EFFECT OF ENDOGENOUS FACTORS ON PROXIMATE COMPOSITION OF NILE TILAPIA (*Oreochromis niloticus* L.) FILLET FROM LAKE ZEWAY

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Abstract

Fish body composition is affected by exogenous and endogenous factors. The exogenous factors would seem to have very limited effects. Endogenous factors govern the majority of principles that determines the body composition of fish. This study was aimed to investigate the influence of age and sex on proximate composition of Nile Tilapia *(Oreochromis niloticus)* fillet. Specimens were collected from Lake Zeway during January 2011. Samples were cool transported to Food Science and Nutrition laboratory of Addis Ababa University. Proximate composition was determined following the procedure of AOAC. Data was analyzed using two way ANOVA. The moisture and fat contents increased from 79.5 to 80.8 and 0.4 to 0.7 % respectively, with increase in age of fish from 4 to 5 years. Conversely, the contents of crude protein, ash and gross energy decreased from 15.8 to 13.3, 1.2 to 1.0 % and 65 to 55.9 kcal/100 g respectively with increase in age of fish from 4 to 5 years. Sex has no significant effect ($p>0.05$) on proximate composition.

Key words: Age, Sex, Nile Tilapia, Proximate composition

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1. Introduction

Fish fillet consists of several components, such as moisture, protein, lipids, vitamins and minerals, all of which contribute to the overall meat composition. Fish body composition is affected by both exogenous and endogenous factors (Huss, 1995). Exogenous factors that affect fish body composition include the diet of the fish (composition, frequency) and the environment in which it is found (salinity, temperature). The main exogenous factor affecting proximate composition is diet. Various studies have examined the effects of temperature, light, salinity, pH and oxygen concentration on the proximate composition of fish but these factors would seem to have very limited effects. On the other hand, endogenous factors are genetic and linked to the life stage, age, size, sex and anatomical position in the fish (Huss, 1995). These endogenous factors govern the majority of principles that determines the composition of fish (Huss, 1995). Proximate composition of body muscles of *Puntius stigma* (male and female) analyzed shows that the moisture content was found to be higher in female, while protein, fat, ash, carbohydrate and minerals contents were higher in male. Moreover, different sexes were observed to have varying chemical composition (Biro *et al*., 2009). Nile Tilapia exhibits sexual dimorphic growth where males grow significantly faster, larger and more uniform in size than females. Males and females had significantly different final weights owing to supplementations of three different oils (Biro *et al*., 2009). There are, therefore, a number of variables that can affect the overall chemical composition of fish meat. Nonetheless, there is little information on the effects of sex and size (age) on the individual chemical components of Nile Tilapia meat. To determine the overall chemical composition of the fish meat, all the aforementioned factors of variations need to be investigated. Therefore, in view of this fact, the present research was carried out to determine proximate composition Nile Tilapia fillet collected from lake Zeway, Ethiopia.

2. Materials and methods

2.1. *Description of the study area*

Lake Zeway is the most northerly rift valley lakes of Ethiopia. The lake is located to 7^o 52' to 8^o 8' N latitude and 38^o 40' to 38^o56' E longitude. It lies between East Shoa and Arsi Administrative zones of Oromia Region about 160 km due South of Addis Ababa, on the left side of Addis-Awassa highway. The altitude of Lake Zeway is 1636 meter above sea level. It is the shallowest of the rift valley lakes with maximum and mean depth 8.95 m and 2.5 m respectively. The lake has an open water area of 434 km^2 and shore line length of 137 km. It has a maximum length of 32 km and maximum width of 20 km. The lake contains five main Islands (Tullu guddo (4.8 km^2), Tsedecha (2.1 km^2), Debresina (0.3 km^2), Funduro (0.4 km^2) and Gelila (0.2 km^2). The lake is fed by the two major rivers i.e. Meki and Katar and has one outflow in the south, the Bulbula river which flows into lake Abiyata. The lake is endowed with different kind of fish species like Nile Tilapia (*Oreochromis niloticus*), African Cat fish (*Clarias gariepinus*), Crucian carp (*Carassus carrassus*), *Labeobarbus intermidus*, *Barbus paludinosis*, *Tilapia zillii* and *Gara* species.

2.2 Sample collection and preparation

Fresh fish was purchased from local fishermen at Bochessa, Korokonch and Menefesha landing sites of Lake Zeway. Live fish was transported to Zeway Fisheries Resources Research Center laboratory layered with flaked ice using ice box. Sex was identified by examining genital papilla located immediately behind the anus. In males the genital papilla has only one opening (the urinary pore of the ureter) through which both milt and urine pass. In females the eggs exit through a separate oviduct and only urine passes through the urinary pore. Immediately after sex identification, length of fish was measured to the nearest 0.1 cm and converted to age using Von Bertalanffy Growth Function.

$$L_t=L_\infty [1-e^{-k\,(t-to)}]$$

In the above Von Bertalanffy Growth Function equation constant parameters like L_∞, K and t_o for female are 30.19cm, 0.25 per year and – 0.27 year respectively and for male are 30.81 cm, 0.27 per year and – 0.12 year respectively have been established. To determine age, 19.08 cm and 22.10 cm for female 20.6 cm and 23 cm length for male were selected and inserted into the above Von Bertalanffy Growth Function. After sexes have identified and age was determined, composite of fish sample from the same age and sex was cleaned, descaled, eviscerated and filleted manually using sterile plastic knife. The fillet was oven dried at 60 ^{0}c for 72 hours then transferred into desiccators and cooled for 30 minutes. Dried fillet was grounded to 0.3 mm size using laboratory mill and the powder was stored in desiccators for further proximate composition analysis.

2.3 Proximate composition analysis

2.3.1Moisture content

Moisture content was determined by Oven drying method following the procedure of AOAC (1998). Empty dishes were dried using air drying oven for 1 hour at 105^0C, transferred to the desiccators (with granular silica gel), cooled for 30 minutes, and were weighed. Replicates of the minced samples were mixed thoroughly and five gram of composite fresh fillet was transferred to the dried and weighed dishes. The dishes and their contents were placed in the drying oven and dried for 3 hrs at 105^0C in an oven until constant weights were obtained, and then the dishes and their contents were cooled in desiccators to room temperature and reweighed. The moisture content was determined by measuring the weight of a sample before and after the water was removed by evaporation:

$$\text{Moisture content} = \frac{\text{Weight of wet sample- Weight of dried sample}}{\text{Weight of wet sample}} \times 100$$

2.3.2 Crude protein

Crude protein in the sample fish fillets were quantified method following the procedure of AOAC (1998) by Kjeldahl methods. 0.5 g of powdered fish fillet was weighed into Kjeldahl digestion flask and then digested by heating at 370 ^{0}C for four hours in the presence of 6 mL Sulfuric acid, 3.5 mL H_2O_2, 3 g of catalyst Copper Sulfate ($CuSO_4$) and Potassium sulfate (K_2SO_4). After digestion was completed, formed clear solution was cooled for 30 minutes and neutralized by addition of 25 mL NaOH (40 %) and diluted using 25mL distilled water. 25 mL of distilled water, 25 mL of Boric acid and 3 drops of Methyl blue was added to receiving flask 250 mL capacity connected to the distiller by tube. The distillation process was terminated when the volume of receiving flask reached between 200 to 250 mL. Note: all reagents were added to the blank except the sample**.** The nitrogen content was estimated by titration of the borate anion formed with 0.1N HCl. The amount of Nitrogen was calculated using the formula:

$$\% \text{ N} = \frac{\text{N HCl x (Volume of HCl titrates sample- Volume of HCl titrates blank)}}{\text{Gram of sample}} \times \frac{14\text{g}}{\text{mole}} \times 100$$

$$\text{Crude protein} = 6.25 \times \text{N}$$

2.3.3 Crude fat

Crude fat was determined method following the procedure of AOAC (1998) by semi continuous solvent extraction methods (Soxhlet method). Accordingly, for all sample categories, 2 g of dried and ground sample was placed in a porous cellulose extraction thimble and thimble was covered with fat free cotton. The thimble was placed in an extraction chamber which is suspended above a flask containing the solvent (50 mL of diethyl ether) and below a condenser. The flask which had dried in drying oven at 105^{0}C containing boiling chips was placed inside the extraction chamber and heated at 55^{0}C and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. At the end of the extraction process, which typically lasts for 3 hours, the flask containing the solvent and lipid was removed, the solvent was evaporated in drying oven at 70 ^{0}C and the mass of lipid remaining was quantified gravimetrically and calculated from the difference in weight of the extraction flask before and after extraction as percentage. The crude fat in the initial sample was calculated as:

$$\text{Fat content} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

Eventually protein and fat content in wet base was recalculated from dry base using the formula:

$$\% \text{ Proximate in wet} = \frac{\% \text{ Proximate in dry (100-Moisture content)}}{100}$$

2.3.4 Gross energy value

Gross energy values (kcal/g) was calculated by overall addition of the protein content multiplied by 4 and the total lipids content multiplied by 9 and using Atwater's conversion factors. The result was expressed as kcal per 100 gram.

$$\text{Gross energy value} = 4 \times \text{protein content} + 9 \times \text{fat content}$$

2.3.5 Statistical analysis

Proximate data were analyzed with two ways ANOVA to evaluate the effects of sex and age. Mean differences were considered statistically significant at $p < 0.05$. Duncan Multiple Range Test technique was used to separate the means.

3 Result and discussions

Table 1 indicates the proximate composition of Nile Tilapia. Sex was found to have no significant (P > 0.05) influence in terms of the four proximate components (moisture, protein, fat and ash) measured in Nile Tilapia fillet collected from Lake Zeway. The major component of fish fillet was moisture. The highest moisture content (80.8 %) was recorded from five years fish and lowest (79.6 %) being from four year old fish. It was found that the moisture contents in different ages of the fish were 80.6 % for male and 79.7 % for female. Moisture showed a tendency to increase with advance in age of the fish. However moisture content was nearly the same for both sexes.

Table1: Mean ± SE proximate composition and gross energy content in kcal /100 g of Nile Tilapia fillet in wet basis

	Parameter	Moisture	Crude protein	Fat	Ash	GE (Kcal/100 g)
Sex	Male	80.6 ± 0.39^a	14.5 ± 0.21^a	0.52 ± 0.02^a	1.14 ± 0.02^a	60.2 ± 0.86^a
	Female	79.6 ± 0.39^a	14.6 ± 0.21^a	0.54 ± 0.02^a	1.17 ± 0.02^a	60.7 ± 0.86^a
Age	Four	79.5 ± 0.39^y	15.8 ± 0.21^x	0.4 ± 0.02^x	1.2 ± 0.02^x	65.0 ± 0.86^x
	Five	80.8 ± 0.39^x	13.3 ± 0.21^y	0.7 ± 0.02^y	1.0 ± 0.02^y	55.9 ± 0.86^y

At each level of parameter, values with different superscripts in the same column are significantly different ($p < 0.05$).

The proximate composition of fish could not undergo any significant change between both sexes (Cornelia, 2012). Different researchers have reported that, moisture content of male fish higher than female fish (Amer *et al*., 1991; Islam and Joadder, 2005; Nargis, 2006; Bhavan *et al*., 2010; Cornelia, 2012). Investigation on proximate composition of fresh water Gobi (*Glossobobius giuris*) revealed that the moisture content has increased as the length of fish increased from 18 cm to 20 cm (Islam and Joadder, 2005). The moisture content of male fish is slightly higher than female corresponding similar age category. As similar to present study, the moisture content of male Gilthead bream fish is higher than female (Wassef and Shehata, 1991). An exploration conducted on moisture content of farmed and wild silver carp and grass carp between different sizes (1 kg grass carp Vs 1.5 kg) revealed significantly ($p<0.05$) lower moisture contents in smaller carps as compared to bigger carps (Ashraf, 2011). The lower moisture content in female Nile Tilapia can be attributed to muscles of female fish contain more organic materials and less water than male (Amer *et al*., 1991).

Content of protein was 14.5 % in male, 14.6 % in female, 15.8 % in four year and 13.3 % in five year fish. Across sex groups, the protein content of the female was slightly higher than the male. Sex has no significant ($p>0.05$) effect on protein content. The decreased in average protein content with further increase in age can be ascribed to either protein was used for energy or converted into fat (Rubbi *et al*., 1984). Study conducted on fatty acids and energetic substrates of two Nile Tilapia maintained in cages for 11 months showed that body mass increased and the protein content decreased (Viera *et al*., 2011). There is higher protein content in medium sized Koi fish and gradually decreased with an increase in age (Nargis, 2006). This decrease in muscle protein could be due to the use of this energetic substrate for growth. The average protein content of the present study was less as compared to others finding may be associated with stage of sexual development and feeding conditions (El-Serafy *et al*., 2005). Juvenile fish contains more protein than the adult (Rubbi *et al*., 1984; Cornelia, 2012). In the present study, smaller fish contained high protein as compared to bigger fish may be due to fish expend a great deal of energy during egg formation. The high tissue protein content in smaller fish may result from high protein content of their diets (Zenebe, 1988).

Lipid content showed inverse patterns of variation to protein in four and five year's fish. Even though sex has no significant ($p>0.05$) effect on lipid content female fish had higher fat (0.54 %) as compared to male (0.52 %). Age significantly ($p<0.05$) affected the fat content but interaction effect of age and sex showed no significant ($p>0.05$) effect. The maximum and minimum lipid extracted was 0.7 % and 0.4 % from female and male fish respectively. Lipid content was increased with increase of fish age, however, the average lipid content shown no significant difference between male and female fish. Larger (older) fish have higher levels of lipid than smaller fish (Toppe *et al*., 2006). The higher lipid content in five year fish as compared to four year may be attributed to the diversified feeding habits of the two groups (Wassef and Shehata , 1991; Caponio *et al* 2004). At any stage of fish, fat content is dependent upon age, the older the fish, the higher the amount of fat. The variation among age in fat content may be fish is in state of development of gonad and spawning, the fish expends great deal of energy with fat as its principal sources (Wassef and Shehata , 1991; Caponio *et al* 2004).

The ash content was 1.14 % in male and 1.17 % in female fish. Age significantly ($p < 0.05$) affected ash content. Ash content decreased from 1.2 % in four year to 1.0 % in five year fish. No significant ($p > 0.05$) variation in ash content was found between male and female fish. The higher ash content in female fish as compared to male fish may be due to increased minerals contents to correct ionic balance during starvation.

The gross energy contents of Nile Tilapia fillet were 60.2 kcal/100 g for male and 60.7 kcal/100g for female fish. Age significantly ($p < 0.05$) affected gross energy content. It was higher (65.0 kcal /100g) in four year fish than in five year fish (55.9 kcal/100 g). Higher gross energy value in female Nile Tilapia as compared to male may be attributed to relatively higher protein and fat in females than males (Amer *et al*., 1991).

4. Conclusions

From the present study it can be concluded that, there is variation between age and sex of Nile Tilapia with regard to moisture content, crude protein, crude fat, crude ash and gross energy content. Female fish contained slightly higher protein and fat as compared to male. Moisture content and fat tend to increase with increase in age but protein and ash tend to decrease with increase in age.

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