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# IMPORTÂNCIA DOS AMINOÁCIDOS AROMÁTICOS FENILALANINA E TIROSINA NA NUTRIÇÃO DE PEIXES

*(Importance of aromatic amino acids phenylalanine and tyrosine in fish nutrition)*

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## RESUMO

Aminoácidos essenciais estão diretamente associados com a fisiologia dos peixes em sistemas de criação, desempenhando um papel essencial no metabolismo e produção de energia dos animais. Baseado no conceito de proteína ideal, a exigência adequada de proteína bruta e a inclusão de aminoácidos essenciais que são necessários nutricionalmente para melhorar a eficiência de utilização de nutrientes, desenvolvimento de órgãos, crescimento e formação de tecido muscular, sistema imunológico, reprodução e o bem-estar dos peixes. A fenilalanina é um aminoácido essencial que desempenha importante papel na biossíntese de outros aminoácidos e no sistema neurotransmissor e hormonal, obtendo de sua hidroxilação o aminoácido não-essencial tirosina. Nesse sentido, o objetivo desta revisão é demonstrar a importância dos aminoácidos fenilalanina e tirosina na nutrição de peixes e sua influência nos parâmetros de desempenho produtivo e reprodutivo de espécies comerciais.

**Palavras-chave:** Piscicultura, desempenho produtivo, respostas fisiológicas, metabolismo aminoácidos, exigências nutricionais.

## ABSTRACT

Essential amino acids are directly associated with the physiology of fish in farming systems, playing an essential role in the metabolism and energy production of animals. Based on the concept of ideal protein, the adequate requirement of crude protein and the inclusion of essential amino acids are required nutritionally in order to improve nutrient utilization efficiency, organ development, growth and formation of muscle tissue, immune system, reproduction, and the well-being of fish. Phenylalanine is an essential amino acid that has an important role in the biosynthesis of other amino acids and the neurotransmitter and hormonal system, obtaining from its hydroxylation the non-essential amino acid tyrosine. In this sense, the purpose of this review is to demonstrate the importance of the amino acids phenylalanine and tyrosine in fish nutrition and their influence on the parameters of the productive and reproductive performance of commercial species.

**Key words:** Fish-farming, growth performance, physiological responses, metabolism, amino acids, nutritional requirements.

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## INTRODUCTION

The principle of animal nutrition is to use food in a balanced way in order to provide the nutritional well-being of farming organisms. For fish, protein sources are important in their development and body composition, aiding in the metabolism and maintenance of tissues and also reduces the fat accumulation in the visceral cavity (FRACALOSSI e CYRINO, 2013; SAKOMURA *et al*., 2014; RODRIGUES *et al*., 2013; SANTOS, 2015).

In artificial fish feeding, regularly protein intake at appropriate levels and stage of life, it is necessary to direct them to the structural formation of vital tissues used for growth, reconstitution, body tissues levels, and reproductive development (LIMA *et al*., 2015). Due to the contribution of amino acids in the animal organism that is crucial to meet the maintenance and production requirements to ensure the optimal fish growth (FURUYA, 2010).

The objective of this review is to demonstrate the importance of the amino acids in fish nutrition and the influence the phenylalanine and tyrosine on the parameters of the productive and reproductive of commercial species.

## DEVELOPMENT

### *Importance of amino acids and their characterization*

In order for protein deposition to take place with maximum efficiency, there should be a balancing of essential and non-essential amino acids with full metabolic and digestive availability to ensure that protein and other molecules are adequately deposited, providing the minimum requirements of all amino acids in the diet formulation. Thus, meeting the requirement for fish maintenance (WILSON, 2002; FURUYA, 2010).

Proteins consist essentially of polymers of α-amino acids, comprising complex organic molecules that have a central carbon (α-carbon) attached to a hydrogen atom, an amine group, a carboxylic group and a specific "R" chemical group, which characterizes each amino acid in its multiple physiological functions to the organism (FRANCISCO JÚNIOR and FRANCISCO, 2006).

The functions of the amino acids (Fig. 01) can be related to the structural and dynamic actions, indispensable for survival, growth, reproductive development, and animal health (PEZZATO *et al*., 2004; WU *et al*., 2013). In low amounts of amino acids, there is a reduction in productivity, leading to the use of the proteins that make up the tissues in order to maintain their vital functions (NRC, 2011). Regarding classification, they can be defined as nutritionally essential or non-essential according to the nitrogen balance (WU, 2013).

In the early stages of life, the animals accumulate nitrogenous compounds like the proteins, until their growth ceases and consequently presenting the ingested N higher than excreted. Thus, the nitrogen balance consists of the difference between the N ingested per day from the food and the N excreted by the feces and urine (SMITH *et al*., 2007).

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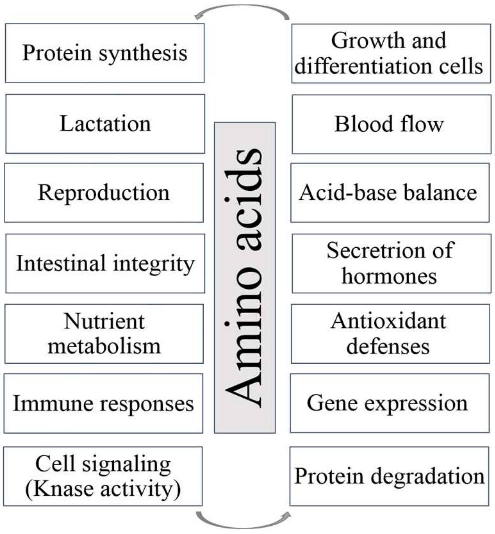


**Figure 01:** Multiple roles of amino acids in animal physiology.

**Source:** Adapted from Wu *et al*., 2013.

Non-essential amino acids are defined as those that can be endogenously produced, via metabolic processes from liver syntheses, such as alanine, aspartic acid, glutamic acid, cysteine, glycine, hydroxyproline, proline, serine, and tyrosine. In turn, essential amino acids are not present in quantities needed by the endogenous metabolism of the organism, it being necessary to supply them through the diet in order to meet their requirements and guarantee adequate protein synthesis and balance in the nitrogen balance (NELSON and COX, 2014; WU, 2014).

Essential amino acids for fish include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Among these, phenylalanine ($C_9H_{11}NO_2$) is classified as an apolar amino acid, presenting an aromatic ring in its side chain (NELSON and COX, 2014). In the Fig. 02A, represents its molecular structure.

Phenylalanine belongs to the group of aromatic amino acids and has its metabolism initiated by oxidation into tyrosine ($C_9H_{11}NO_3$), becoming its precursor. Tyrosine is converted by the enzyme phenylalanine hydroxylase (Fig. 02), being dependent on the tetrahydrobiopterin coenzyme originated in the liver and kidneys. Phenylalanine, however, cannot be converted back into tyrosine (LI *et al*., 2009). Under normal conditions, there is a balance between catabolism and anabolism of phenylalanine to tyrosine. This factor contributes to the body maintenance associated with the other essential amino acids (BENDER, 2012; NELSON and COX, 2014).

The synthesis processes of phenylalanine and tyrosine occur in divergent metabolic pathways when it refers to plants and bacteria and to animals. In the first case, amino acids are synthesized from the formation and action of chorismic acid, providing the formation of aromatic amino acids, via the shikimate, in which a benzene ring is constructed (CZELUSNIAK *et al*., 2012). In animals, in turn, hydroxylation of phenylalanine occurs, catalyzed by the enzyme phenylalanine hydroxylase to form tyrosine (NELSON and COX, 2014).

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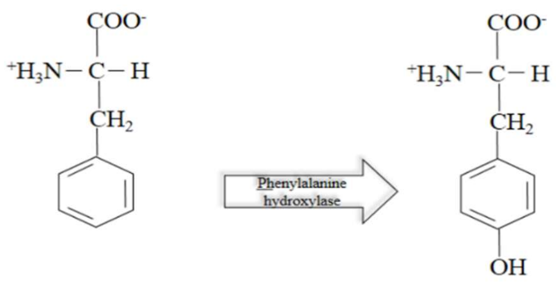


Phenylalanine

Tyrosine

(A)

**Figure 02:** Molecular structure of phenylalanine (A) and transformation of phenylalanine into tyrosine (B).

**Source:** Adapted from Wu *et al*., 2013; Nelson and Cox, 2014.

Phenylalanine catabolism (Fig. 03) occurs due to the action of the hepatic enzyme known as phenylalanine hydroxylase, the first enzyme in the catabolic reaction, which directs its conversion to the amino acid tyrosine, belonging to the group of non-essential amino acids. The following reaction occurs by the oxidation of phenylalanine and tetrahydrobiopterin from molecular oxygen, subsequently giving rise to tyrosine and dihydrobiopterin. In this case, tyrosine cannot be converted back to phenylalanine (BENDER, 2012).

The catalysis is promoted by dihydrobiopterin reductase, following a reduction by NADH or NADPH and being the last most important physiological reductant due to its higher cellular ratio, directs the tetrahydrobiopterin regeneration from the quinonoid form of the dihydrobiopterin. When there is an inefficiency of the enzymatic activity that promotes the catabolism of phenylalanine, its accumulation occurs, which, through transamination, can generate phenylpyruvate, and the increase of its byproducts may lead to pathological situations, causing neuroregulatory retardation in several organs (NELSON and COX, 2014).

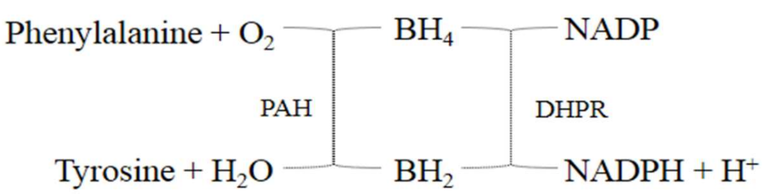


**Figure 03:** Phenylalanine catabolism process and its interaction process with tyrosine.

**Source:** Saldanha, 2007.

**Abbreviations:** Prephenate dehydrogenase (NADP); Nicotinamide-adenine dinucleotide phosphate (NADPH); Tetrahydrobiopterin (BH4); Dihydrobiopterin (BH2); Phenylalanine hydroxylase (PAH); Dihydropteridine reductase (DHPR).

In the initial stage of the tyrosine catabolism (Fig. 04), a transamination occurs, followed by a dioxygenase reaction, becoming irreversible, as the incorporation of the

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oxygen in the reaction causes a formation of the intermediate compound homogentisate. With the presence of ascorbate (ascorbic acid), the ferrous ion retention of the reduced dioxygenase occurs. And, by an oxygenation reaction, the paired hydroxyls of the phenyl group become prone to the aromatic ring opening, in which the action of the homogentisate dioxygenase enzyme acts to catalyze the reaction of two oxygen atoms with those of carbons adjacent to the ring, thus forming a ketone and the carbonyl belonging to a carboxylate (BENDER, 2012).

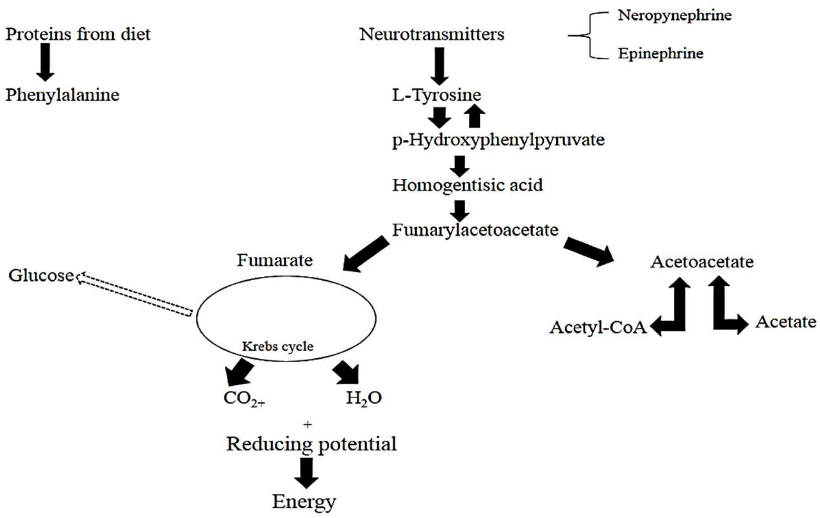


**Figure 04:** Tyrosine catabolism process and its final products.

**Source:** Saldanha, 2007.

Subsequently, complex isomerization reactions for the maleylacetoacetate form the intermediate fumarylacetoacetate, in which fumarate is the trans isomer of the four-carbon dicarboxylate, its derivative being exergonic hydrolysis, to form fumarate and acetoacetate (NELSON and COX, 2014).

The nature of the products generated in the metabolic pathways of phenylalanine to tyrosine during its catabolism is classified as glycol-ketogenic, as the molecules can be converted to acetoacetyl-CoA or acetyl-CoA and intermediates of the Krebs cycle or glucose (NELSON and COX, 2014). Similar to most amino acids, its catabolism occurs in the liver, and the resulting metabolites are directed via excretion (urea or ammonia) or converted into energy by glucose in order to be oxidized to continuous tissue activity (WU, 2013).

Phenylalanine acts in the body in regulatory functions alongside other essential amino acids, playing a key role in necessary maintenance, as well as participating in tyrosine biosynthesis (NELSON and COX, 2014). It is involved in regulatory processes dependent on hormone action and some neurotransmitters (LI *et al*., 2009).

The main sites of action of phenylalanine are indirectly linked to the regulation of gene transcription, cognitive functions, maintenance of brain activity, cell differentiation and growth of animals (LIAPI *et al*., 2008). There is also a production of catecholamines due to the process of hydroxylation of tyrosine, which acts as neurotransmitters and hormonal receptors that stimulate the reduction of stored fat, intending it for body maintenance and the

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intracellular domain of tyrosine kinase determining growth factors (KIM, 1993; NELSON and COX, 2014).

Tyrosine acts as a common precursor to important hormones and neurotransmitters, including thyroxine (T4), triiodothyronine (T3), adrenaline, noradrenalina, dopamine, and melanin (LI *et al*., 2009; SHNITKO *et al*., 2016). As a consequence, the hormonal activities of norepinephrine and dopamine tend to play a critical role in adapting the animal to environmental changes. Also, the nutritional imbalance in amino acids such as phenylalanine and tyrosine can influence consumption and food selection due to modulation in the synthesis and metabolism of neurotransmitters (FULLER, 2004).

**Effects of phenylalanine and tyrosine on fish**

In fish, dietary protein levels should adequately provide the minimum concentration of amino acids essential for metabolic maintenance and growth, considering the intrinsic characteristics of each cultive species (PEZZATO *et al*., 2004). In this sense, studies indicate that phenylalanine and tyrosine are considered essential amino acids for the development of fish, due to their performance in increasing the consumption of artificial food and, subsequently, improving their growth, immunological response, and the control of the survival of the animals (KIM *et al*., 2012).

The fishes, as other animals, need a balanced of essential and non-essential amino acids. The requirement of essential amino acids, as is the case of phenylalanine in fish diets, is based on the amount of crude protein in the formulations of the diets, quality of the protein used, and factors related to food habits and life stages (TACON and COWEY, 1985).

The minimum concentrations phenylalanine, when replaced by tyrosine (40-60%), varies according to the cultivable species. It is suggested that tyrosine may provide a portion of the total requirement of aromatic amino acids, sparing phenylalanine and subsequently providing the balance of total amino acids with a minimum percentage of protein in the diets, without affecting the optimal development of the fish (FURUYA, 2010; NRC, 2011; KLEIN *et al*., 2014).

The dietary requirements of phenylalanine were determined in several species of freshwater fish, such as *Ctenopharyngodon idella* in the juvenile stage, which presents an estimated dietary requirement of phenylalanine of 8.31 g $kg^{-1}$ in diet (2.75 g 100 $g^{-1}$ protein) (FENG *et al*., 2015). For *Oreochromis niloticus*, the consumption of 4.84% of dietary protein (CYRINO *et al*., 2002) is required for phenylalanine. For *Labeo rohita*, this inclusion is recommended in the range from 2.16% (1.16% phenylalanine + 1.0% tyrosine) to 5.55% (3.05% phenylalanine + 2.5% tyrosine) (ABIDI and KHAN, 2007). For *Cirrhinus mrigala*, 12.5 g $kg^{-1}$ phenylalanine and 8.5 g $kg^{-1}$ tyrosine are recommended, corresponding to 21.3 g $kg^{-1}$ dietary protein (AHMED, 2009). For *Astyanax fasciatus*, the recommended amount is 4.66% phenylalanine + tyrosine in the diet (FURUYA *et al*., 2015). In turn, the total requirement of phenylalanine and tyrosine for *Catla catla* fingerlings was established at 16.9 g $kg^{-1}$ dry feed (10.1 g $kg^{-1}$ phenylalanine + 6.8 g $kg^{-1}$ tyrosine) (ZEHRA and KHAN, 2014). In marine fish such as *Oncorhynchus tshawytscha*, in the fingerlings phase, 2.1% of phenylalanine (0.4% supplied by tyrosine) (CHANCE *et al*., 1964) was required. For *Chanos chanos* juveniles, phenylalanine was required at 1.90% of the dry diet (4.22% dietary

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protein), as well as 0.45% for tyrosine (BORLONGAN, 1992). Finally, the phenylalanine requirement for *Oncorhynchus mykiss* fingerlings was 0.7% of the dry diet or 2.0% of dietary protein (KIM, 1993).

Studies with native Brazilian species such as *Astyanax altiparanae* and *Piaractus mesopotamicus* reveal that the nutritional requirements in essential amino acids were estimated in order to maximize protein utilization, leaving the formulations of the diets less costly considering the species and the farming environment (BICUDO *et al*., 2009; ABIMORAD *et al*., 2010; ABIMORAD and CASTELLANI, 2011).

According to Furuya (2010), the need for phenylalanine and tyrosine in tilapia feeding may be reduced as the animals grow, although for the physiological functions of the fish are strictly necessary.

The digestion of the nutrient components of the diets depends on the correlation with the activity of the digestive enzymes, and the phenylalanine promotes beneficial effects on the activities of digestive enzymes, being related to the synthesis and secretion of enzymes that consequently improves the digestive and absorptive capacity of the digestive system of fish (LI *et al*., 2015).

The influence of phenylalanine on the performance in certain sweet-water species acts as a growth promoter. When used in adequate levels, it triggers an increase in weight gain, as well as improving food and protein efficiency; This is due to the improvement of the digestive capacity and absorption of the fish promoted by phenylalanine (RONNESTAD *et al*., 2007).

In the study by Li *et al*., (2015), phenylalanine promotes an increase in the activity of digestive enzymes (trypsin, chymotrypsin, lipase, and α-amylase) and pancreatic enzymes, resulting in a better use and absorption of the foods of the artificial diets. Nevertheless, increases in supplementation levels cause a deficit due to the accumulation and oxidation factors, forming toxic metabolites (BORLOGAN 1992), in addition to an imbalance about the other amino acids (AHMED, 2009; ZEHRA and KHAN, 2014; REN *et al*., 2015). According to Mozanzadeh *et al*. (2018), the digestive enzymatic secretion for *Sparidentex hasta* changes when the effects of other essential amino acids and phenylalanine are in imbalance, causing deficiencies in growth and body composition.

The physiological changes in the fish can be triggered by the level of phenylalanine dietary, influencing the concentrations of phenylalanine and plasma glucose in the activity of the alkaline phosphatase enzyme. Also, it interferes in the chemical composition of the entire body, reflecting in the contents of moisture, crude protein, and lipids (REN *et al*., 2015). Danuwat *et al*. (2016), for *Pangasius bocourti*, indicated the chemical composition and amino acid for the whole body, establishing the requirement of dietary amino acids for species, guiding studies on the changes in the body concentration of phenylalanine according to age and the possible promoter effect of the anabolism, influencing fish growth.

Tyrosine supplementation exerts a sparing effect on phenylalanine because the organism can produce tyrosine, as opposed to phenylalanine, which would be responsible for the synthesis while tyrosine would be intended for energy production, thereby reducing its dietary requirement in fish without negatively affecting the performance of the animals

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(ZEHRA and KHAN, 2014; REN *et al*., 2015). Thus, tyrosine may become essential when the level of phenylalanine in the diet meets the minimum requirement (KIM, 1993).

The effects of phenylalanine on physiological responses also trigger better efficiency in the immune state, expression of cytokine-regulated genes, junction proteins and antioxidant enzymes in the intestinal tract, and hepatopancreas (LI *et al*., 2015; FENG *et al*., 2015). It plays a role in protecting the structural integrity of fish gills due to the expression of antioxidant enzyme genes, inhibiting the action of oxidative damage (FENG *et al*., 2015). Precursor of dopamine and neurotransmitters which acts as an anxiolytic in responses to stress during handling of fish (LI *et al*., 2009; ZEHRA and KHAN, 2014; CALHEIROS *et al*., 2019). It also acts as a cofactor in the production of phenylacetic acid, which promotes antimicrobial action in artificial diets (WANG *et al*., 2018).

The process of synthesis and secretion of digestive enzymes is closely related to the action of insulin, which increases the activity of the enzyme intestinal alkaline phosphatase and sodium and potassium ions in the enzymatic activity of cells, with the important role of performing maintenance, regulating metabolic and growth effects. In the development of digestive organs, the effect through the insulin receptor has an intrinsic activity with the tyrosine kinase. Besides, the effect of thyroid hormones on the growth of fish should be considered (WEN *et al*. 2009; BAKKE *et al*., 2010).

Studies on the trophic chain of stable nitrogen isotopes of the essential amino acids, including phenylalanine in *Fundulus heteroclitus*, indicate their positions and the possible application of essential compounds in changes in basal values and their interference in animal feeding behavior (McMAHON *et al*., 2015).

The effect of growth on the use of phenylalanine at appropriate levels is targeted at the body protein synthesis, which influences the growth of fish musculature, *i.e*., it triggers the growth of new muscle fibers in a process referred to as hyperplasia (YAMASHIRO *et al*., 2016). The influence of amino acids on protein synthesis, in particular, phenylalanine on muscle development from increased metabolic rate and/or available amino acid concentration, provides greater weight gain, resulting in fish growth (HOULIHAN *et al*., 1995).

The coloration and pigmentation physiology, in freshwater fishes, directed to communication, camouflage or behavior of strategic alterations, which occurs from the synthesis of L-tyrosine captured by adrenergic neurons, potentiated as pigmentary responses of animals (BALDISSEROTTO *et al*., 2014), is related to the action of tyrosine in the production of melanin (eumelanin).

The balance of amino acids in the diets is necessary to ensure maximal absorption, their balance being crucial to the optimal productive performance of the fish. Currently, the search for alternative foods with the aim of reducing the cost of diets and maintain the proportion of amino acids essential determines the levels of phenylalanine for practical diets (Fig. 05). The protein and amino acid requirements, based on the natural matter of the diets as a standard source of protein, express the quantities and proportions of essential and non-essential amino acids in the diets (FURUYA, 2010).

Diets, when formulated from total amino acids, may provide an excess of amino acids that will undergo deamination, reflecting energy expenditure for nitrogen excretion.

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However, the formulation of diets with the use of digestible amino acids increases their accuracy, minimizes excess nutrients, promotes the reduction of production costs, especially with the use of foods that have a variable amino acid composition (NRC, 2011). Therefore, the knowledge of the digestibility coefficients of the amino acids phenylalanine and tyrosine, allow the new formulations of rations that more efficiently meet the requirements of the fish.

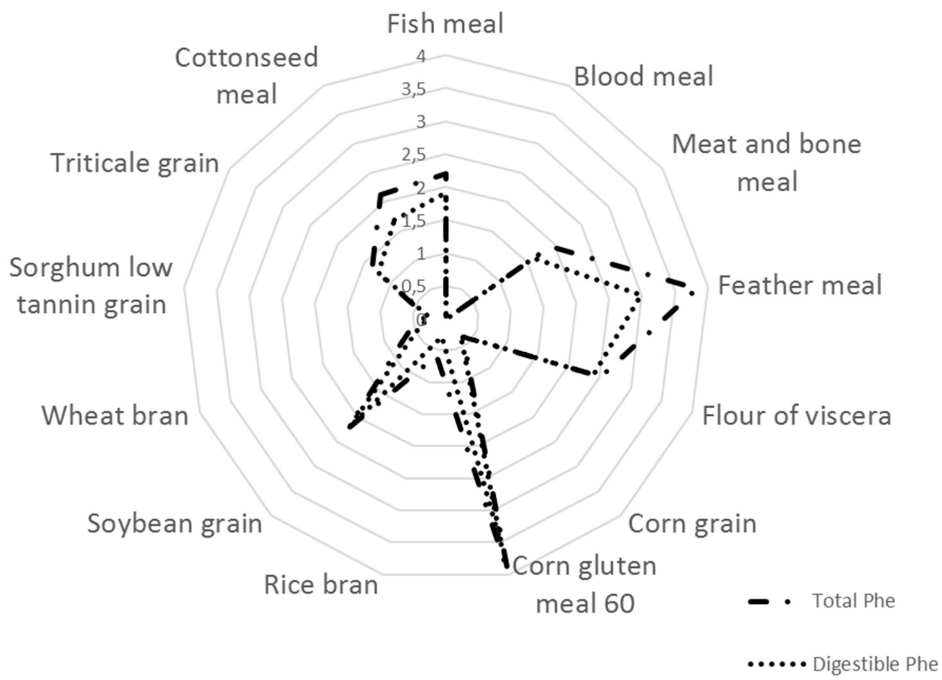


**Figure 05:** Total and digestible phenylalanine in feed formulations of fish diets.

## FINAL CONSIDERATIONS

The nutritional importance of phenylalanine, whether from protein sources of animal or vegetable origin or in its crystalline amino acid form, should meet the optimal requirements of the target species, as it affects the balance of the physiological processes that promote the growth of the fish. Although the research provides guidance on its effect, additional studies are recommended in order to elucidate its action better.

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