonine and 24 % crude protein, respectively. The highest significant ($p \leq 0.05$) different of feed conversion showed in fish fed on diet 1 containing 16 % crude protein level (2.78). At the end of the experiment, the results were divided into three clusters. Fish fed on diets 5, 9, 4, 10, 8 and 3, containing protein level from 32 % to 48 % CP, had a significant ($P \leq 0.05$) better FCR than the other groups of fish. Fish fed on diets 7 and 2 containing 24 % crude protein were intermediate. The higher FCRs were observed in group of fish fed on diets 1 and 6 with FCR of 2.44 and 2.36, respectively. In general, the FCR data followed the same general pattern as weight gain.

Table 25: Feed conversion ratio (FCR) dependent on test diets and age (genotype 2)

Diet	FCR (g/g)		
	28 days	56 days	70 days
1	2.06 ^{c} ±0.10	2.35 e ±0.12	2.44 ^{c} ±0.33
2	1.47 ^{b} ±0.00	1.71 ^{d} ±0.18	1.76 ^{b} ±0.24
3	1.24 ^{ab} ±0.00	1.41 ^{bc} ±0.00	1.34 ^{a} ±0.01
4	1.08 ^{a} ±0.00	1.26 ^{ab} ±0.10	1.26 ^{a} ±0.10
5	1.09 ^{a} ±0.00	1.18 ^{a} ±0.00	1.12 ^{a} ±0.04
6	2.16 ^{c} ±0.13	2.50 ^{e} ±0.00	2.36 ^{c} ±0.20
7	1.96 ^{c} ±0.36	1.88 ^{d} ±0.14	1.66 ^{b} ±0.03
8	1.29 ^{ab} ±0.00	1.47 ^{c} ±0.00	1.26 ^{a} ±0.20
9	1.18 ^{a} ±0.00	1.26 ^{ab} ±0.00	1.13 ^{a} ±0.00
10	1.08 ^{a} ±0.00	1.25 ^{ab} ±0.00	1.26 ^{a} ±0.00

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4. 2. 3. Protein Efficiency Ratio (PER)

Protein efficiency ratio were calculated for each group and are presented in table 26. After 28 days of the experimental beginning the PER values were 2.11 and 2.12 for fish on diets 5 and 10 fed containing 48 % crude protein. Statistical analysis revealed that fish fed on diet 5 and 10 had a significant ($p \leq 0.05$) lower PER value than groups 6, 2 and 1 fed on diets containing 24 % and 16 % with PER values 3.02, 3.17 and 3.17, respectively. After 56 days, the fish fed on diet 1 showed a significant ($p \leq 0.05$) higher PER than the other groups. The groups of fish fed on diets 10 and 5 had a significant ($p \leq 0.05$) lower PER than the other groups, mainly diet 10 with a PER value of 1.82. At the end of the experimental period the PER values showed that the fish fed on diet 10 had also a significant ($p \leq 0.05$) lower PER than

the other groups of fish with a PER value of 1.82 without no significant differences between diets 10 and 5 with the same protein level (48 %) and diet 4 with 40 % crude protein level. The PER found to be 2.05 and 2.39 for diets 5 and 4, respectively. The fish fed on diet 6 containing 16 % crude protein had a significant ($p \leq 0.05$) higher PER value of 2.77, no significant differences were found between diet 6 and diets 8, 7, 1, 2, 3 and 9.

In general, the PER influenced by the dietary protein level when dietary protein level increased the PER value decreased.

Table 26: Protein efficiency ratio (PER) dependent on test diets and age (genotype 2)

Diet	PER (g/g)		
	28 days	56 days	70 days
1	3.17 ^{a} ±0.16	2.78 ^{a} ±0.14	2.71 ^{a} ±0.35
2	3.17 ^{a} ±0.21	2.73 ^{ab} ±0.30	2.67 ^{a} ±0.39
3	2.81 ^{bc} ±0.00	2.46 ^{bcd} ±0.00	2.59 ^{a} ±0.00
4	2.56 ^{cd} ±0.11	2.20 ^{edf} ±0.18	2.23 ^{abc} ±0.32
5	2.11 ^{e} ±0.00	1.95 ^{hf} ±0.02	2.05 ^{bc} ±0.11
6	3.02 ^{ab} ±0.18	2.60 ^{abc} ±0.10	2.77 ^{a} ±0.30
7	2.37 ^{ed} ±0.40	2.43 ^{cde} ±0.19	2.75 ^{a} ±0.15
8	2.62 ^{d} ±0.00	2.30 ^{ed} ±0.13	2.75 ^{a} ±0.55
9	2.32 ^{ed} ±0.10	2.16 ^{df} ±0.00	2.42 ^{ab} ±0.12
10	2.12 ^{e} ±0.00	1.82 ^{h} ±0.00	1.82 ^{c} ±0.00

1* Mean values in the same column withthe same superscript are not significantly different ($p \leq 0.05$).

4. 2. 4. Productive Protein Value (PPV)

The productive proteins value are presented in table 27. The highest PPV after 28 days, was found in fish fed on diet 2 containing 24 % crude protein level and a CP:E ratio of 15.4. The lowest value of PPV was found in fish fed on diet 5 containing 48 % crude protein and a CP:E ratio 30.7. The PPV seemed to be affected by the dietary fat content. The statistical analyses showed that there are no significant differences found between all the fish

groups throughout the first bulk of the experiment. After 56 days, the fish of groups 3 and 2 containing 32 % and 24 % crude protein level and CP:E ratios of 20.5 and 15.4, had a significant ($p \leq 0.05$) higher PPV than fish fed on diets 10 and 5 containing 48 % crude protein level and CP:E ratios of 27.3 and 30.7, respectively. Diets 10 and 5 had a significant ($p \leq 0.05$) lower PPV than the other groups of fish, except in diet 9. The results of PPV between the groups 7, 1, 3, 6, 9, 2 and 8 showed no significant differences.

At the end of the experiment (70 days) the results indicates the same pattern as in the second period of the experiment. The highest PPV was found in diet 8, followed by diets 7 and 1 with a PPV value of 49.7, 48.1 and 47.2, respectively. The lowest PPV was found in diet 10 and 5 (33.3 and 38.5), respectively. The statistical analysis revealed that fish fed on diet 8 containing 32 % crude protein level had a significant ($p \leq 0.05$) higher PPV than fish fed on diets 10 and 5 containing 48 % crude protein. The results of PPV showed no significant differences between diets 7, 1, 3, 6, 9, 2 and 8.

Table 27: Productive protein value (PPV) dependent on test diets and age (genotype 2)

Diet	PPV (%)		
	28 days	56 days	70 days
1	36.2 ^{a} ±5.9	37.3 ^{ab} ±2.8	47.2 ^{abc} ±5.4
2	42.8 ^{a} ±2.9	41.4 ^{a} ±5.4	43.3 ^{abc} ±4.3
3	42.0 ^{a} ±1.8	41.6 ^{a} ±1.7	45.6 ^{abc} ±1.0
4	40.3 ^{a} ±2.4	38.47 ^{a} ±3.1	39.8 ^{bcd} ±4.6
5	33.2 ^{a} ±6.1	32.6 ^{bc} ±4.3	38.5 ^{cd} ±2.4
6	36.1 ^{a} ±7.9	40.4 ^{a} ±2.5	45.1 ^{abc} ±3.1
7	35.5 ^{a} ±8.9	39.0 ^{a} ±2.7	48.1 ^{ab} ±2.3
8	38.4 ^{a} ±5.4	41.4 ^{a} ±3.7	49.7 ^{a} ±10.6
9	36.1 ^{a} ±3.9	35.8 ^{abc} ±1.9	43.6 ^{abc} ±3.5
10	33.7 ^{a} ±4.0	30.9 ^{c} ±0.9	33.3 ^{d} ±2.5

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4.2.5. Net Protein Utilization (NPU)

The result of net protein utilization are presented in table 28. After 28 days of the experimental period the fish fed on diet 1 containing 16 % crude protein had a significant ($p \leq 0.05$) higher NPU than the other groups, followed by diet 6. The lowest significant ($p \leq 0.05$) differences in the NPU value resulted in diet 5 containing 48 % crude protein level . After 56 days, the fish fed on diets 6 and 1 containing 16 % crude protein with different levels of energy density had a significant ($p0 \leq 0.05$) higher NPU than the other groups, followed by group 2 fed on a diet containing 24% crude protein level. The lowest significant ($p \leq 0.05$) NPU value was observed in diets 10 and 5 containing 48 % crude protein level with different levels of energy.

Table 28: Net protein utilization (NPU) dependent on test diets and age (genotype 2)

Diet	NPU (%)		
	28 days	56 days	70 days
1	61.8 ^{a} ±6.2	49.0 ^{ab} ±2.9	56.1 ^{a} ±5.6
2	59.1 ^{c} ±2.6	48.3 ^{ab} ±5.1	48.3 ^{abc} ±4.5
3	53.6 ^{abc} ±2.0	46.2 ^{bc} ±2.0	49.0 ^{abc} ±1.1
4	49.3 ^{bcd} ±2.3	42.2 ^{cd} ±3.0	42.4 ^{cd} ±4.4
5	40.6 ^{d} ±5.8	35.9 ^{ef} ±3.8	41.0 ^{cd} ±1.9
6	61.0 ^{a} ±8.4	52.4 ^{a} ±2.4	54.4 ^{ab} ±3.1
7	50.1 ^{bcd} ±10.8	46.0 ^{bc} ±3.3	53.4 ^{ab} ±2.3
8	49.8 ^{bcd} ±5.1	45.7 ^{bc} ±3.3	53.3 ^{ab} ±10.4
9	44.8 ^{cd} ±3.9	39.7 ^{ed} ±1.9	46.3 ^{bc} ±3.4
10	41.2 ^{d} ±3.2	33.7 ^{f} ±1.1	35.3 ^{d} ±2.3

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

At the end of the experiment the results of NPU had the same pattern as observed in the first period of the experiment. However, the statistical analysis of NPU results showed no significant differences between diets 6, 7, 8, 3, 2 and diet 1.

4. 2. 6. Body composition

Data concerning the whole body composition are presented in table 29. The whole body composition were significantly ($p \leq 0.05$) differences by the level and component of the diets. These result were higher than the crude protein contents of the initial fish carcass. The lowest crude protein content showed in the group fed on diet 6 and 2. Statistical analysis showed that no significant differences in crude protein among the test diets except diet 6, 2. Fat content in final groups were statistically effected by the level of energy density in the diets. The highest fat content showed in the group of fish 1 and lowest fat content showed in group of fish 4, 5. The fat content higher that the fat content in the initial fish carcass, effected by the level of energy density in the diets. Crude ash content of final groups were significantly effected by test diets and feed intake. The highest crude ash showed in group of fish 6 and 1. Dry matter content were higher than the initial fish carcass. Statistical analysis showed also the same pattern. The data concerning about the whole body composition after 28 and 56 days are documented in Appendix table A5, A6, respectively.

Table29: Whole body composition of fish at the start and end of experiment (genotype 2)

	Dry matter (%)	Crude protein (%)	Ether extract *[1] (%)	Ash (%)
Initial	28.8	16.3	8.1	4.4
1	40.3 ^{a} ±0.9	17.0 ^{bc} ±0.2	18.1 ^{a} ±0.2	5.2 ^{a} ±0.4
2	37.1 ^{b} ±0.8	16.2 ^{c} ±0.9	16.1 ^{ab} ±1.0	4.8 ^{ab} ±0.1
3	36.9 ^{b} ±2.8	17.4 ^{abc} ±0.2	14.9 ^{b} ±2.8	4.6 ^{b} ±0.1
4	33.4 ^{c} ±1.9	17.5 ^{ab} ±0.5	11.0 ^{c} ±1.6	4.9 ^{ab} ±0.1
5	34.1 ^{bc} ±1.0	18.4 ^{a} ±0.2	11.1 ^{c} ±1.0	4.6 ^{b} ±0.2
6	35.2 ^{bc} ±1.5	16.3 ^{c} ±1.1	13.7 ^{bc} ±1.2	5.2 ^{a} ±0.3
7	35.2 ^{bc} ±2.9	17.2 ^{abc} ±0.5	13.0 ^{bc} ±2.4	5.0 ^{ab} ±0.4
8	35.4 ^{bc} ±0.9	17.7 ^{ab} ±0.8	13.1 ^{bc} ±1.5	4.6 ^{b} ±0.0
9	36.0 ^{bc} ±1.3	17.7 ^{ab} ±0.5	13.7 ^{bc} ±1.5	4.6 ^{b} ±0.2
10	37.2 ^{b} ±0.4	18.0 ^{ab} ±0.7	14.4 ^{b} ±1.1	4.8 ^{ab} ±0.1

1* Calculated as (fat + NFE)

2* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4.2.7. Nutrient deposition

The data concerning protein and fat deposition after 28 days, 56 days and the final of the experimental fish are presented in table 30 and 31, respectively. The crude protein deposition after 28 days of the experiment period was significantly ($p \leq 0.05$) higher in groups fed on diets 10, 5, 4 and 9, containing crude protein level 48 % and 40 %, respectively, followed by group 3 and 8 containing 32 % crude protein level supplemented with threonine. The lowest crude protein deposition was observed in group 1 and 6. The lowest fat deposition was observed in the group fed on diet 6. The highest fat deposition was observed in diet 10. There were no significant effects observed between the other groups of fish. After 56 days, the highest crude protein deposition was observed in fish on diets 10, 4, 5, 3, 9 and 3 containing crude protein levels of 48 %, 40 % and 32 %, respectively. The lowest protein deposition was observed in group 1 and 6 containing 16% crude protein. The highest fat deposition was observed in group 10 followed by group 3, 9, 4 and 2. The lowest fat deposition was observed in group 6, followed by group 5. There were no significant differences found between group 2 and 7. The statistical analysis of fat deposition showed no significant differences between the diets 3, 9, 4, 2 and 8. At the end of the experimental period (70 days), the highest crude protein deposition was observed in fish on diets 10, 5, 4, 9, 8 (+Thr) and 3 containing 48 %, 40 % and 32 % crude protein levels, respectively. The lowest crude protein deposition was observed in group 1 and 6 containing 16% crude protein level with two different levels of energy, followed by diet 2 and 7 containing a crude protein level of 24 % with two different levels of energy. Fat deposition indicate the same pattern as crude protein deposition.

In general, the crude protein gain and fat gain was effected by the dietary protein, energy density and feed intake. increasing of energy level in the diet gives the highest level of protein and fat gain. The increase of feed intake also gives increases in protein and fat gain.

Table 30: Protein gain dependent on test diets and age (genotype 2)

Diet	Initial CP quantity (g)	Protein gain (g)		
		28 days	56 days	70 days
1	2.00	1.41 ^{e} ±0.21	3.18 ^{d} ±0.21	5.29 ^{b} ±0.49
2	2.05	2.63 ^{cd} ±0.22	6.07 ^{c} ±1.03	8.66 ^{b} ±0.84
3	2.02	3.62 ^{abc} ±0.13	9.09 ^{ab} ±0.00	13.58 ^{a} ±0.63
4	2.03	4.49 ^{a} ±0.34	10.92 ^{a} ±1.36	15.54 ^{a} ±2.88
5	2.02	4.53 ^{a} ±1.10	10.02 ^{ab} ±2.66	16.11 ^{a} ±3,.36
6	1.99	1.45 ^{e} ±0.30	3.42 ^{d} ±0.30	4.88 ^{b} ±0.48
7	2.01	2.45 ^{d} ±0.43	5.61 ^{c} ±0.50	8.98 ^{b} ±1.12
8	2.04	3.40 ^{bcd} ±0.59	8.35 ^{b} ±1.32	13.87 ^{a} ±3.70
9	2.02	4.14 ^{ab} ±0.45	9.26 ^{ab} ±0.64	15.01 ^{a} ±1.78
10	2.01	4.59 ^{a} ±0.93	11.19 ^{a} ±1.82	17.17 ^{a} ±3.98

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$)

Table 31: Fat gain dependent on test diets and age (genotype 2)

Diet	Initial fat quantity (g)	Fat gain (g)		
		28 days	56 days	70 days
1	1.00	2.60 ^{ab} ±0.44	5.36 ^{d} ±0.14	6.72 ^{cd} ±0.39
2	1.02	3.71 ^{a} ±0.64	7.69 ^{bc} ±0.99	9.69 ^{bc} ±2.18
3	1.00	3.86 ^{a} ±0.39	8.51^{b} ±0.66	12.43 ^{ab} ±2.96
4	1.02	3.57 ^{a} ±0.72	7.92 ^{b} ±1.03	9.96 ^{abc} ±2.35
5	1.00	3.19 ^{ab} ±1.10	4.70 ^{ed} ±1.94	9.86 ^{bc} ±1.36
6	0.99	1.85 ^{b} ±0.64	3.34 ^{e} ±0.93	4.86 ^{d} ±1.27
7	1.00	2.58 ^{ab} ±0.24	5.88 ^{cd} ±0.45	7.38 ^{cd} ±2.26
8	1.02	3.30 ^{ab} ±0.30	7.62 ^{bc} ±0.87	10.90 ^{abc} ±3.63
9	1.00	3.75 ^{a} ±1.44	8.44 ^{b} ±1.17	12.26 ^{ab} ±1.97
10	1.00	4.15 ^{a} ±1.22	10.98 ^{a} ±1.30	14.37 ^{a} ±3.26

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4. 2. 8. Calculation of maximum growth capacity

The data concerning of maximum N-deposition capacity are presented in table 32.The growth capacity was calculated as the maximum of nitrogen deposition by using a model from GEBHARDT (1966). The results show that the daily maximum N- deposition for Red Tilapia (male : female = 80 : 20) between 12 to 41 g body weight at day 28 of the experiment, resulted in 379 mg N/BW $_{kg}^{0.67}$ for fish fed on diets 1- 5 containing 16 % to 48% crude protein level with the same energy level of 15.6 MJ ME/kg. 372 mg N/BW $_{kg}^{0.67}$.was found for fish fed on diets 6 – 10 containing 16 % to 48 % crude protein with adapted energy levels between 13.6 and 17.6 MJ ME/kg, respectively. After 56 days of the experiment the groups fed on diets 1 – 5 containing 16 % to 48 % crude protein and body weight between 12g to 78g resulted in 335 mg N/BW $_{kg}^{0.67}$ and 288 mg N/BW $_{kg}^{0.67}$ after diets 6 – 10 with adapted energy levels between 13.6 and 17.6 MJ ME/kg, respectively. At the end of the experiment, the maximum N-deposition capacity for fish between 12 g and 106 g body weight fed on diet 1 - 5 with energy level of 15.6 MJ ME/kg was 300 mg N/BW $_{kg}^{0.67}$. However, maximum N-deposition capacity was 289 mg N/BW $_{kg}^{0.67}$ for fish body weight between 12g and 106 g after diets 6 – 10 containing 16 % to 48 % crude protein level with adapted energy levels. The maximum N-deposition data are presented in table 32.

In general, with increasing age of fish the daily N-deposition capacity was decreased. Besides that some changes were observed corresponding to energy content and sex ratio in the tank culture. The N-deposition curve at the end of experiment (70 days)are presented in figure 4 and figure 5.

Table 32: Calculation of maximum N-deposition capacity dependent on test diets and age (genotype 2)

Deit	28 days	56 days	70 days
Isoenergetic diets	379 mg N/BW $_{kg}^{0.67}$	335 mg N/BW $_{kg}^{0.67}$	300 mg N/BW $_{kg}^{0.67}$
Adapted diets	372 mg N/BW $_{kg}^{0.67}$	288 mg N/BW $_{kg}^{0.67}$	289 mg N/BW $_{kg}^{0.67}$

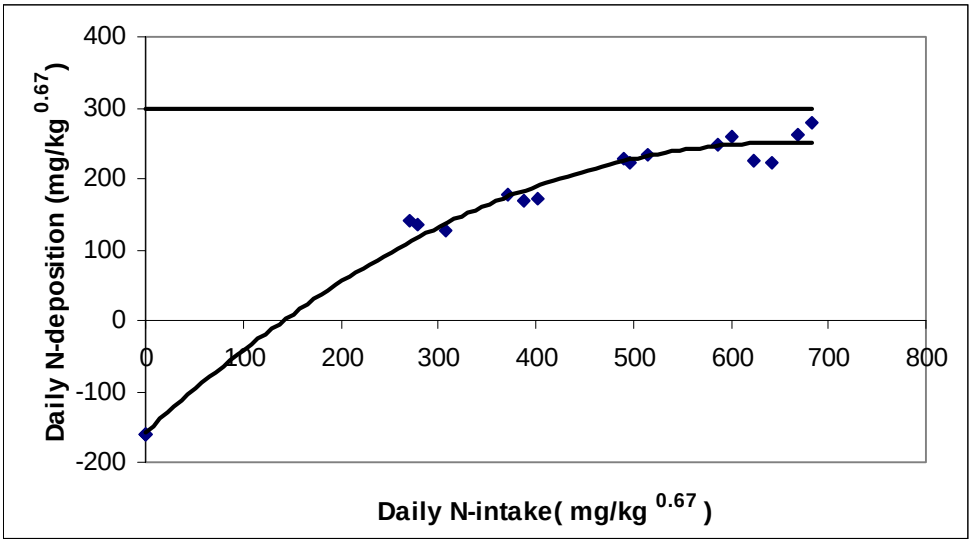

Figure 4: N- deposition curve at the end of the experiment for isoenergetic diets (genotype2).

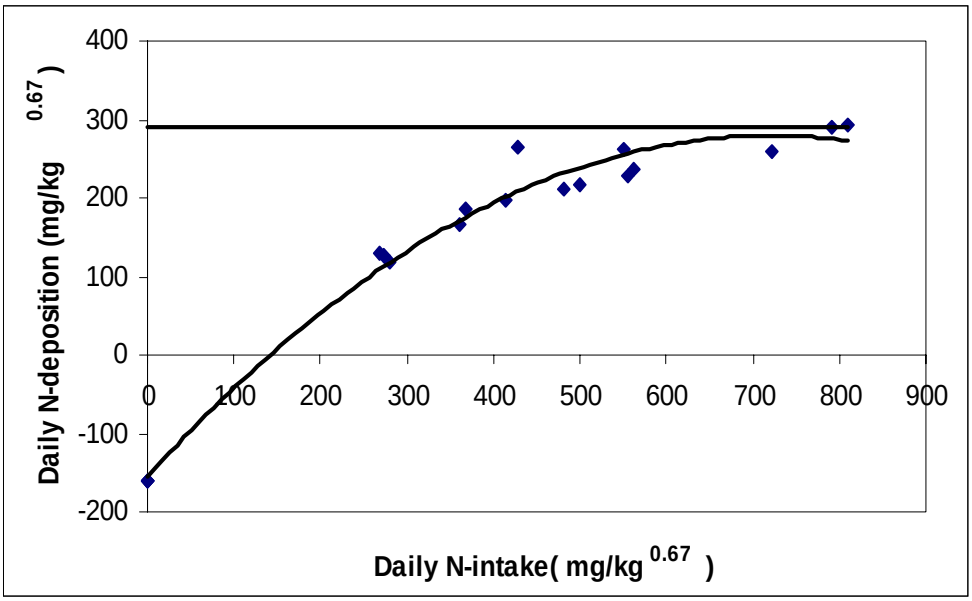

Figure 5: N-deposition curve at the end of the experiment for adapted energy diets (genotype 2).

4.3. Experiment 3

The third experiment was conducted with hybrid of Nile Tilapia female X Red Tilapia male which were produced under conditions of commercial fish farm. No mortality was observed through the experiment.

4.3.1. Feed intake and growth performance

The growth data and feed intake are presented in table 33.The average body weight (12.3±0.1g) between groups of genotype Tilapia (*Oreochromis niloticus* x *O.mosambicus* x *O.hornorum*) at the start of the experiment was not significantly ($p \leq 0.05$) different, indicating that groups were randomly divided and homogenous. After 28 days of the experimental start, the average body weights were significantly ($p \leq 0.05$) affected by the increasing dietary protein levels in the diet. The body weight and final weight gain of fish fed on different protein level fell into three clusters. The fish fed on diets 10,5 and 9, which were containing 48 % and 40 % crude protein with different energy levels, respectively, had a significant ($p \leq 0.05$) higher body weight than after the other treatments, also no significant differences were found between diet 4 and diets 5 and 9 fed the same levels of protein, followed by the groups fed on diets 8 and 3 containing 32 % crude protein level. Group of fish 2, 7 fed on diet 7 and 2 containing 24 % crude protein with two levels of energy were intermediate. Diets 1 and 6 containing 16 % crude protein had a significant ($p \leq 0.05$) lower body weight . After 56 days, the diets 8, 4, 3, 5, 10 and 9 had a significant ($p \leq 0.05$) higher body weight than the other treatments, followed by fish in groups 2 and 7 fed on diet containing 24 % crude protein with two energy levels. Diets 1 and 6 which contained 16 % crude protein level had significant ($p \leq 0.05$) lower body weight. At the end of the experiment (70 days), the fish fed on diets 4, 5, 8, 3, 10 and 9 had higher final body weights than the other groups. The groups fed on diets 1, 2, 6 and 7 had a significant ($p \leq 0.05$) lower body weight. The data of weight gain as percent of the initial body of fish are also presented in table 33. It is shown, that the increasing of final body weight was effected by the increasing of dietary protein level. The feed intake had also the same pattern as body weight and final weight gain.

Table 33: Growth parameters dependent on test diets and age (genotype 3)

Diet	Initial body weight (g)	Body weight (g)			Feed intake (g)	Weight gain as % of the initial body weight
		28 days	56 days	70 days	70 days	
1	12.2	25.3 ^{e} ±0.5	40.0^{c} ±2.0	47.6 ^{b} ±2.5	76.8^{d} ±2.7	286.7^{b} ±25.1
2	12.2	31.0 ^{d} ±1.7	51.6 ^{b} ±2.3	60.3 ^{b} ±3.5	87.8^{cd} ±1.8	389.2^{b} ±28.3
3	12.2	36.6 ^{c} ±1.1	68.6 ^{a} ±8.6	84.0 ^{a} ±14.4	106.9^{ab} ±13.1	580.3^{a} ±125.5
4	12.2	38.3 ^{bc} ±1.5	67.0 ^{a} ±5.5	78.6 ^{a} ±9.3	97.1^{bc} ±14.1	541.8^{a} ±81.3
5	12.3	41.6 ^{ab} ±2.5	69.0 ^{a} ±1.7	82.6 ^{a} ±5.0	87.9^{cd} ±3.0	574.2 ^{a}±46.1
6	12.3	24.0 ^{e} ±0.0	40.0 ^{c} ±1.7	48.6 ^{b} ±5.5	78.0^{d} ±7.0	297.9 ^{b}±46.1
7	12.3	29.3 ^{d} ±1.5	47.3 ^{bc} ±2.8	63.0 ^{b} ±5.5	87.8^{cd} ±4.2	389.2 ^{b}±44.5
8	12.3	35.3 ^{c} ±0.5	64.6 ^{a} ±8.7	83.0 ^{a} ±16.0	113.9^{a} ±6.3	574.1^{a} ±137.7
9	12.3	41.0 ^{ab} ±3.0	71.3 ^{a} ±4.9	84.3 ^{a} ±7.0	99.6^{abc} ±9.1	583.4^{a} ±48.8
10	12.4	42.0 ^{a} ±3.6	70.6 ^{a} ±5.1	84.0 ^{a} ±6.2	100.1^{abc} ±6.1	584.7^{a}± 48.7

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

The differences in final body weight were reflected by differences in specific growth rate (SGR) which are presented in table 34. After 28 days average SGR was significant ($p \leq 0.05$) higher in the groups fed on diets 4, 5, 9 and 10 containing 40 % and 48 % crude protein levels, respectively, with different energy levels compared to the other fish groups. Intermediate were fish groups fed on diets 3 and 8 (+Thr), containing 32 % crude protein, followed by groups 2 and 7 fed on diets containing 24 % crude protein level. The lowest ($p \leq 0.05$) SGR showed in the groups 1 and 6 with a crude protein level of 16 %. After 56 days, the fish on the diets 4, 8, 3, 5, 9 and 10 also had a significant ($p \leq 0.05$) higher SGR than the other treatment. However, group 1 and 6 had also significant ($p \leq 0.05$) lower SGR than the other groups. At the end of the experimental period the data of SGR showed the same pattern as the results of SGR from the second period. There were no significant ($p \leq 0.05$) effects between the group of fish with diets 4, 8, 3, 5, 9 and 10. The SGR was found to be 2.64 to 2.74 higher than in the other fish groups. The lowest results of SGR were obtained with diets 1 and 6 containing 16 % crude protein level . In general the SGR was clearly effected by the level of dietary protein and feed intake.

Table 34: Specific growth rate (SGR) dependent on test diets and age (genotype 3)

Diet	SGR (g/d)		
	28 days	56 days	70 days
1	2.58 ^{e} ±0.00	2.10 ^{c} ±0.10	1.92 ^{c} ±0.00
2	3.07 ^{d} ±0.29	2.55 ^{b} ±0.10	2.26 ^{b} ±0.00
3	3.83 ^{bc} ±0.16	3.04 ^{a} ±0.24	2.72 ^{a} ±0.26
4	4.09 ^{ab} ±0.15	3.07 ^{a} ±0.00	2.64 ^{a} ±0.19
5	4.37 ^{a} ±0.15	3.09 ^{a} ±0.00	2.72 ^{a} ±0.00
6	2.39 ^{e} ±0.00	2.12 ^{c} ±0.00	1.97 ^{c} ±0.15
7	3.05 ^{d} ±0.23	2.56 ^{b} ±0.15	2.30 ^{b} ±0.12
8	3.74 ^{c} ±0.13	2.94 ^{a} ±0.28	2.69 ^{a} ±0.30
9	4.27 ^{a} ±0.19	3.13 ^{a} ±0.10	2.74 ^{a} ±0.10
10	4.41 ^{a} ±0.27	3.12 ^{a} ±0.12	2.74 ^{a} ±0.00

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4.3.2. Feed Conversion Ratio

As given in table 35 the average FCR after 28 days indicate that the feed conversion ratio improved when the level of protein was 32% and more. The group of fish fed on diets 5 had a significant ($p \leq 0.05$) better FCR than the other groups, with a level of FCR 0.93.The result with fish on diets 10 and 9 were not significantly different from diet 5, with a level of FCR near to 1:1 (1.05 and 1.10), respectively. Groups 3 and 8 had also significant differences in the FCR ($p \leq 0.05$) comparing to the other groups, except diet 2 had no significant difference to diet 8. Groups fed on diets 6 and 1 had a significant ($p \leq 0.05$) higher FCR than the other groups. After 56 days, the fish fed on diets 5, 9, 4, 10 and 3 were significantly ($p \leq 0.05$) better FCR with an average of 1.20 to 1.40 .No significant differences were found between groups of fish 2 and 7 fed on diets containing 24 % crude protein with different energy density. The fish fed on diet 1 had a significant ($p \leq 0.05$) higher level of FCR (2.15). At the end of the experiment, group 5 had a significantly ($p \leq 0.05$) better FCR than groups 1, 2, 6 and 7 fed on

diet containing 16 % and 24 % crude protein level with different energy density . No significant differences were observed between diet 5 and diets 9, 10, 4 and 3. The highest levels of FCR data were 2.15 and 2.17 for fish fed on diets 6 and 1 intimating the lowest level of feed efficiency.

Table 35: Feed conversion ratio (FCR) dependent on test diets and age (genotype 3)

Diet	FCR (g/g)		
	28 days	56 days	70 days
1	2.05 ^{f} ±0.14	2.15 ^{d} ±0.14	2.17 ^{e} ±0.00
2	1.49 ^{ed} ±0.10	1.67 ^{b} ±0.12	1.83 ^{d} ±0.10
3	1.24 ^{bc} ±0.00	1.40 ^{a} ±0.11	1.50 ^{abc} ±0.02
4	1.13 ^{b} ±0.00	1.35 ^{a} ±0.00	1.47 ^{abc} ±0.17
5	0.93 ^{a} ±0.00	1.20 ^{a} ±0.00	1.25 ^{a} ± 0.12
6	2.17 ^{f} ±0.20	1.99 ^{cd} ±0.00	2.15 ^{e} ±0.15
7	1.55 ^{e} ±0.11	1.88 ^{bc} ±0.00	1.74 ^{cd} ±0.11
8	1.32 ^{cd} ±0.00	1.66 ^{bc} ±0.36	1.65 ^{bcd} ±0.30
9	1.10 ^{ab} ±0.00	1.32 ^{a} ±0.00	1.38 ^{ab} ±0.00
10	1.05 ^{ab} ±0.00	1.39 ^{a} ±0.00	1.40 ^{ab} ±0.10

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4.3.3. Protein Efficiency Ratio

Protein efficiency ratio were calculated for each group and are presented in table 36. After 28 days, the PER value was 2.17 for fish fed on the diet containing 48 % crude protein, followed by the fish on diets 4, 5 and 9 containing 40 %,48 % CP, respectively. Statistical analysis reported that fish fed on diet 10 had a significantly ($p \leq 0.05$) lower PER than groups 6, 2 and 1 which were fed diets containing 24 % and 16 % and PER values of 3.01, 3.12 and 3.20, respectively. After 56 days fish fed on diets 6 and 1 showed a significant ($p \leq 0.05$) higher PER than the other fish groups, while the fish fed on diets 10 and 5 had a significant ($p \leq 0.05$) lower PER than the other groups. At the end of the experimental period the PER

values showed that the fish fed on diet 10 had also a significantly ($p \leq 0.05$) lower PER than the other groups of fish. No significant differences were observed between diets 10, 5, 4 and 9 with 40 and 48 % crude protein level in the diets. The PER was found to be 1.85, 1.91 and 1.98 for diets 5, 4 and 9, respectively. The group fed on diet 6 and 1 containing 16 % crude protein with two different levels of energy had a significant ($p \leq 0.05$) higher PER value of 3.04 and 3.01.

Table 36: Protein efficiency ratio (PER) dependent on test diets and age (genotype 3)

Diet	PER (g/g)		
	28 days	56 days	70 days
1	3.20 ^{a} ±0.22	3.05 ^{ab} ±0.20	3.01 ^{a} ±0.13
2	3.12 ^{ab} ±0.22	2.79 ^{bc} ±0.21	2.54 ^{b} ±0.15
3	2.81 ^{bc} ±0.13	2.50 ^{cd} ±0.19	2.32 ^{bc} ±0.19
4	2.47 ^{ed} ±0.20	2.06 ^{e} ±0.14	1.91 ^{ed} ±0.23
5	2.47 ^{ed} ±0.14	1.92 ^{ef} ±0.12	1.85 ^{ed} ±0.18
6	3.01 ^{ab} ±0.26	3.27 ^{a} ±0.00	3.04 ^{a} ±0.22
7	2.94 ^{ab} ±0.21	2.43 ^{d} ±0.10	2.62 ^{b} ±0.16
8	2.56 ^{cd} ±0.00	2.10 ^{e} ±0.41	2.09 ^{cd} ±0.37
9	2.48 ^{ed} ±0.00	2.07 ^{e} ±0.00	1.98 ^{cde} ±0.00
10	2.17 ^{d} ±0.10	1.64 ^{f} ±0.00	1.64 ^{e} ±0.12

1* Mean values in the same column with the same super script are not significantly different ($p \leq 0.05$).

4.3.4. Productive protein value

The data concerning of productive protein value are presented in table 37. The highest PPV after 28 days of the experiment was found in fish fed on diet 6 containing 16 % crude protein and a CP: E ratio of 11.70 and the lowest value of PPV was found in fish group fed on diet 10 containing 48 % crude protein with CP:E ratio of 27.30. The PPV seemed to be affected by dietary fat content. The statistical analysis showed that no significant differences were found between all other fish groups through the first part of the experiment, except diet

10 with a significant (p≤0.05) lower result of PPV. After 56 days, the fish of groups 7 and 6 containing 24 % and 16 % crude protein and CP:E ratios 16.50 and 11.70 had a significant (p≤0.05) higher PPV than fish fed on diet 10 containing 48% crude protein with CP:E ratio of 27.30. Diet 10 had a significant (p≤0.05) lower PPV than the others groups of fish. The results of PPV between fish on diets 9, 4, 3, 8 showed no significant differences.

At the end of the experimental period (70 days), the results showed that fish of group 6 had a significant (p≤0.05) higher PPV than the other groups with a PPV value of 51.2.The lowest PPV was found after diet10 with a PPV value of 28.7, followed by the groups after diets 4, 5 and 9 with PPV values of 34, 34.1 and 34.9, respectively. The statistical analysis showed that no significant difference between diets 10,4,5 and 9 could be observed.

Table 37: Productive protein value (PPV) dependent on test diets and age (genotype 3) .

Diet	PPV (%)		
	28 days	56 days	70 days
1	41.0 ^{abc} ±7.6	37.1 ^{ab} ±2.6	45.1 ^{b} ±2.2
2	43.0 ^{abc} ±8.2	35.5 ^{bc} ±1.5	40.6 ^{bc} ±4.3
3	43.4 ^{abc} ±1.1	30.4 ^{bc} ±3.7	40.3 ^{bc} ±2.6
4	43.3 ^{abc} ±2.8	30.1 ^{bc} ±5.7	34.0 ^{cd} ±5.0
5	39.9 ^{bc} ±0.8	31.2 ^{bc} ±4.8	34.1 ^{cd} ±3.2
6	48.5 ^{a} ±1.8	42.5 ^{a} ±8.2	51.2 ^{a} ±3.7
7	46.1 ^{ab} ±4.6	43.5 ^{a} ±3.9	43.1 ^{b} ±3.4
8	42.9 ^{abc} ±1.4	31.0 ^{bc} ±8.3	35.7 ^{c} ±5.2
9	41.2 ^{abc} ±0.5	28.1 ^{bc} ±4.5	34.9 ^{cd} ±0.8
10	37.5 ^{c} ± 1.3	24.8 ^{c} ±3.6	28.7 ^{d} ±2.3

1* Mean values in the same column with the same superscript are not significantly different (p ≤ 0.05).

4.3.5. Net Protein Utilization

The results of net protein utilization are presented in table 38. The NPU decreased by the increasing of protein level. After 28 days, the fish fed on diet 6 containing 16 % crude protein had a significant ($p \leq 0.05$) higher NPU than the other groups. A significant lower ($p \leq 0.05$) NPU value was observed with diet 10 containing 48 % crude protein level, followed by diet 5 with the same protein level. After 56 days, the fish fed on diets 6 containing 16 % crude protein the NPU was significantly ($p \leq 0.05$) higher than in the others groups, followed by group 2 fed on diet containing 24 % crude protein. The lowest NPU value was observed after diet 10 containing 48 % crude protein. The results showed no significant differences between diets 10, 9, 4, 5, 3 and 8.

Table 38: Net protein utilization (NPU) dependent on test diets and age (genotype 3)

Diet	NPU (%)		
	28 days	56 days	70 days
1	65.6 ^{b} ±7.5	48.2 ^{ab} ±2.7	53.7 ^{b} ±1.9
2	59.8 ^{bc} ±8.2	42.6 ^{bc} ±1.7	45.9 ^{c} ±4.2
3	55.0 ^{cd} ±1.3	34.9 ^{cd} ±3.9	43.6 ^{cd} ±2.2
4	52.8 ^{cd} ±3.1	33.9 ^{cd} ±6.1	36.9 ^{df} ±5.1
5	48.3 ^{ed} ±0.4	34.6 ^{cd} ±5.0	36.7 ^{df} ±3.3
6	74.2 ^{a} ±3.3	54.3 ^{a} ±7.7	59.7 ^{a} ±3.7
7	63.6 ^{b} ±4.3	50.5 ^{ab} ±3.6	48.3 ^{bc} ±3.1
8	54.1 ^{cd} ±1.3	35.0 ^{cd} ±8.4	38.7 ^{ed} ±5.1
9	49.9 ^{ed} ±1.0	31.7 ^{d} ±4.2	37.7 ^{ed} ±0.9
10	44.8 ^{e} ±0.9	27.6 ^{d} ±3.4	31.0 ^{f} ±2.3

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

At the end of the experimental period (70 days), the results of NPU show the same pattern as in the first period of the experiment. However, the statistical analysis of NPU showed no significant differences between diets 10, 5 and 4.

4.3.6. Body Composition

Data concerning the whole body composition are presented in table 39. The whole body composition was altered significantly ($p \leq 0.05$) by the diets. Dietary protein level up to 32 % (+Thr) and 48 % crude protein resulted in an increase of protein content comparing with initial fish carcass. The lowest crude protein showed in the group fed on diet 1. Statistical analysis showed that high significant differences in crude protein among the test diets 3, 4, 5, 8, 9 and 10. Fat content in final groups were statistically effected by the level of energy density in the test diets. The highest fat content was observed in the fish after diet 1 and lowest fat content was shown by the group after diet 5. The fat content was higher than the fat content of the initial fish carcass effected by level of energy density in the test diets except group 5. Crude ash content of final groups were significantly effected by test diets and feed intake. The highest crude ash content was shown in groups of fish after diet 6. Dry matter contents had no significant differences among the different test diets except diet1.The data concerning whole body composition after 28 and 56 days are documented in table A7and A8, respectively, in the appendix.

Table 39: Whole body composition of fish at the start and end of experiment (genotype 3)

	Dry matter (%)	Crude protein (%)	Ether extract *[1] (%)	Ash (%)
Initial	31.8	16.6	10.9	4.3
1	40.4 ^{a} ±2.3	15.3 ^{f} ±0.2	20.2^{a} ±2.2	4.9 ^{b} ±0.2
2	34.6 ^{b} ±3.2	15.9 ^{e} ±0.5	14.2 ^{b} ±2.5	4.5 ^{b} ±0.3
3	34.2 ^{b} ±0.7	17.1^{bc} ±0.2	12.7 ^{b} ±1.0	4.4 ^{b} ±0.2
4	34.4 ^{b} ±1.0	17.6 ^{ab}±0.4	12.2 ^{bc} ±0.8	4.6 ^{b} ±0.1
5	31.3 ^{b} ±1.1	18.1^{a} ±0.1	8.5 ^{c} ±0.7	4.7 ^{b} ±0.2
6	34.0 ^{b} ±3.2	16.6 ^{cd}±0.2	11.7 ^{bc} ±2.7	5.6 ^{a} ±0.4
7	32.8 ^{b} ±1.2	16.4 ^{ed}±0.2	11.6 ^{bc} ±0.9	4.7 ^{b} ±0.0
8	32.8 ^{b} ±0.8	17.0 ^{bcd} ±0.4	12.5 ^{b} ±2.3	4.6 ^{b} ±0.5
9	33.1 ^{b} ±3.7	17.4 ^{b}±0.4	11.0 ^{bc} ±3.1	4.7 ^{b} ±0.3
10	32.4 ^{b} ±2.2	17.2 ^{bc}±0.2	10.7 ^{bc} ±2.1	4.-4 ^{b} ±0.2

1* Calculated as (fat + NFE)

2* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

4.3.7. Nutrient deposition

The data concerning the protein and fat deposition at 28, 56 days and at the end of the experimental period are presented in tables 40 and 41. The crude protein deposition after 28 days was significantly ($p \leq 0.05$) higher in the group fed on diets 10, 9, 5 and 4 containing crude protein level 48 % and 40 % respectively, followed by the groups 3 and 8 containing a crude protein level of 32 % supplemented with threonine. The lowest crude protein deposition was observed in fish on diets 1 and 6.The lowest fat deposition was observed in fish fed on diet 6 and the highest fat deposition was observed after diet 9. There are no significant effects of fat deposition between groups 9, 10, 8 and 3. After 56 days, the highest crude protein deposition was observed in fish after diets 5, 10, 9, 4 and 8 containing crude protein levels of 48 %, 40 % and 32 %, respectively. The lowest protein deposition was observed in group of fish 1 and 6 containing 16 % crude protein. The highest fat deposition was observed in group 8, followed by the other fish groups, however the lowest fat deposition was observed in group 6. The statistical analysis of fat deposition analysis showed that no significant differences between all groups fed on the experimental diets, except in diet 6 with a significant ($p \leq 0.05$) lower fat deposition could be observed.

At the end of the experimental period (70 days),the results show the same pattern as in the second period, also the highest crude protein deposition was observed in fish of group 10, 5, 4, 9, 8 (+Thr) and 3 containing 48 %. 40 % and 32 %, crude protein, respectively. The lowest value of crude protein deposition were found in fish on diets 1 and 6 containing 16 % crude protein level with two different levels of energy, followed by diet 2 and 7 containing crude protein levels of 24 % with two different levels of energy. Fat deposition showed also the same pattern as crude protein deposition.

In general, the crude protein gain and fat gain were effected by the dietary protein level, energy density and feed intake. An increase of energy level of the diets gives an highest level of protein gain and fat gain. An increase of feed intake gives an increase in protein gain and fat gain, too.

Table 40: Protein gain dependent on test diets and age (genotype 3)

Diet	Initial CP Quantity (g)	Protein gain (g)		
		28 days	56 days	70 days
1	2.00	1.66 ^{d} ±0.32	3.40 ^{e} ±0.25	5.30 ^{c} ±0.45
2	1.99	2.56 ^{c} ±0.48	5.01^{e}d ±0.00	7.65 ^{bc} ±0.91
3	2.00	3.74 ^{b} ±0.00	6.80 ^{bcd} ±0.77	12.41 ^{a} ±2.30
4	1.98	4.58 ^{a} ±0.19	7.88^{abc} ±0.67	11.83 ^{a} ±1.91
5	1.99	4.75 ^{a} ±0.38	9.18^{a} ±0.82	12.97 ^{a} ±0.88
6	1.97	1.90 ^{d} ±0.18	3.62 ^{e} ±0.83	6.13 ^{bc} ±0.79
7	2.01	2.65 ^{c} ±0.31	6.25^{cd} ±0.85	8.30 ^{b} ± 1.33
8	1.98	3.84 ^{b} ±0.18	7.76 ^{abc}±2.01	12.03 ^{a} ± 2.38
9	2.00	4.75 ^{a} ±0.43	8.11^{abc} ±2.11	12.68 ^{a} ± 1.21
1 0	1.99	5.12 ^{a} ±0.53	8.84 ^{ab} ±1.70	12.52 ^{a} ± 1.18

1* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

Table 41: Fat gain dependent on test diets and age (genotype 3)

Diet	Initial fat quantity (g)	Fat gain (g)		
		28 days	56 days	70 days
1	1.3	3.40 ^{abc} ±0.40	5.93 ^{ab} ±1.15	8.26 ^{ab} ±0.75
2	1.3	3.30 ^{bc} ±0.50	6.65 ^{a} ±0.24	7.24 ^{ab} ±1.74
3	1.3	3.54 ^{abc} ±0.26	6.49 ^{ab} ±2.23	9.39 ^{a} ±1.91
4	1.3	3.15 ^{bc} ±0.36	5.75 ^{ab} ±0.24	8.31 ^{ab} ±1.80
5	1.3	2.95 ^{c} ±1.11	4.60 ^{ab} ±0.81	5.73 ^{bc} ±0.56
6	1.3	1.88 ^{d} ±0.19	3.83 ^{b} ±0.91	4.32 ^{c} ±0.90
7	1.3	2.65 ^{cd} ±0.40	6.71 ^{a} ±1.13	5.99 ^{bc} ±1.15
8	1.3	3.57 ^{abc} ±0.65	7.24 ^{a} ±2.16	8.91 ^{a} ±1.69
9	1.3	4.38 ^{a} ±0.69	6.17 ^{ab} ±2.29	7.77 ^{ab} ±1.96
10	1.3	4.20 ^{ab} ±0.45	5.82 ^{ab} ±0.65	7.63 ^{ab} ±1.32

1* Mean values in the same column having the same superscript are not significantly different ($p \leq 0.05$).

4.3.8. Calculation of growth capacity

The growth capacity was calculated as the maximum of nitrogen deposition by using model from (GEBHARDT 1966).The results show that the daily maximum N- deposition for hybrid Tilapia (O. niloticus X Red Tilapia) from 12 to 42 g body weight at day 28 of the experiment, resulted in 443 mg N/BW $_{kg}{}^{0,67}$ for fish fed on diets 1 - 5 containing 16 % to 48 % crude protein with the same energy level of 15.6 MJ ME/kg. 407 mg N/BW $_{kg}{}^{0.67}$ were found for fish fed on diets 6 – 10 containing 16 % to 48 % crude protein level with adapted energy levels of 13.6 to 17.6 MJ ME/kg. After 56 days, the hybrid of Tilapia with body weight from 12 g to 70 g, fed on diets 1-5 containing 16 % to 48 % crude protein level with the same energy level of 15.6 MJ ME/kg resulted in 233 mg N/BW $_{kg}{}^{0.67}$. 217 mg N/BW $_{kg}{}^{0.67}$ were found for fish fed on diets 6 – 10 containing 16 % to 48 % crude protein level with adapted energy levels of 13.6 to 17.6 MJ ME/kg, respectively.

At the end of the experimental period, the hybrid of Tilapia with body weight 12 g to 84 g fed on diets 1-5 containing 16 % to 48% crude protein level with the same energy level of 15.6 MJ ME/kg resulted in 250 mg N/BW $_{kg}{}^{0.67}$. 232 mg N/BW $_{kg}{}^{0.67}$ were calculated for fish fed on diets 6 – 10 containing 16 % to 48 % crude protein level with adapted energy levels of 13.6 to 17.6 MJ ME/kg, respectively. The maximum N-deposition data are presented in table 42.

In general the daily N-deposition capacity decreased with age of fish also some changes were observed corresponding to energy density the diets 6 - 10. The N-deposition curve are presented in figure 6 and figure 7, respectively.

Table 42: Calculation of maximum N-deposition capacity dependent on test diets and age (genotype 3)

Diet	28 days	56 days	70 days
Isoenergetic diets	443 mg N/BW $_{kg}{}^{0.67}$	233 mg N/BW $_{kg}{}^{0.67}$	250 mg N/BW $_{kg}{}^{0.67}$
Adapted diets	407 mg N/BW $_{kg}{}^{0.67}$	217 mg N/BW $_{kg}{}^{0.67}$	232 mg N/BW $_{kg}{}^{0.67}$

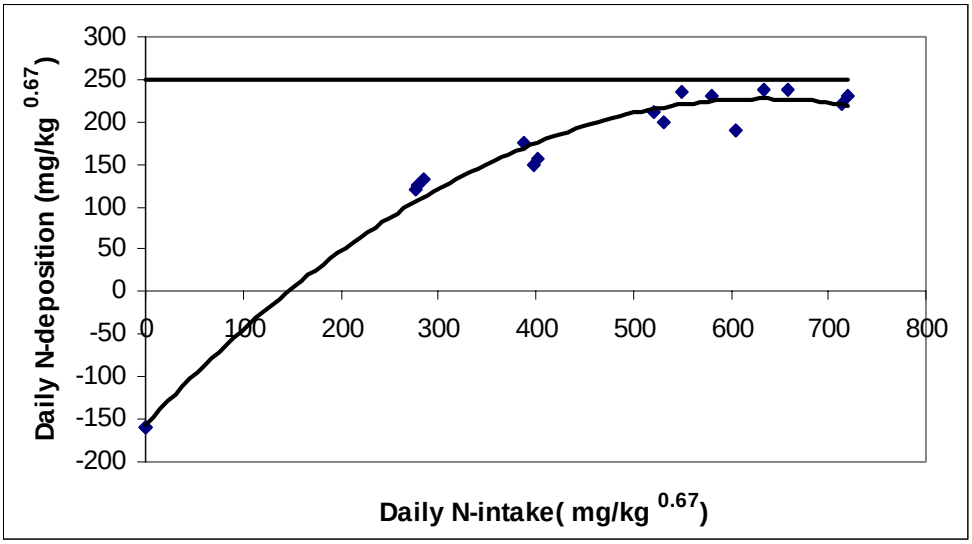

Figure 6: N-deposition curve at the end of the experiment for isoenergetic diets (genotype 3).

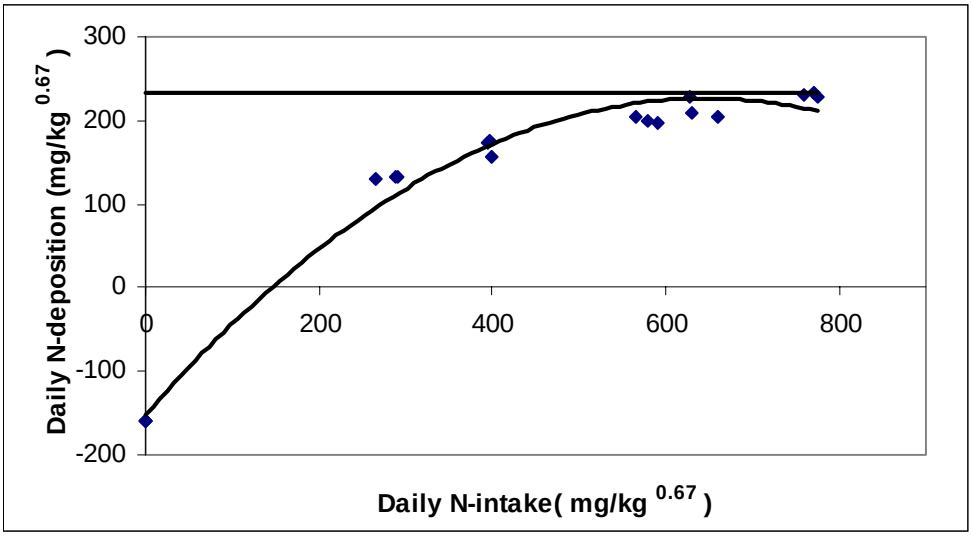

Figure 7: N-deposition curve at the end of the experiment for adapted energy diets (genotype 3).

5. DISCUSSION

The present investigations were carried out for the description of physiological response of different Tilapia genotypes to different protein supply in combination with different energy density in the diet. Based on the results of these experiments we create basic data for the use in an exponential N-utilization-model and furthermore the estimation of growth capacity of different Tilapia genotypes. The aim of the following discussion is to give a comparable and general explanation about these aspects.

5.1. The effect of dietary protein intake on growth performance

The results of the present study showed that the fish fed on all diets were growing, but in general, growth rate, weight gain and weight gain percentage were strongly related to dietary protein levels and feed intake. As given in table 13, 23 and 33, respectively, the results indicated that final body weight, weight gain and weight gain percentage increased by the increasing of dietary protein levels in the diets. The statistical analysis of the results of the experiments showed that the body weight, weight gain, weight gain percentage and also feed intake increased significantly up to 40 % crude protein with P:E ratio ranged from 22.80 to 29.10 g protein/MJ ME for genotype 1 and 32 % crude protein with P:E ratio from 19.30 to 29.10 g protein/MJ ME for genotype 2 and 3, respectively. The results underline a more pronounced sensitivity of genotype 1 corresponding to a higher protein supply mainly with diets 4, 5, 9 and 10.The daily N-deposition data also show the same pattern. The pattern of growth data is similar to those reported for some Tilapia genotypes. JAUNCEY (1982) found that the optimum protein level was 40 % for *O. mossambicus* at P:E ratio 27.9 g protein/MJ ME. MAGAUZE (1990) found that the optimum protein level required for producing maximum growth for *O.niloticus* was found to be 41 % at a P:E ratio ranged from 26.7 - 29.5 g protein/MJ ME. Also ABDELGHANY(2000) reported optimum crude protein level required for producing maximum growth for *O. niloticus* was 35 % - 40 % at a P:E ratio 13.01 g protein/MJ ME. These studies are in close agreement with that reported in our study mainly with genotype 1. However, the results of experiment 2 and 3 are also similar to those reported

by VIOLA and ZOHAR (1984) who found that optimum growth was indicated with protein level of 30 - 35 % for hybrid (*O. niloticus* x *O. aureus*). ROSS (1982) reported the same protein level for *O. mossambicus*. This pattern is different with the results, which was reported by SHIAU and HAUNG (1989) the authors found that optimum protein level was 24 % for Tilapia hybrid (*O. niloticus* x *O. aureus*) rearing in the sea water, while WANG et al (1985b) reported as optimum of protein at level 29 % and a P:E ratio of 15.4 mg protein /kJ GE. The difference between the results of the studies and those of our study may be attributed to the environmental conditions of the experiments such as temperature, salinity , size of fish, energy density of the diet or to the physiological state of fish and feed intake.

The difference between the results with different genotypes in our study may be due to the different sex ratio between male and female in tank culture, which was obtained mainly for genotype 2 and 3 after 4 weeks of the experimental period. The feed intake which was required for optimum growth was effected by the sex ratio in tank culture. DE SILVA and RADMPOLA (1990) reported that optimal protein level for growth of *O. niloticus* both male and female was 30% and the greatest percentage of female spawning occurred at 25% crude protein level.

HICKLING (1968), KUO (1969), PURGININ et al. (1975) reported that the all male Tilapia were growing faster than the other Tilapia genotype. In Tilapia low protein rations induce females to mature earlier, produce more eggs relative to their body size and spawn over a longer period of time. This in agreement with MINRONOVA (1978), who showed that reducing feed of uniform quality induced reproduction in female *T. mossambicus*. When the dietary level is optimal for growth a greater proportion of the population spawns.
The effect of dietary protein level of diets on the growth performance on different Tilapia genotype is shown in figure 8.

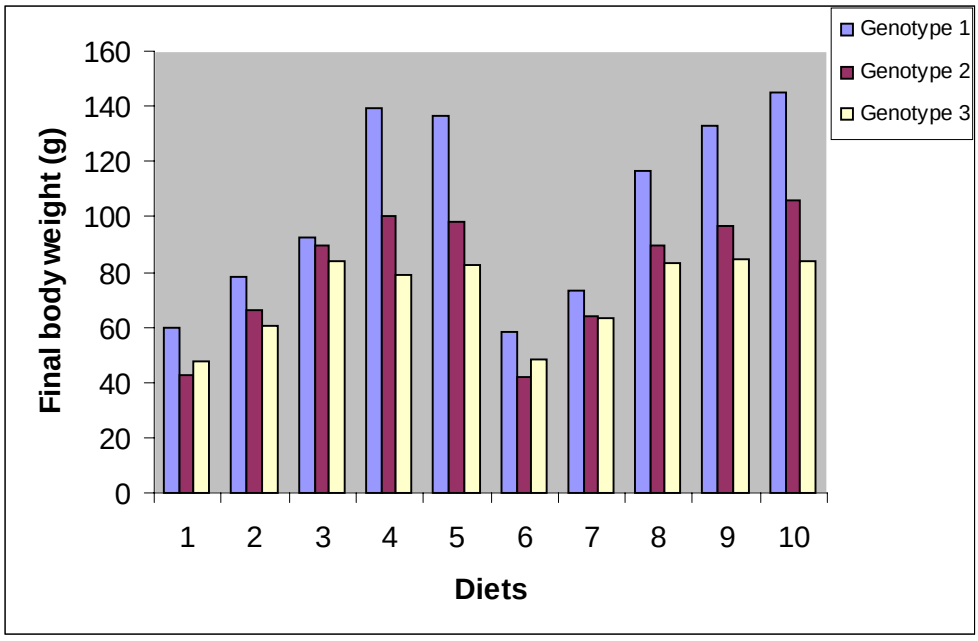

Figure 8: The effect of dietary protein level of the diets on growth performance of different Tilapia genotypes (70 days).

The present study indicated also that weight gain and specific growth rate were increased gradually with increasing dietary protein level. As given in table 14, 24 and 34 respectively, statistical analysis showed the same pattern such as weight gain. This pattern is similar to those reported for brown trout (POSTON, 1975), for rainbow trout (LEE and PUTNAM, 1973), for Yellow tail (TAKEDA et al., 1975), for penaeus merquiensis (SEDGWICK, 1979) and for Tilapia (KAUSHIK et al., 1994) where the growth increased with further increase in dietary protein level and P:E ratio above the optimum. Maintenance of growth rate or weight gain at the higher protein levels above the optimum depends to a large extent on the energy content of the diet. When energy level in the diet is enough to compensate the energy losses in catabolizing and excreting the excess of protein growth will be maintained and not decrease. Table 43 shows the N-deposition data of different Tilapia genotypes (see 5. 5.).

5.2. Effect of dietary protein and protein energy ratio on feed conversion

As given in table 15, 25 and 35 respectively, the results of feed conversion ration indicate that the quality of the diet was poor at low levels of protein and improved when the protein level was 40 % and more for genotype1.The results showed also that diet 8 supplemented with the amino acid threonine was no significant differences with diets containing 40 % and more for genotype 1. The present results of FCR of experiment 1 ranged from 1.0 to 1.18 with protein level of 40 % at P:E ratio 22.80 – 25.90 g protein/MJ ME. These results were similar and in agreement with the result reported by EL SAYED and TESIMAA (1992) who found that feed conversion ratio was 1.1 for Tilapia *O. niloticus* . ABDELGAHANY (2000) found also the same feed conversion ratio (1.11). From the other hand, the results from experiments 2 and 3 showed also positive relationship between dietary protein and feed conversion ratio. It was improved, when protein level was 32 % and more, with level of FCR ranged from 1.12 to 1.34 for experiment 2 (genotype 2) and FCR level from 1.25 to 1.65 for experiment 3 (genotype 3) . The feed conversion ratio effected linearly as protein content in the diet increased , it means that the diets were better utilized as the protein content in the diet increased. This result is in agreement with the work from SIDDIQUI et al. (1988) with *O.niloticus,* SHIAU and HAUNG (1989) with a hybrid of (*O. niloticus* x *O. aureus*). The present results from all three experiments were not significantly effected by the different energy levels used.

The difference between the results of the experiments may be also due to the changes on feed intake in the experiment 2 and 3, which were effected by the sex ratio between male and female animals in the tank culture. Figure 9 shows the effect of dietary protein level on feed conversion ratio of different Tilapia genotypes.

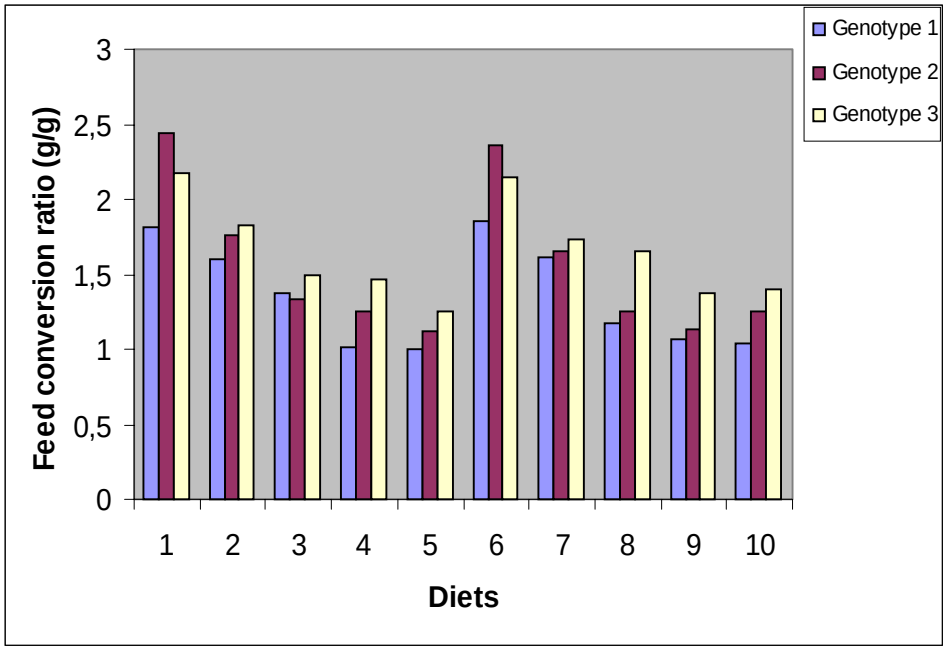

Figure 9: Relationship between dietary protein level of the diets on feed conversion ratio of different Tilapia genotypes (70 days).

5. 3. The effect of dietary protein levels on protein utilization

The parameters of PER, PPV and NPU were used to describe the protein utilization . The results of PER were presented in table 16, 26 and 36. The results of PER indicated that PER values decreased steadily with the increasing of dietary protein levels. This result indicates that maintenance requirements for dietary protein in Tilapia appear very low and Tilapia form flesh with a low protein content when fed on low protein diets. Otherwise there is a very efficient utilization of dietary protein as limited protein supply, the result of experiment 1 (genotype 1) at the end of experiment was better than the others experiment with ranged of PER between 3.62 and 2.19, 2.77 and 1.82 for experiment 2 (genotype 2),

3.06 and 1.64 for experiment 3 (genotype 3). These results were in agreement with many workers. MAZID et al.(1979) found that PER of *Tilapia zillii* decreased linearly from 3.21 to 1.29 as protein level in crease from 21.7 to 53.6 %. In addition JAUNCEY (1982) reported that PER of Tilapia mossambicus declines with increasing protein level above 16 % an P:E ratio 12.3g protein/MJ ME. ABDELGHANY (2000) reported that PER decreased linearly from 4.9 to 2.0 with increasing of dietary protein for *O.niloticus* above 16 % to 50 % an P:E ratio 13.01 g protein /MJ ME. Numerous studies have reported similar results for various fish species (OGINO and SIATO, 1970; DABROWSKI, 1977; SHIAU and HAUNG, 1989; SHYONG et al., 1998). Figure 10 shows the effect of dietary protein level of the diets on protein efficiency ratio of different Tilapia genotypes (70 days).

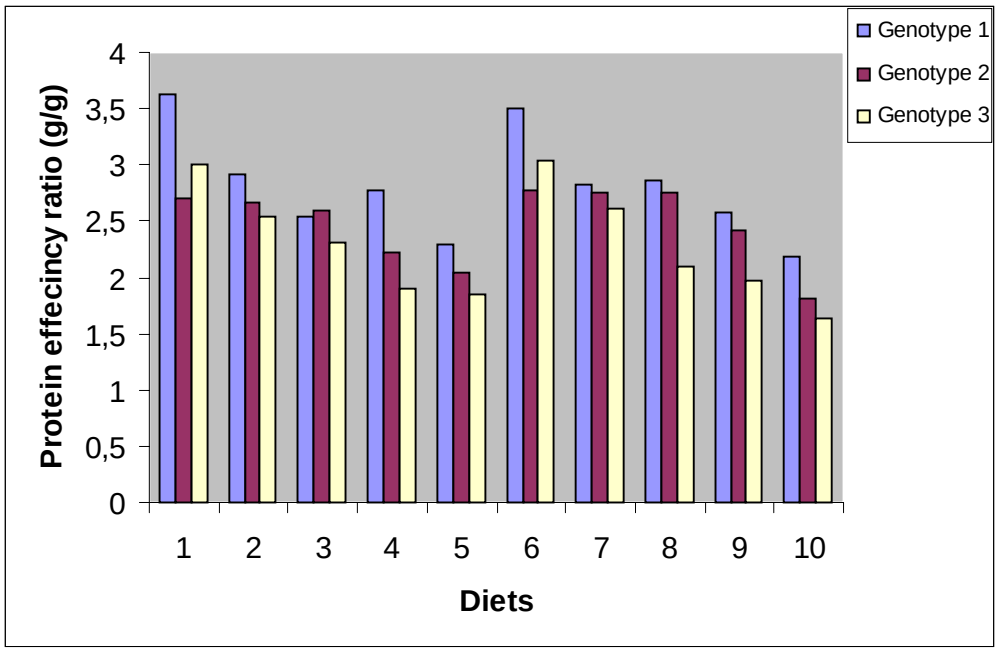

Figure 10: The effect of dietary protein level of the diets on protein efficiency ratio of different Tilapia genotypes (70 days).

The results of productive protein value (PPV) were presented in table 17, 27 and 37. The effect of dietary protein level appeared clearly when dietary protein utilization was measured in terms of PPV. PPV decreased substantially with increasing dietary protein levels, without effect of P:E ratio on PPV. PPV is a better indicator of the feed protein quality than PER. PPV ranged between 33.8 and 52.4 % for experiment 1 (genotype 1), the highest PPV at the end of

experiment 1was found in fish fed a diet containing 16 % crude protein and P:E ratio 11.60 g protein /MJ ME. The PPV ranged between 33.3 and 47.2 for experiment 2 (genotype 2), the highest PPV was found to be 47.2 % with protein level of 16 % at P:E ratio 10.30 g protein /MJ ME. The PPV ranged between 28.7 and 51.2 for experiment 3 (genotype 3), the highest PPV was found to be 51.2 % with protein level of 16 % at P:E ratio 11.60 g protein /MJ ME. These results are in general agreement with many authors. JAUNCEY (1982) reported that PPV of juvenile Tilapia decreased (49.9 % - 22.0 %) with increasing dietary protein content (8% - 56 %). MOORE et al. (1988) reported that PPV of *Acipenser transmontanus*, fed diets containing 34.0 - 52.7% protein showed a linear decrease, but the PPV of fish fed diets containing 20.0 - 43.0 % crude protein showed no significant difference. FASSBENDER (1990) found that PPV decreased from 42.6 % to 32.7 % when carp were fed on diets with increasing protein levels from 26 % to 57%. A similar result in carp was also reported by KIM et al. (1995) who showed that low dietary protein level and restrictive feeding resulted in better protein utilization. On the other hand, the statistical analysis of PPV indicated that no significant differences between groups at different levels of energy density, except diet 6 with 16 % crude protein and low fat content (genotype 3). This finding was different from results reported from ECKHARDET et al. (1982) who found that PPV was effectively improved in the diets with lower protein (28%) content and with increasing dietary fat up to 12%.

Figure 11 summarizes the effects of dietary protein level on productive protein value of different Tilapia genotypes.

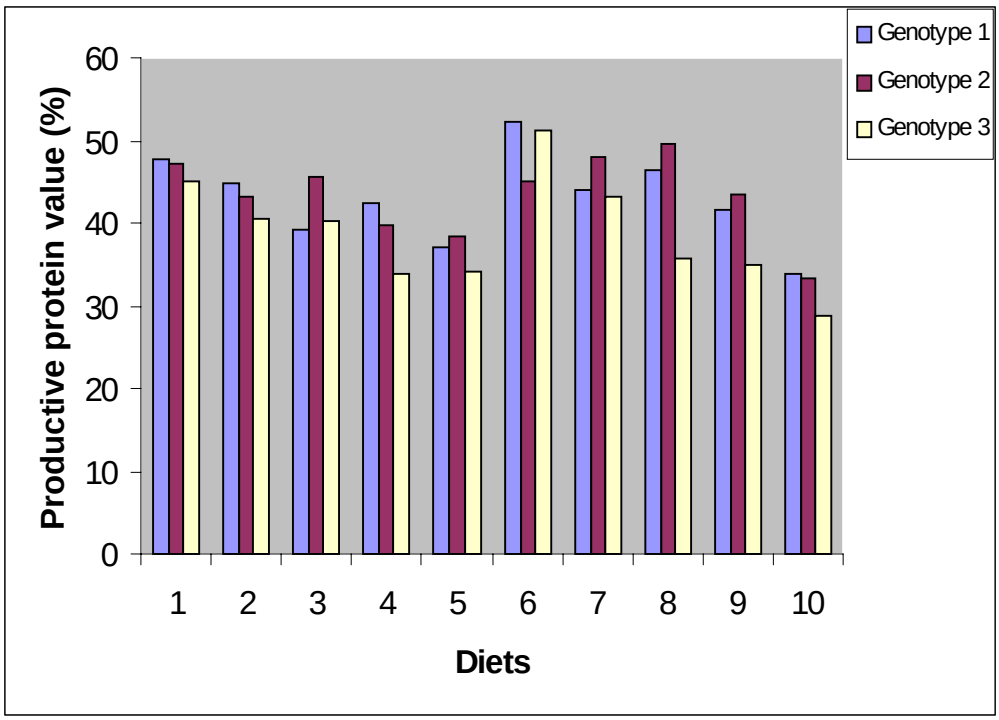

Figure 11: The effect of dietary protein level on productive protein value of different Tilapia genotypes (70 days).

The results of net protein utilization (NPU) were presented in table 18, 28 and 38. These data indicated that as the proportions of dietary protein increases, the value of NPU falls. These finding are in agreement with those reported for carp by OGINO et al. (1976) and TAKEUCHI (1979). At low dietary protein levels, the amino acid composition and total quantity of the protein is the limiting factor and maximal NPU are obtained. At a higher level of protein supply, a greater proportion of the protein is utilized as energy . However, high dietary protein levels are necessary to grow reasonably fast. This results are also in agreement with those reported by SHIAU and HUANG (1989) who reported that NPU of hybrid Tilapia reached maximum (36.05 %) when fish fed a diet containing 24 % protein , increasing dietary protein level up to 56 % caused a linear reduction of NPU. ABDELGAHNY (2000) reported also that NPU was 54.4% at a protein level of 15 % and decreased linearly with increasing protein level. In our experiments the results tended in the same, more than thus no statistically

significant effects on NPU were found between the different energy levels used. Figure 12 shows the effect of dietary protein level on net protein utilization of different Tilapia genotypes (70 days).

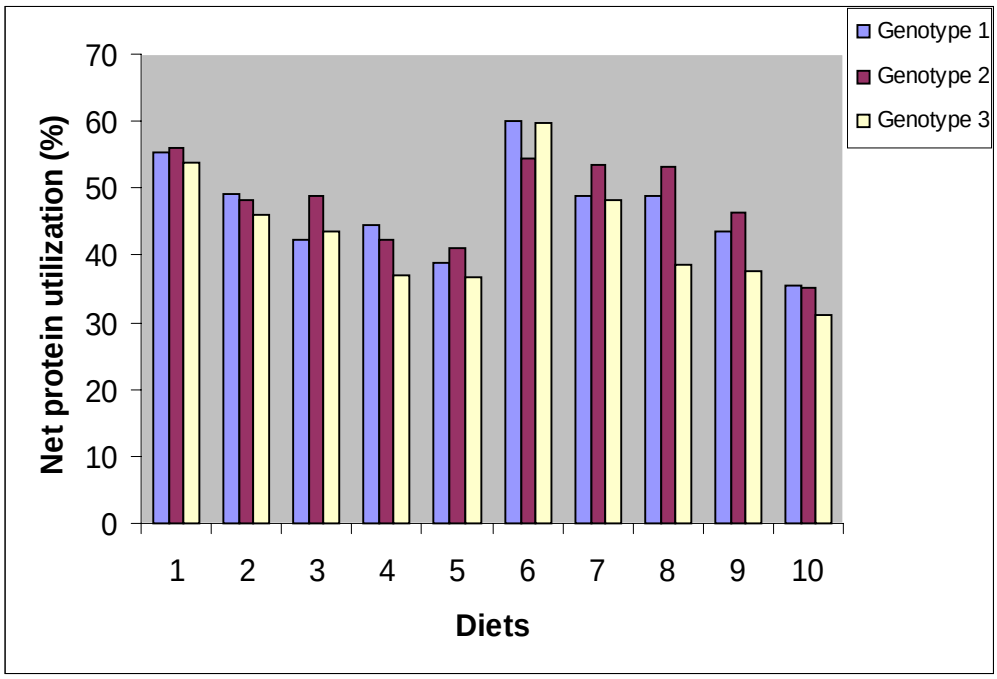

Figure 12: The effect of dietary protein level on net protein utilization of of different Tilapia genotypes (70 days).

5.4. The effect of dietary protein level on body composition and nutrient deposition

The results of the effect of body composition were presented in table 19, 29 and 39. These results indicated no relationship between dietary protein content and body protein content, mainly with experiment 1 (genotype 1). This results are in agreement with many authors. TAKEUCHI et al. (1979) found no effect of dietary protein level on protein content of fish body . Other workers observed no clear relationship between dietary protein level and body protein content (WINFREE and STICKNEY, 1981; TAKEDA et al., 1975). In contrast, JAUNCEY (1982), ATACK et al. (1979), SHIAU and HUANG (1989), MAGOUZ (1990) and ABDELGAHNY (2000) indicated that the increasing of dietary level at each energy level

resulted in increasing body protein content. This findings are in close agreement with result of experiment 2 and 3 (genotype 2,3) respectively, which reported also the same trend. The body fat content was found to be inversely related to body protein content, mainly with isoenergatic diets and no clear trend was observed with the diets adapted in energy, may be due to the energy levels balanced with the protein levels in the diets. This relationship was established by several studies in different fish species, in rainbow trout (LEE and PUTNAM, 1973), in channel catfish (GARLING and WILSON, 1976). The crude ash content at the end of experiments were significantly effected by test diets and feed intake. On the other hand the data of protein gain and fat gain were presented in table 20,21 for (experiment 1), table 30,31 (experiment 2) and table 40,41(experiment 3) . The results reported that the protein and fat gain increased steadily with increasing of protein content and energy content in the test diets. This results are in agreement with work from MAGOUZ (1990) and ABDELGAHNY (2000). High fat gain was observed by experiments 2 and 3 respectively. To explain this results OSMAN (1988) and MAGOUZ (1990) indicated that Tilapia have the ability to reserve the excess of dietary energy in the inedible part of the body . This may be desirable for the consumer. They also reported from the analysis of fish body fractions that, the viscera contain 42 - 77 % fat (OSMAN 1988) and 18 - 43 % fat (MAGOUZ 1990) respectively. Carp can also deposit high quantities of fat not only in viscera but also in the carcass. In our study, the whole body of fish was used to determine the body composition.

5.5. The effect of genotype on maximum N-deposition capacity

The protein deposition capacity was calculated as the upper theoretical limit for nitrogen deposition of each genotype. The results of realised N- deposition were presented in table 43 and the maximum daily N-retention capacity in table 44. These results indicated that N-deposition was increased significantly up to 40% crude protein in the diets for experiment 1 (genotype 1) and up to 32% for experiments 2, 3 (genotype 2, 3) respectively, after application of the isoenergetic diets (1- 5). The adapted energy density did not effect the course and level of N-deposition capacity. However, the results show a more pronounced sensitivity of different genotypes corresponding to a higher level of protein supply, mainly with diets 4, 5, 9 and 10 containing 40 to 48 % crude protein for genotype 1 and up to 32 %

for genotype 2 and 3. The results of daily N-deposition capacity indicate some differences between the genotypes under study. However the results were not clear enough for final calculations relating to the different genotypes. Furthermore it is possible to assume that these difference between the genotypes may have consequences on amino acid requirements. It is known that male fish of *O.niloticus* grow faster than other Tilapia hybrid. This observation is in agreement with HICKLING (1968), KUO (1969) and PURGININ et al. (1975) who reported that male Tilapia growing faster than the other Tilapia genotype. Therefore, the protein and amino acid supply must be related to cover the requirement of each different genotype to improve the N-deposition.

Table 43: N-deposition data of different Tilapia genotypes dependent on test diets (70 days) and percentage of N-deposition capacity

Diets	CP(%)	N-deposition (mg/d)					
		Genotype 1		Genotype 2		Genotype 3	
1	16	14^{a}	[42]	12^{a}	[43]	12^{c}	[49]
2	24	23^{c}	[60]	20^{a}	[57]	18^{bc}	[65]
3	32	28^{c}	[68]	31^{b}	[74]	28^{a}	[83]
4	40	45^{ed}	[83]	35^{b}	[78]	27^{a}	[83]
5	48	46^{e}	[86]	37^{b}	[84]	30^{a}	[90]
6	16	16^{ab}	[49]	11^{a}	[40]	13^{bc}	[53]
7	24	22^{b}	[59]	20^{a}	[58]	19^{b}	[66]
8	32	38^{d}	[77]	32^{b}	[77]	28^{a}	[84]
9	40	44^{ed}	[83]	34^{b}	[78]	29^{a}	[85]
10	48	47^{e}	[85]	39^{b}	[85]	29^{a}	[85]

* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$)

[ ] % of N-deposition capacity

Table 44: Maximum daily N-retention capacity dependent on test diets of different Tilapia genotype (70 days)

Diets	Genotype 1	Genotype 2	Genotype 3
Isoenergetic diets	452 mg $N/BW_{kg}^{0.67}$	460 mg $N/BW_{kg}^{0.67}$	410 mg $N/BW_{kg}^{0.67}$
Adapted diets	465 mg $N/BW_{kg}^{0.67}$	449 mg $N/BW_{kg}^{0.67}$	392 mg $N/BW_{kg}^{0.67}$

5.6. Calculation of threonine efficiency and threonine requirement

The estimation of N-retention capacity gives results which are higher than real N-retention under in vivo conditions. So we prefer the term "theoretically maximum of N-retention capacity", indicating that this value is really an upper limit for this parameter.

In spite of this fact, these A-values or corresponding C-values are generally useful as the "genotype-factor" within our physiological based N-utilization model $y = \mathbf{A (1 - e^{-bx})}$ - **NMR** (GEBHARDT, 1966) (see 3.5). These values can be identified and used for further calculations within the model, mainly for evaluation of amino acid efficiency and amino acid requirements. The efficiency threonine from experiment 1 (genotype 1)will be used as example for requirement calculation. Based on LIEBERT and GEBHARDT (1988) the requirement of limiting amino acid is predictable as follows:

$$x_{Thr} = [\ln A - \ln (A-y)] : (16 : bc^{-1})$$

Where

x_{Thr} = Thr- requirement for defined percentage of N-retention capacity (mg $N/BW_{kg}^{0.67}$)

A= N-retention capacity (460 mg $N/BW_{kg}^{0.67}$/d)

C= N-deposition capacity (300 mg $N/BW_{kg}^{0.67}$ /d)

bc^{-1}= efficiency of limiting amino acid threonine (0.000874)

The efficiency of threonine (bc^{-1}) was calculated based on experimental results over 70 days after application of diet 3 with threonine as limiting amino acid (c_{Thr} = 3.54 g/16g N).

Threonine requirement (x_{Thr}) was calculated for 80% of N-retention capacity:

x_{Thr} = 119 mg / $BW_{kg}^{0.67}$/d

Assuming a mean body weight (BW = 60 g) the threonine requirement can be expressed in (mg /d):

x_{Thr} = 18 mg /d.

Furthermore assuming feed intakes of 5% and 3% of the body weight , threonine requirement can be expressed per kg of feed:

- Feed intake 5%→ 6g Thr /kg feed
- Feed intake 3%→ 10g Thr / kg feed

. This result is in agreement with requirement of Tilapia after NRC (1993).

6. CONCLUSIONS

According to our findings the proper balance between protein and energy in the diet will occur at a protein/energy ratio that ranged between 22.90 and 29.10 g protein/MJ ME . The optimum protein level for production of maximum growth was 40% for the first genotype and reduced to 32 % for genotype 2 and 3 respectively, with P:E ratio ranged between 19.30 g protein /MJ ME and 29.10 g protein / MJ ME. These results are important with respect to the new interests in Tilapia culture and the fact that nutrient requirements are not exactly determined. One of the most important aspect of this study is that the physiological response of different Tilapia genotypes with the calculation of daily N-deposition capacity for Tilapia genotypes indicates a higher capacity of protein deposition for genotype 1 and genotype 2 (300 mg $N/BW_{kg}^{0.67}$) and lower capacity of protein deposition for genotype 3 (250 mg $N/BW_{kg}^{0.67}$).These model calculations are very important for the evaluation of amino acid efficiency and amino acid requirements. These findings are equally important for either the developing countries or the third world.

To understand their importance we will take Egypt as an example of the third world. Egypt is an agricultural land. The consumption of animal products per person lies under the world rate. This is as a result of many factors that could affect the animal production in Egypt.

The success of intensive Tilapia production in Egypt depends mainly on two factors, the selection of fast growing fish genotypes and the estimation of the requirements of these genotypes . The second problem is feed supplementation. The current shortage in the protein supply for both terrestrial animals and fish is in direct relation to the fact that protein is the most expensive nutrient in the feed. It is necessary to know the exact protein level and the amino acid requirements for the optimum utilization of protein. This will of course help in formulating well balanced mixed diets for economic feeding of Tilapia.

Further investigation are required:

1- To prove these results by using practical diets.
2- Estimation of the N-deposition capacity of female Tilapia in comparison to male Tilapia.
3- Estimation of N-deposition capacity at different fish density.

7. SUMMARY

The present study was carried out in three experiments with three different genotype of Tilapia. The first genotype was pure male *Oreochromis niloticus*, the second was red Tilapia *Oreochromis mosambicus* x *Oreochromis hornourm* and the third genotype was hybrid *O. niloticus* x (*O. mosambicus* x *O. hornourm*). All of three experiments were conducted to determine the growth capacity and maximum N-retention of Tilapia, family Chialiade, also to find out the physiological response of different Tilapia genotypes to different protein supply in combination with different energy density. To reach this purpose 10 semi purified diets were formulated with fish meal and wheat gluten (3 : 1 constant ratio) for a N-rise experiment combining 5 crude protein levels ranging from 16% - 48 % crude protein. The semi purified diets 1 to 5 were isoenergetic with an energy level of 15.6 MJ ME/kg and protein energy ratio ranged between 10.30 and 29.10 g protein /MJ ME. The semi purified diets 6 to 10 were containing 16 to 48 % crude protein level with an adapted energy level of 13.60 to 17.60 ME MJ/kg and protein energy ratio ranged from 11.60 to 25.90 g protein /MJ ME. Threonine was calculated to be the first limiting amino acid (except diet 8 with threonine supplementation). Each experiment conducted 10 weeks .The initial average body weight of fish in each experiment was 12.3±0.1g , 12.4±0.1g and 12.3±0.1g for genotype 1, genotype 2 and genotype 3, respectively. The fish were stocked in each experiment at 25 fish per tank in three replicates in a recirculating filtered rearing system at 27 - 28 ºC. The fish were fed semi ad libitum by the hand ranging between 7 % of body weight and 4 % at the end of each experiment. At the end of each experimental period a total number of 90 fish was analyzed for body composition. The effect of dietary treatments should be evaluated based on growth rate, body composition, nutrient deposition, feed and nutrient utilization. Furthermore, the results of protein deposition were used for estimations of protein deposition capacity based on an exponential N-utilization model for growing animal (GEBHARDT , 1966).

The results of these experiments can be summarised as follows :

1. Genotype 1(*O.niloticus*) was growing faster than the other Tilapia genotypes.
2. The optimum protein level required for producing maximum growth for male *O. niloticus* was found to be 40% at a P:E ratio ranged from 22.80 – 23.90 g protein /MJ ME.
3. The optimum protein level required for producing maximum growth for red Tilapia (genotype 2) and hybrid between *O.niloticus* and red Tilapia (genotype 3) were found to be 32 % at P:E ratio 19.30 g protein /MJ ME.

4. The calculation of maximum daily N-deposition was 300 mg N/$BW_{kg}^{0.67}$ for *O.niloticus* and red Tilapia and 250 mg N/ $BW_{kg}^{0.67}$ for the hybrid (genotype 3) respectively.
5. The calculation of threonine requirement for *O. niloticus* with an average of 60 g body weight 6g threonine / kg feed assuming a feed intakes of 5 % of body weight.
6. The results indicate that the N-utilization model used is also appropriate tool for description of growth processes in fish.

8. APPENDIX

Table A1:Composition and proximate analysis of the standard diet fed to the fish as starter feed

Composition	g/kg
Fish meal	500
Wheat	420
Sunflower	40
Mineral mixture	20
Vitamin mixture	20
Analysis*	
Dry matter(%)	89.4
Crude protein	39.1
Ether extract	10.5
Ash	12.4
Crude fiber	1.2
NFE	36.8
Gross energy (kJ/g DM)	19.7

* according to MAGOUZ (1990)

Table A2: Physic-chemical characters of water of different experiments

Experiment	Temperature C°	pH	NH_4 ppm	NO_2 ppm	NO_3 ppm
1	27.5	7.1	0.246	0.168	25.5
2	28.1	7.3	0.030	0.133	30.3
3	27.8	7.5	0.090	0.150	27.5

*value given as an average for total experimental period

Table A3: Whole body composition of fish at the start and after 4 weeks of experiment 1(genotype 1)

Diet	Dry matter (%)	Crude protein (%)	Ether extract *[1] (%)	Ash (%)
Initial	23.8	15.3	3.8	4.6
1	28.2 [a] ±3.4	15.0 [ab] ±0.9	8.8[a] ±1.8	4.4 [a] ±1.1
2	26.5[ab] ±0.6	14.4 [bc] ±0.4	7.9 [ab] ±0.3	4.1 [a] ±0.4
3	25.4 [abc]±1.8	14.7 [abc] ±0.9	6.6 [bc] ±0.5	4.1 [a] ±0.3
4	23.5 [bc] ±1.2	14.4 [bc] ±0.4	5.3 [cd] ±0.1	3.7 [a] ±1.1
5	24.1 [bc] ±1.2	15.9[a] ±0.7	4.7 [c] ±0.2	3.6 [a] ±0.7
6	23.1 [c] ±0.9	13.4 [c]±0.9	5.3 [cd] ±1.0	4.3 [a] ±0.4
7	24.6 [bc] ±0.9	14.3 [bc]±0.8	6.3 [cd] ±0.3	4.1 [a] ±0.2
8	25.2 [bc] ±0.6	15.1 [ab] ±0.3	6.3 [c] ±0.7	3.7 [a] ±0.3
9	25.4 [abc] ±0.1	15.6 [ab] ±0.3	6.1 [cd] ±0.1	3.7 [a] ±0.3
10	24.6 [bc] ±1.7	15.5 [ab] ±0.2	5.4 [cd] ±1.1	3.7 [a] ±0.4

1* Calculated as (fat + NFE)

2* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

Table A4: Whole body composition of fish at the start and after 8 weeks of experiment 1 (genotype 1)

Diet	Dry matter (%)	Crude protein (%)	Ether extract *[1] (%)	Ash (%)
Initial	23.8	15.3	3.8	4.6
1	29.1 [a] ±2.8	14.9 [b] ±0.7	9.8 [a] ±3.2	4.3 [ab] ±0.2
2	27.9 [a] ±0.5	15.3 [ab] ±0.3	8.6 [ab] ±0.3	4.0 [ab] ±0.3
3	27.0 [ab] ±1.2	16.2 [ab] ±0.3	6.7 [bc] ±0.8	4.0 [ab] ±0.5
4	27.5 [ab] ±0.4	16.3 [ab]±0.5	7.4 [abc] ±0.6	3.7 [b] ±0.3
5	24.7 [b] ±2.9	15.9 [ab] ±1.7	4.9 [c] ±1.5	3.9 [b] ±0.5
6	27.8 [a] ±1.2	15.4[ab] ±0.4	7.5 [abc] ±1.1	4.8 [a] ±0.4
7	26.5 [ab] ±0.4	15.3 [ab] ±0.5	6.9 [bc] ±0.4	4.2 [ab] ±0.2
8	26.8 [ab] ±1.1	15.3 [ab] ±1.1	7.5 [abc] ±1.0	4.0 [ab] ±0.5
9	27.3 [ab] ±1.0	16.2 [ab] ±0.2	7.3 [abc] ±1.3	3.9 [b] ±0.4
10	28.3 [a] ±0.8	16.5[a] ±0.5	7.8 [ab] ±1.2	3.9 [b] ±0.6

1* Calculated as (fat + NFE)

2* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

Table A5: Whole body composition of fish at the start and after 4 weeks of experiment 2 (genotype 2)

Diet	Dry matter (%)	Crude protein (%)	Ether extract *[1] (%)	Ash (%)
Initial	28.8	16.3	8.1	4.4
1	32.6 [a] ±1.5	13.7 [b] ±0.9	14.6[ab] ±1.4	4.3 [a] ±0.3
2	33.6 [a] ±1.9	14.6 [ab]±0.5	14.8 [a] ±1.3	4.2 [a] ±0.3
3	32.7 [a] ±32.7	15.4 [ab] ±0.5	13.3 [abc] ±1.5	4.0[a] ±0.2
4	30.9 [a] ±1.7	15.8 [a]±0.4	11.1 [bc] ±1.3	4.0 [a] ±0.1
5	29.9 [a] ±3.7	15.9 [a] ±1.6	10.2 [c] ±1.9	3.8 [b] ±0.3
6	30.7 [a] ±2.5	14.3 [ab]±0.7	11.8 [abc] ±1.9	4.6 [a] ±0.1
7	32.5 [a] ±0.8	15.5 [ab]±1.3	12.4 [abc] ±0.6	4.5 [a] ±0.1
8	31.5 [a] ±1.5	15.2 [ab] ±1.3	12.1 [abc] ±0.7	4.1 [a] ±0.2
9	32.4 [a] ±3.1	15.7[a] ±0.7	12.2 [abc] ±3.8	4.4 [a] ±0.6
10	32.8[a] ±2.6	16.0[a] ±0.8	12.4 [abc] ±1.5	4.3 [a] ±0.5

1* Calculated as (fat + NFE)

2* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

Table A6: Whole body composition of fish at the start and after 8 weeks of experiment 2 (genotype 2)

Diet	Dry matter (%)	Crude protein (%)	Ether extract *[1] (%)	Ash (%)
Initial	28.8	16.3	8.1	4.4
1	36.6 [a] ±0.9	14.4 [d] ±0.3	17.7[a] ±0.4	4.5 [a] ±0.4
2	36.3 [a] ±0.9	15.4 [cd] ±0.2	16.5 [ab] ±0.8	4.3 [a] ±0.3
3	35.3 [ab] ±0.5	16.7[ab] ±0.7	14.3 [bcde] ±0.6	4.3 [a] ±0.0
4	33.9 [abc] ±2.1	17.3 [a]±0.7	12.0 [fe] ±1.4	4.5 [a] ±0.2
5	31.8 [c] ±2.5	16.5[abc] ±0.9	10.7 [f] ±1.8	4.5 [a] ±0.1
6	33.0 [bc] ±2.6	15.8 [bc]±0.7	12.6 [edf] ±1.9	4.5 [a] ±0.1
7	35.3 [ab] ±0.4	16.1 [bc]±0.2	14.6 [bcd] ±0.5	4.6 [a] ±0.3
8	36.3 [a] ±1.4	17.3 [a] ±0.4	14.6 [bcd] ±1.6	4.3 [a] ±0.1
9	34.4 [abc] ±1.2	16.4 [abc]±0.0	13.7 [bcde] ±0.9	4.2 [a] ±0.4
10	36.2 [a] ±1.5	16.8 [ab]±1.2	15.4 [bc] ±1.5	4.0 [a] ±0.5

1* Calculated as (fat + NFE)

2* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

Table A7: Whole body composition of fish at the start and after 4 weeks of experiment 3 (genotype 3)

Diet	Dry matter (%)	Crude protein (%)	Ether extract *[1] (%)	Ash (%)
Initial	31.8	16.6	10.9	4.3
1	37.5 [a] ±0.3	14.4 [d] ±1.1	18.6[a] ±1.5	4.5 [d] ±0.4
2	36.1 [ab] ±1.3	15.5 [bcd] ±0.4	15.9 [b] ±1.5	4.7 [ab] ±0.2
3	33.6 [cd] ±1.1	15.8[abc] ±0.5	13.6[bc] ±0.8	4.3 [bcd] ±0.0
4	32.9 [d] ±0.5	16.9 [a]±0.3	11.6 [cd] ±0.6	4.3 [bcd] ±0.2
5	30.4 [e] ±1.7	16.1[ab] ±0.7	10.3 [d] ±2.4	4.1 [d] ±0.0
6	33.2 [cd] ±0.8	14.9 [cd]±1.1	13.5 [bc] ±0.7	4.8 [a] ±0.0
7	34.2 [bcd] ±0.4	15.9 [abc]±0.3	13.6 [bc] ±0.5	4.7 [ab] ±0.0
8	35.1 [bc] ±1.4	16.5 [ab] ±0.4	13.9 [bc] ±1.6	4.6 [ab] ±0.4
9	34.9 [bc] ±0.7	16.5 [ab]±0.0	14.0 [bc] ±0.9	4.3 [abcd] ±0.0
10	34.1 [cd] ±0.5	16.8 [a]±0.4	13.1 [bc] ±0.6	4.2 [cd] ±0.2

1* Calculated as (fat + NFE)

2* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

Table A8: Whole body composition of fish at the start and after 8 weeks of experiment 3 (genotype 3)

Diet	Dry matter (%)	Crude protein (%)	Ether extract *[1] (%)	Ash (%)
Initial	31.8	16.6	10.9	4.3
1	35.5 [a] ±1.9	13.4 [ab] ±0.1	18.0[a] ±1.9	4.1 [a] ±0.0
2	32.8 [a] ±2.6	13.6 [ab] ±0.7	15.5 [ab] ±1.3	3.7 [a] ±0.6
3	27.9 [a] ±3.1	12.9 [b] ±2.1	11.6 [cde] ±4.3	3.3 [a] ±0.8
4	28.9 [a] ±3.8	14.8 [ab]±1.8	10.6 [de] ±1.1	3.5 [a] ±0.9
5	29.1 [a] ±1.9	16.2 [ab] ±1.2	8.6 [e] ±1.1	4.3 [a] ±0.1
6	31.1 [a] ±5.1	13.9 [ab]±1.8	12.7 [bcd] ±1.8	4.3 [a] ±1.6
7	35.4 [a] ±1.5	15.7 [ab] ±0.3	15.3 [abc] ±0.9	4.4 [a] ±0.3
8	32.2 [a] ±4.4	15.1 [ab] ±2.4	13.1 [bcd] ±1.7	3.9 [a] ±1.3
9	29.1 [a] ±4.4	14.1 [ab]±1.9	10.4 [de] ±2.4	3.9 [a] ±0.5
10	32.4 [a] ±2.3	15.3 [ab]±1.3	10.1 [de] ±0.4	3.6 [a] ±0.9

1*Calculated as (fat + NFE)

2* Mean values in the same column with the same superscript are not significantly different ($p \leq 0.05$).

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