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DETERMINATION OF LYSINE IN FILETS OF "NILE TILAPIA" *(OREOCHROMIS NILOTICUS)*

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Abstract: Lysine is an essential amino acid, important in the composition of collagen, used in the fortification of cereals and protein supplements, in the production of medications, and in the production of animal feed. Given the many uses of this amino acid, “Nile tilapia” (*Oreochromis niloticus*) fillets become a potential source of lysine. Therefore, the objective of this study was to determine the percentage of lysine in fresh “Nile tilapia” fillets collected at different stages of the species’ development. For this purpose, duplicate samples of fillets were collected. Protein analysis was performed using the Kjeldahl method. The percentage of lysine was obtained by correlating the protein content determined by the method with the sample’s nutritional information. It was found that the juvenile stage has the highest lysine concentration due to increased hormonal activity during this developmental period of the species.

Keywords: Nile tilapia, protein, lysine, Kjeldahl method.

INTRODUCTION

According to O’Neil et al (2001, p. 1010), lysine derives from the Greek word “Lysitine,” which means “loss.” It is a product of urea following treatment with barium hydroxide, where its degradation produces two molecules of glutamate and one of acetate. Through methylation and the synthesis of N-trimethyllysine, it produces carnitine, an important protein transporter found in muscles and certain organs such as the liver. It is an essential amino acid of basic nature, one of the most scarce in the food chain.

According to Ross et al. (2003), it is very important in the composition of collagen and can be used for various purposes, such as in the fortification of cereals and protein supplements, in the production of medications, as a supplement in animal feed, and in cosmetics. Given these uses, lysine proves to be of great importance in the food and pharmaceutical industries. In this context, the “Nile tilapia” (*Oreochromis niloticus*) becomes a potential food source of this amino acid, precisely because it contains a large amount of protein. Thus, the objective of this study was to determine the percentage of lysine by correlating it with the results obtained using the Kjeldahl method.

LITERATURE REVIEW

Lysine

Lysine (Lys), according to O’Neil et al (2001, p. 1010), derives from the Greek “Lysitine,” which means “loss.” It consists of a –COOH group (carboxylic acid), a chiral carbon, and an amine moiety, and is therefore a basic amino acid (Figure 1).

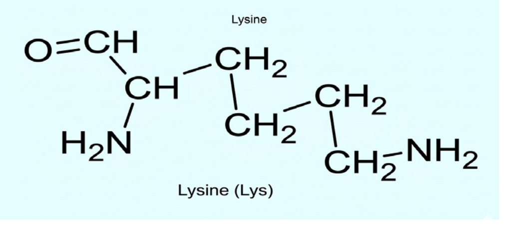

Figure 1. Structural formula of Lysine.

Available at: <http://www.educadores.diaadia.pr.gov.br/arquivos/File/tvmultimidia/imagens/2011/quimica/lisina.jpg>. Accessed on: October 12, 2014.

According to Ross et al. (2003), its degradation produces two molecules of glutamate and one of acetate. Through methylation and the synthesis of N-trimethyllysine, it produces carnitine, an important protein transporter found in muscles and in some organs such as the liver. As described by RABITO, E. I (2007, p. 19):

6-N-trimethylylisine is formed by the methylation of lysine residues in proteins such as myosin, actin, and histones [...] this is hydroxylated to 3-hydroxy-6-N-trimethylylisine, and hydroxytrimethylylisine is cleaved to butyrobetaine, which is finally hydroxylated to carnitine. The enzyme responsible for the conversion of butyrobetaine to carnitine (butyrobetaine hydroxylase; δ-butyrobetaine-2-oxoglutarate oxygenase) is present in the liver of all mammals.

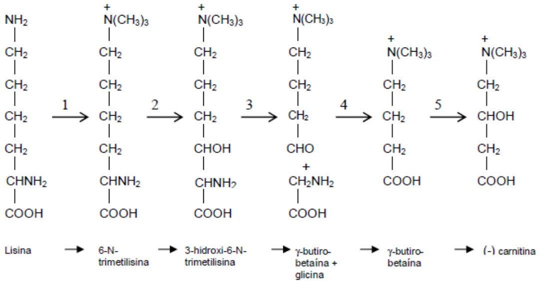

Figure 2. Synthesis of N-trimethylisylcholine to form carnitine. Source: RABITO (2007, p. 19).

This enzyme is also found in the kidneys and brain. The conversion of trimethylisyl to butyrobetaine occurs in the liver, while the hydroxylation of butyrobetaine to carnitine occurs exclusively in hepatocytes (RABITO, 2009). In humans, synthesis is approximately 1–2 μmol of carnitine/kg/day, derived from lysine and methionine, and accounts for 3.9% of collagen composition (ANGELINI, 2011).

Importance of Lysine for the Body

Particularly important in the composition of collagen, an abundant fibrous protein found in connective tissue, tendons, bones, and muscles. According to Ross et al. (2003), collagen fibers are arranged differently depending on their functional type, and according to Angelini (2011), lysine accounts for 3.9% of collagen's composition (ANGELINI, 2011).

Collagen is necessary to support cellular structure, such as in the extracellular matrix or the vitreous humor, and to provide great strength, such as in tendons (tightly twisted into parallel fibers).

In the cornea, collagen is stacked in such a way as to transmit light with minimal scattering. Bone collagen consists of fibers arranged at such an angle to one another that it exhibits high mechanical strength in any direction. According to CHAMPE, P. C. et al (2006), there are more than 20 types of collagen, which, like other proteins, have similar domains within their polypeptide chains. It is typically composed of three polypeptide chains held together by hydrogen bonds. These amino acid chains combine to form the various types of collagen found in tissues.

TYPE	TISSUE DISTRIBUTION
	Fibril-forming collagens
I	Skin, bones, tendons, blood vessels, cornea
II	Cartilage, intervertebral disc, vitreous humor
III	Blood sangüíneos, pele fetal
	Network-forming collagens
IV	Basement membrane
VII	Below squamous stratified epithelium
	Fibril-associated collagens
IX	Cartilage
XII	Tendons, ligaments, some other tissues

Figure 3. The most abundant types of collagen in the human body.

Source: CHAMPE, P. C. et al. Biochemistry I. 3rd ed. Porto Alegre/RS. Artmed, 2006, p. 44.

According to CHAMPE, P. C. et al. (2006), type I collagen fibers are found in structural elements that exhibit high tensile strength (e.g., tendons and the cornea), while fibers formed by type II collagen molecules are restricted to cartilaginous structures (e.g., those of the nose). Fibrils derived from type III collagen are prevalent in more elastic tissues, such as vascular walls. Type IV and V collagens form a three-dimensional mesh, rather than distinct fibrils. Type IV molecules associate to form a sheet or mesh, which constitutes the majority of basement membranes. (These are thin, sheet-like structures that provide mechanical support to adjacent cells and function as a semipermeable filtration barrier for macromolecules in organs such as the kidney and lung) and type IX and XII collagens bind to the surface of collagen fibrils, linking these fibrils to one another and to other components of the extracellular matrix.

According to the Department of Medicine and Nutrition, Brazilian Vegetarian Society (2012), a 70-kg human requires a 2,000-kcal diet. In this diet, 28.5 kcal/kg/day are consumed, and 56 g of protein (0.8 g/kg/day) are required; this includes 2,660 mg of lysine, 1,000 mg of calcium, 8 mg of iron, and 11 mg of zinc. For a diet of nearly 2,000 kcal, the same study recommends the following distribution of food groups:

Since lysine is more abundant in animal-based foods, vegetarians should use the portion calorie values adopted by the Ministry of Health. If the diet consists exclusively of whole grains (which have the lowest lysine content) totaling 14 servings, a 2,100 kcal diet planned for a 70 kg man will provide 72.21 grams of protein, which corresponds to 1.03 g/kg/d. In this diet, the required lysine intake would be 2,660 mg (RDA: Recommended Dietary Allowance), and he would receive 2,646 mg. Two servings of beans (equivalent to 4 tablespoons per day, or 110 kcal) and 13.26 servings of grains (1,990 kcal)will already provide the necessary lysine intake of 2,660 mg per day, with 2,506 mg coming from the grains and 498 mg from the beans, for a total of 3,004 mg of lysine.

Food group	Number of Servings
Grains, Tubers, Roots, and Derivatives	6
Beans	1
Fruits	3
Vegetables	3
Milk and dairy products	3
Meat and eggs	1
Oils, fats, and oilseeds	1
Sugars and sweets	1
Fish (as a substitute for meat and eggs)	2

Table 1. Recommended diet for humans based on a 2,000 kcal diet

Source: Department of Medicine and Nutrition (2012) and Ministry of Fisheries and Aquaculture (2014).

Diseases caused by lysine deficiency Disorders of the urea cycle metabolism

According to CHAMPE, P. C. et al (2006), the urea cycle is described as follows:

> The catabolic phase involving the removal of amino groups (generally through transamination and subsequent oxidative deamination), forming ammonia and the corresponding α-ketoacid—the "carbon skeletons" of amino acids. A portion of the free ammonia is excreted in the urine, but most of it is used in urea synthesis, which is quantitatively the most important pathway for the elimination of nitrogen from the body. In the second phase of amino acid catabolism [...], the carbon skeletons of the α-ketoacids are converted into common intermediates of the energy-producing metabolic pathways. These compounds can be metabolized to CO2 and H2O, glucose, fatty acids, or ketone bodies via the central pathways of metabolism.

There are five main enzymes involved in the cycle (Enteropeptidase, Trypsinogen, Chymotrypsinogen, Pro-elastase, Pro-Carboxypeptidase A and B); errors in the cloning or formation of these enzymes can cause mutations, which in turn destabilize the nitrogen release process. These disorders can be identified through prenatal diagnosis and for the purpose of conducting population studies. NADAI, C. P. et al (2006) estimate that urea cycle disorders occur in 1 in every 25,000 births. It is likely that 2% to 4% of the population are heterozygous for a urea cycle defect.

Hyperargininemia

This is an excessive increase in the concentration of arginine in the blood; this occurs because: arginase is the final enzyme in the urea cycle that catalyzes the hydrolysis of arginine into urea and ornithine. Ornithine then returns to the mitochondria to participate in another ammonia detoxification cycle.

There are two types of arginases: one expressed in the liver and red blood cells, and the other found in renal mitochondria. The liver-encoded arginase is responsible for this arginase deficiency, mapped to the long arm of chromosome 6. It is characterized by a heterogeneous disorder resulting from point mutations in the arginase type I gene.

This leads to the urinary excretion of arginine, lysine, cystine, and ornithine, which may suggest a probable diagnosis of cystinuria.

According to NADAI, C. P. et al (2006), it is a rare disorder that causes many abnormalities in the development and function of the central nervous system. It presents with late-onset clinical manifestations that are more gradual and inexorable in their progression. Patients generally remain asymptomatic during the first months or years of life. However, developmental delay is progressive, and seizures are very common.

Cystinuria

Abnormal transport of cystine (a disulfide derivative of the amino acid cysteine) between cells and the extracellular matrix causes cystinuria, one of the most common inherited metabolic disorders.

NADAI, C. P. et al (2006) describe it as a genetically heterogeneous disorder caused by a defect in the transport of dibasic amino acids that affects the epithelial cells of the gastrointestinal tract and renal tubules. As a result, cystine, lysine, arginine, and ornithine are excreted in the urine in abnormal amounts.

According to NADAI, C. P. et al (2006), recent molecular findings have identified the genes responsible for this disease: SLC3A1 and SLC7A9. Accurate phenotypic and/or genotypic identification of cystinuric patients allows for better prevention and treatment of this condition.

And complications in **Ehlers-Danlos Syndrome** or **Cutis Elastica.** This syndrome is a rare hereditary connective tissue disorder that results in excessive joint flexibility, inguinal hernia, increased skin elasticity, and cardiovascular system lesions such as perimembranous ventricular septal defect and mitral or tricuspid valve prolapse. LOPES, D. O. et al (2005).

This is caused by a defect in the pro-collagen peptidase enzyme responsible for removing the non-helical ends of pro-collagen, which results in the formation of defective collagen fibrils, as well as a mutation in the gene encoding the lysyl-hydroxylase, necessary for the post-translational modification of lysine to hydroxylysine, resulting in decreased collagen molecule strength in the syndrome. LOPES, D. O. et al (2005).

LOPES, D. O. et al (2005) state that this genetic alteration generally causes abnormalities in collagen fibers and can progress to the formation of aneurysms, vascular ruptures, and arteriovenous fistulas, as well as severe systemic vascular stenoses.

Obtaining Lysine

Lysine can be obtained in two ways:

Synthetic: obtained from urea after treatment with barium hydroxide, through hydrolysis. As represented by the reaction below:

2Ba(OH2) + 8CO(NH2)2 → 2BaCO3 + C6H14N2O2 + 4H2O + 3NH3 + 11N

Where barium carbonate, lysine, water, ammonia, and nitrogen are formed. This is confirmed by TAYLOR et al. (2010, p. 13) and BAILEY, R. D. (1907). Another method involves chemical conversion through the synthesis of diaminopimelic acid.

This conversion is described by GORTON, B. S. (1961); where the synthesis of diaminopimelic acid containing a portion of D-diaminopimelic acid is converted into lysine by treatment with monodescarboxylase enzymes (which can be obtained from the microorganism *Aerobacter aerogenes*, which must be in an aqueous solution of 0.1% to 3% diaminopimelic acid, between 20 °C and 45 °C with a pH of 6.5 to 8.5, at a ratio of 5 to 20 parts per 1000 parts of the enzyme solution) to produce a mixed reaction containing lysine and unchanged D-diaminopimelic acid. This acid is then recovered through racemization of its reaction products with aqueous acid solutions at 230°C, until this racemization of diaminopimelic acid with monodescarboxylase enzymes converts 62–70% of this racemic mixture into lysine.

Natural: obtained through food. Especially from meat, milk, and dairy products. Lysine is one of the essential amino acids, meaning an amino acid that cannot be synthesized by the body and must therefore be obtained through diet.

However, it is one of the most limited in the food chain, particularly in grains and legumes when compared to animal-based products (Table 2):

Applications of Lysine in Industry

According to O'Neil et al. (2001, p. 1010), lysine can be used as: a supplement in cereals, a supplement in protein products (whey protein), a supplement in animal feed, as described by Gonçalves et al. (2009), and in the production of medications (such as Dolamin Flex®, for example; by Farmoquímica/SA (2010), a medication used for pain management, for the relief headaches, muscle pain, and joint pain) and in cosmetics (Biotiful Hair® Phytocomplex for hair toning).

Thus, it spans various industrial sectors for the production of diverse products and byproducts of this amino acid. It is found in "Nile tilapia" fillet (*Oreochromis niloticus*) at a ratio of 1.810 g of lysine per 100 g of fresh fillet (USDA, 2002). Thus, "Nile tilapia" fillet becomes a potential food source of this amino acid. Since this species is easy to manage and breed TILAPIA (Oreochromis niloticus)

Nile tilapia (*Oreochromis niloticus*), according to AYROZA, L. M. S. (2009), is a fish belonging to the cichlid family, native to the Nile River basin in East Africa, which is widely distributed in tropical and subtropical regions, such as Israel, Southeast Asia (Indonesia, the Philippines, and Taiwan), the Americas (the U.S., Mexico, and Panama), and throughout South America. In

Food Group	Average lysine content (mg) per 100 g of the dietary group	Standard Deviation	Average lysine content per serving (in mg)	Calories per serving
Cereals	452	221	189	150
Whole-grain whole and refined	212	107	89	150
Beans	1552	182	249	55
Oilseeds	653	351	79	73
Vegetables	121	58	68	15
Vegetables	584	31	32	15
Starchy foods	563	11	70	150
Fruits	43	28	48	70
Cheese	1,794	762	722	120
Cow's milk	268	0	495	120
Egg (whole)	912	0	1212	190
Meat	1373	339	786	190
Tilapia fillet	1810	0	1859	96

Table 2. Average lysine content in different food groups

Source: Average lysine content in different food groups. Adapted from the Department of Medicine and Nutrition. Brazilian Vegetarian Society. Dietary Guide for Vegetarian Diets for Adults. São Paulo. 2012 and Full Report (All Nutrients) 15261, Fish, tilapia, raw. 1Nutrient Data Laboratory, ARS, USDA National Food and Nutrient Analysis Program Wave 6l, Beltsville MD. 2002

Brazil, it was introduced in 1971 by the National Department of Works Against Drought (DNOCS) into reservoirs in the Northeast, spreading throughout the country. SILVA, R. D. et al. (2012) It is noted that this is an omnivorous species that readily accepts various types of feed, being docile and easy to manage at all stages of cultivation, hardy, prolific, and capable of early reproduction, with high-quality fillet meat.

One of the main physiological characteristics of the species, according to AYROZA, L. M. S. (2009), is its resistance to diseases caused by pathogens, as well as its tolerance to low levels of dissolved oxygen in the water. Its best productive performance is achieved at water temperatures between 26°C and 28°C. At slaughter, fillets of excellent quality are obtained, accounting for 37% of the carcass (600 g total fish weight). A distinctive feature is the absence of bones in the meat, and it has a very appealing flavor.

Brazil is the sixth-largest producer of farmed tilapia in the world, and the "Nile tilapia" (*Oreochromis niloticus*) alone accounted for 132,000 tons in 2009, representing approximately 39% of the total production of freshwater aquaculture fish (BRASÍLIA/DF. Portal Brasil. Ministry of Fisheries. 2011).

Development Stages of Tilapia

As a species that is easy to manage (NOGUEIRA et al. 2007), tilapia is highly resistant to diseases and parasites and tolerates low levels of dissolved oxygen in the water (up to 0.25 ppm), making this species highly recommended for farming. According to NOGUEIRA et al. (2007), the farming of this species is divided into three production phases:

- **First phase:** the fry phase. The fry, with an average weight of 1.0 gram, are then stocked in 5-mm mesh net pens for about 21 days to reach an average weight of 10.0 grams. After this period, the fry undergo a 24-hour fast and are then transferred to 8-mm mesh net tanks. The duration of stay in the 8-mm mesh net tank is 30 to 40 days, until they reach approximately 200 to 250 grams.
- **Second phase:** In this phase, the fish are referred to as juveniles. They are stocked in net tanks with 17–19 mm mesh at a density of 500 fish/m³ until they reach 200 to 250 grams, at which point they undergo grading, which may vary according to each company's quality standards. All of this takes place over a period of about two months. Afterward, they are stocked at a density of 250 fish/m³ until they reach the average harvest weight.
- **Third phase:** known as the harvest phase. In this phase, the fish are adults and have reached the average harvest weight, which is around 700 grams. This takes about two months to occur. The main critical points in this phase are: stocking density, the uniformity of the stocked juveniles, the removal of dead fish, feed management, and selection. The survival rate averages between 90% and 95%.

Also according to NOGUEIRA et al (2007), the most commonly used net tanks today are those with a useful volume of 4 m^3 and 6 m^3. "Measuring 2.0 m x 2.0 m, with a height ranging from 1.20 m (1 m submerged) to 1.80 m (1.5 m submerged)." Under these conditions, an average of 150 to 200 kg/m^3 of fish can be raised. Thus, it can be concluded that each net tank can produce an average of 800 to 1,200 kg of fish. An encouraging figure given the average annual fish production of 1.2 million tons in Brazil (BRASÍLIA/DF. Portal Brasil. Ministry of Fisheries. 2011), where this species accounts for 39% of national production

Tilapia fillet as a source of lysine

Thus, "Nile tilapia" (*Oreochromis niloticus*) contains approximately 1,810 mg of lysine per 100 g of fresh fillet (USDA, 2009), making it a potential source of this amino acid. As shown in (Figure 4):

MATERIALS AND METHODS

Materials

This study was conducted at the Chemistry Laboratory of the University Center of Votuporanga – UNIFEV, in Votuporanga.

Fillets of "Nile tilapia" from different developmental stages (fingerling, juvenile, adult) were collected in duplicate from a fish processing plant. The boneless, skinless fillets were removed and ground in a food processor for three minutes to form a homogeneous paste.

Methods

Protein content was determined using the Kjeldahl method, in accordance with EMBRAPA Technical Circular No. 63 (2006), attached. Lysine was determined by correlating the amount of protein found with the sample's nutritional information.

RESULTS AND DISCUSSION

Based on the results obtained for the percentage of Total Nitrogen, calculations were performed to determine the percentage of Total Protein for each developmental stage of the species (Tables 1 and 2).

Based on the results of the average Total Protein percentages for each developmental stage of the species, calculations were performed to obtain the lysine percentage, as shown in Figure 4.

Thus, we can conclude that the juvenile stage has a higher lysine concentration than the other developmental stages of the species. According to ALMEIDA (2013), puberty in this species occurs during the juvenile stage. During this period, hormones exert their functions in the hypothalamic-pituitary axis, which regulates, in a highly synchronized manner, all endocrine events involved in the gonadal function of fish; this includes the production and synthesis of amino acids, such as lysine, for example. Thus, when we compare the percentage of lysine found in "tilapia fillet" with that of other species, we observe that the amount of lysine found is lower (Graph 1).

Such differences in lysine concentration among the species presented above, when compared to that of tilapia, likely occur due to osmoregulation among them.

According to THIEMANN (2014), in freshwater fish species, excess water is excreted by the kidneys, and salts are maintained at appropriate concentrations through absorption by specialized cells in the gills; thus, the kidneys of freshwater fish have much

Amino Acids

Tryptophan [1]	g	0.210	--	--	0.244
Threonine [1]	g	0.950	--	--	1.102
Isoleucine [1]	g	0.930	--	--	1.079
Leucine [1]	g	1.603	--	--	1.859
Lysine [1]	g	1.810	--	--	2.100
Methionine [1]	g	0.593	--	--	0.688
Cystine [1]	g	0.220	--	--	0.255
Phenylalanine [1]	g	0.810	--	--	0.940
Tyrosine [1]	g	0.680	--	--	0.789
Valine [1]	g	0.970	--	--	1.125
Arginine [1]	g	1.277	--	--	1.481
Histidine [1]	g	0.470	--	--	0.545
Alanine [1]	g	1.220	--	--	1.415
Aspartic acid [1]	g	2.297	--	--	2.665
Glutamic acid [1]	g	3.213	--	--	3.727
Glycine [1]	g	1.043	--	--	1.210
Proline [1]	g	0.757	--	--	0.878
Serine [1]	g	0.813	--	--	0.943

Figure 4. Table of amino acid composition in 100 g of "Nile tilapia" (*Oreochromis niloticus*) fillet, where lysine appears with a concentration of 1.810 g. USDA- Agricultural Research Service United States Department of Agriculture. National Nutrient Database for Standard Reference. Full Report (All Nutrients): 15261, Fish, tilapia, raw. 2009.

Since this yield is higher when compared to plant-based foods, as shown in Table 2 on page 22.

	Fry	Juvenile	Adult	Slaughter
Tube 1	2.47%	2.68%	2.58%	2.61%
Tube 2	2.68%	2.71%	2.63%	2.58%
Average	2.57%	2.69%	2.60%	2.59%

Table 1. Percentage of Total Nitrogen for samples collected at different developmental stages of the species.

	Fry	Juvenile	Adults	Slaughter
Tube 1	14.37%	15.64%	15.05%	15.21%
Tube 2	15.59%	15.82%	15.35%	15.05%
Average	14.98%	15.73%	15.20%	15.13%

Table 2. Percentage of total protein per sample according to the species' developmental stages.

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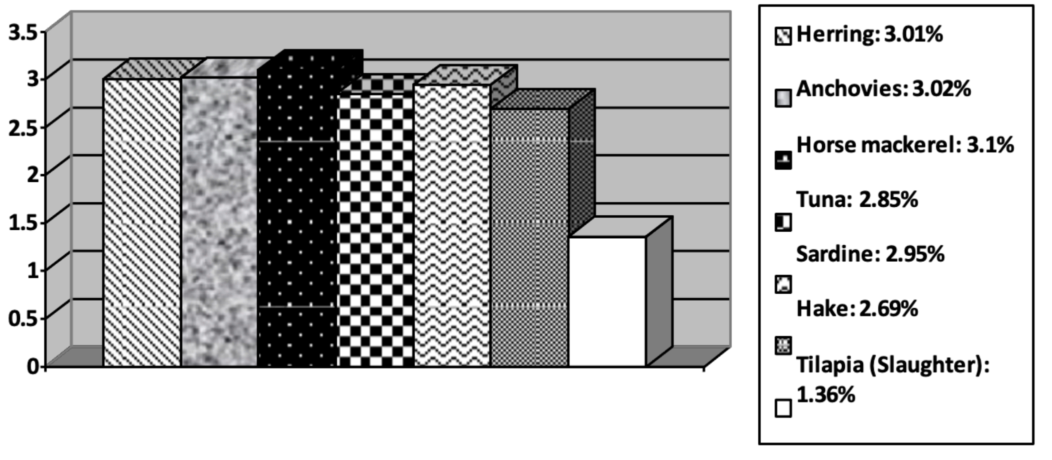
Fry	Juvenile	Adult	Slaughter
1.34%	1.42%	1.37%	1.36%

Table 3. Lysine percentage in "tilapia fillet" for each developmental stage of the species per gram.

Source: Adapted from USDA- Agricultural Research Service United States Department of Agriculture. National Nutrient Database for Standard Reference. Full Report (All Nutrients): 15261, Fish, tilapia, raw. 2009.



Graph 1. Comparison of the percentage of lysine found in "tilapia fillet," according to each developmental stage of the species, with other fish species.

Source: Adapted from HOUSE, H. et al. International Association of Fish Meal Manufacturers – IAFMM. USA. 1970.

more developed glomeruli to filter large amounts of water, excreting excess nitrogen in the form of ammonia. In marine species, deficiency is prevented by the ingestion and absorption of seawater, along with salts, by the intestine, and excess salts are eliminated by secretory cells in the gills; therefore, the glomeruli are reduced and little water is eliminated, with excess nitrogen being excreted in the form of urea.

Ammonia is the end product of protein degradation in the body, and it is used as a nitrogen source for urea formation. Thus, ammonia plays a role in the synthesis and degradation of certain amino acids, including lysine (CHAMPE, P. C. et al., 2006). This explains the difference in lysine concentration found in the species mentioned above, since herring, anchovies, tuna, sardines, and hake are typical saltwater species, whereas tilapia is a freshwater species.

CONCLUSION

Therefore, the juvenile stage presented the highest lysine concentration when correlated with the other developmental stages of the species. This occurred because puberty in the species takes place during the juvenile stage. During this period, hormones exert their functions in the hypothalamic-pituitary axis, which regulates in a highly synchronized manner all endocrine events involved in the gonadal function of fish; this includes the production and synthesis of amino acids, such as lysine. Freshwater fish also excrete excess nitrogen in the form of ammonia, reducing the total protein content in their metabolism and consequently lowering the lysine concentration.

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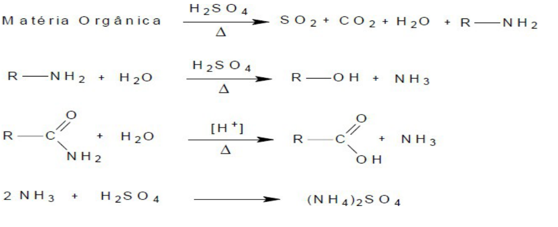
ANEXO

Método de Kjeldahl

Conforme a Circular Técnica Nº 63. EMBRAPA (2006) o Método de Kjeldahl é utilizado para determinação de nitrogênio total, baseado na decomposição da matéria orgânica através da digestão da amostra a 400C com ácido sulfúrico concentrado, em presença de sulfato de cobre como catalisador que acelera a oxidação da matéria orgânica. O nitrogênio presente na solução ácida resultante é determinado por destilação, seguida de titulação com ácido diluído.

Ainda segundo a Circular Técnica Nº 63. EMBRAPA (2006), o método é divido nas seguintes fases:

- **Digestão:** o carbono contido na matéria orgânica é oxidado e o dióxido de carbono se desprende. Durante este processo a solução passa de uma coloração escura para um verde claro. Existe o nitrogênio sob a forma de amina, amida e nitrila que são transformados em amônia a qual reage com o ácido sulfúrico formando sulfato de amônio.



- **Destilação:** o sulfato de amônio é tratado com hidróxido de sódio em excesso ocorrendo a liberação de amônia. Ao adicionar hidróxido de sódio deve-se utilizar algumas gotas de solução indicadora no destilador, para garantir o excesso de base. A amônia que desprende na reação é coletada num frasco contendo ácido bórico com o indicador. O processo é considerado terminado quando toda a amônia se desprendeu. A solução contendo ácido bórico com o indicador que no inicio apresentava a coloração rosa adquire a coloração azulada á medida que se forma o borato de amônio.

- **Titulação:** o borato de amônio é titulado com uma solução padrão de ácido clorídrico de titulo conhecido ate a viragem do indicador.

$$(NH_4)_2SO_4 + 2\ NaOH \xrightarrow[\Delta]{} 2\ NH_4OH + Na_2SO_4$$

$$NH_4OH \xrightarrow[\Delta]{} NH_3 + H_2O$$

$$H_3BO_3 + NH_3 \longrightarrow NH_4H_2BO_3$$

$$NH_4H_2BO_3 + HCl \longrightarrow H_3BO_3 + NH_4CL$$

O nitrogênio total (NT) é determinado pela seguinte equação:

$$NT = \frac{(Va - Vb) \times F \times 0{,}1 \times 0{,}014 \times 100}{P1}$$

Onde:

NT – teor de nitrogênio total na amostra, em percentagem;

Va – volume da solução de ácido clorídrico gasto na titulação da amostra, em mililitros; Vb – volume da solução de ácido clorídrico gasto na titulação do branco, em mililitros; F – fator de correção para o ácido clorídrico 0,01 mol/L;

P1 – massa da amostra (em gramas).

Na determinação da proteína bruta, multiplica-se o valor do nitrogênio total encontrado pelo método de Kjeldahl por um fator que converte o nitrogênio em proteína. Convencionalmente, em amostras de alimentos para animais: plantas forrageiras, rações concentradas, entre outros materiais, a proteína bruta (PB) é expressa pelo fator 6,25, considerando que a maioria das proteínas contém nas suas moléculas aproximadamente 16% de nitrogênio.

PB = NT x FN

Onde:

PB – teor de proteína bruta na amostra, em percentagem; FN – 6,25.