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# Optimizing nutrition in rainbow trout: evaluation of alternative ingredients in feed formulation

# Evaluación de materias primas alternativas en la formulación de alimentos para trucha arcoíris (*oncorhynchus mikyss*)

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**Abstract**

*Trout farming is a significant primary sector activity in Mérida, Venezuela; however, it has experienced a notable decline in recent years, largely due to difficulties in accessing suitable and affordable feed. The formulation of balanced diets for Rainbow Trout (Oncorhynchus mykiss) relies heavily on fishmeal and fish oil as essential raw materials. These ingredients are vital because they offer an optimal amino acid profile and contain Polyunsaturated Fatty Acids, which enhance the Feed Conversion Rate for this carnivorous salmonid species. However, the production of fishmeal and fish oil has been increasingly constrained by stringent regulations on trawling, which aim to address its detrimental effects on marine ecosystems and the competition for food resources among human populations. To address these challenges, several feed formulations have been proposed that theoretically fulfill the nutritional requirements of Rainbow Trout while considering the limitations concerning the inclusion of specific raw materials and the physiological factors pertinent to the species. To aid in the formulation calculations, a computer program named Trout* ***Formulation.exe*** *has been developed. This tool is designed to optimize feed composition in accordance with these nutritional requirements and constraints. As a result, six formulations are proposed that theoretically fulfill the nutritional requirements of Rainbow Trout, utilizing alternative raw materials instead of the two traditional ingredients. These alternatives include Black Soldier Fly (Hermetia illucens) larvae meal reared on fish industry waste substrate, as well as Amaranth (Amaranthus spp.) and Buckwheat (Fagopyrum esculentum) meal. It was determined that while a complete elimination of fish meal and fish oil from the formulation is not feasible—since they play a critical role in ensuring palatability and maintaining the ω6/ω3 balance essential for the proper development of the trout—the formulation incorporating up to 55% Black Soldier Fly larvae meal achieves a theoretical reduction of only 13% in the inclusion of Super Prime fish meal.*

**Keywords:** concentrated trout feed, black soldier fly larvae meal, amaranth, buckwheat, formulation.

**Resumen**

*La Truchicultura, como actividad del sector primario en el estado Mérida-Venezuela, ha decaído fuertemente en los últimos años, principalmente debido a la dificultad para acceder al alimento adecuado y a costos asequibles. La harina y aceite de pescado, son las materias primas clave en las formulaciones de alimento balanceado para Trucha Arcoíris (Oncorhynchus mykiss,) debido a su equilibrio aminoacídico y de Acidos Grasos Poliinsaturados, que aumentan la Tasa de Conversión Alimenticia de este salmónido carnívoro. Sin embargo, su producción está comprometida por las crecientes regulaciones sobre la pesca de arrastre, que afectan negativamente el equilibrio biológico de mares y océanos y compiten con la alimentación humana. En esta investigación, se plantean diversas formulaciones que cumplen teóricamente con los requerimientos nutricionales de la Trucha Arcoíris, considerando las limitaciones de inclusión de ciertas materias primas por razones fisiológicas. Para facilitar los cálculos de formulación, se creó un programa computacional denominado* ***Formulación Truchas.exe****. Como resultado, se proponen 6 formulaciones que cumplen teóricamente con los requerimientos nutricionales de la Trucha Arcoíris, utilizando materias primas alternativas como la harina de larva de*

*Mosca Soldado Negra (Hementia Illucens) criada en substrato de residuos de la industria del pescado, harina de Amaranto (Amaranthus spp.) y harina de Trigo Sarraceno (Fagopyrum esculentum). Aunque no es viable eliminar completamente la harina y el aceite de pescado en la formulación, ya que garantizan la palatabilidad y el equilibrio ω6/ω3 necesario para el desarrollo adecuado de la trucha, se encontró que una formulación con hasta un 55% de harina de larva de Mosca Soldado Negra podría reducir teóricamente hasta un 13% la inclusión de harina de pescado de tipo Súper Prime.*

**Palabras clave**: alimento concentrado para truchas, harina de larva de mosca soldado negra, amaranto, trigo sarraceno, formulación.

## 1 Introduction

Regarding salmonid feed, the trend has been oriented towards the formulation of concentrated fish feed from raw materials of plant and animal origin as an alternative to fishmeal, in order to achieve sustainable aquaculture over time. It has been reported (Makkar et al., 2014) that insect meal could potentially replace between 25% and 100% of fish meal in fish feed, mainly because the general levels of essential amino acids in insect meals are good for this purpose. In the case of vegetable meals, they provide a good source of starch which is useful for the feed pelleting process (Tacon, 1989) and some also provide an excellent source of amino acids and essential fatty acids which, in the latter case, can reduce the dependence on fish meal and fish oil as the main source of protein and lipids, respectively. Amino acids are the basic components of proteins and the amount of these elements present in the feed is decisive in determining the "quality" of the protein, which constitutes its value as a primary component of the diet (Guerrero-Muñoz, 2012). Of the 20 amino acids found in proteins, fish can metabolize 10 and therefore the others must be supplied in the diet, the latter are known as essential amino acids. For salmonids such as rainbow trout (Oncorhynchus mykiss), an absolute requirement of 10 ami-amino acids has been demonstrated: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (National Research Council, 1993).

Nutritional deficiencies caused by low amino acid intake in aquaculture species and animals are very difficult to diagnose due to the general state of alteration that is evident in the animal (Castro and Avilia, n.d.). Faced with a nutritional problem of this type, the main indicators shown by the animal are: a deterioration in conversion efficiency (feed/weight), a reduction in growth rate and a much higher mortality than expected (Guerrero-Muñoz, 2012). However, in certain fish species, such as rainbow trout, a deficiency of methionine or tryptophan also leads to the development of pathologies, because these amino acids not only constitute proteins, but are also used for the synthesis of other essential compounds (National Research Council, 1993).

In the case of dietary lipids, they must be important sources of essential fatty acids (EFAs), which are necessary for proper growth and development and also aid in the absorption of fat-soluble vitamins. Dietary lipids, mainly in the form of triacylglycerols, are hydrolyzed by digestive enzymes to a mixture of free fatty acids and 2-monoglycerides. These compounds are then absorbed and used for the synthesis of various cellular components such as fatty acids with double bonds at the ω6 (linoleic series) and ω3 (linolenic series) positions (Tacon, 1989; or catabolized for energy (National Research Council, 1993). Rainbow trout show an exclusive requirement of fatty acids belonging to the ω3 series in their diet: Linolenic acid (18:3ω3), Eicosapentaenoic acid (EPA) (20:5ω3) and Docosahexaenoic acid (DHA) (22:6ω3) (Tacon, 1989). Although ω6 fatty acids may be essential, their required level in the diet is unknown and evidence indicates that a high ratio of ω6 to ω3 fatty acids in a trout diet may actually be detrimental to the growth of the fish (Takeuchi & Watanabe, 1977). Feeding rainbow trout with experimental diets deficient in EFA has resulted in decreased growth and survival, as well as poor feed conversion efficiency (Tacon, 1989). Other effects of stress include dermal signs (broken fins), shock syndrome, myocarditis and increased mortality.

In order to obtain raw materials that meet the nutritional requirements of trout, these must have a composition that satisfies the needs to achieve their optimum development. In this sense, the required levels of the necessary macro and micronutrients that a concentrated feed must have to achieve a good development of Rainbow Trout, in its juvenile stage, are reported in Table 1.

In their natural habitat, rainbow trout consume insects that fall on aquatic surfaces; it is for this reason that alternatives to fish meal can be found with the use of insect meal. Black soldier fly larvae meal (*Hermetia illucens*) has been successfully implemented in concentrated diets for rainbow trout (Kroeckel et al., 2012), confirming that the good balance of essential amino acids contained in this meal satisfies the nutritional requirements of trout. Thanks to the ease with which this insect can be reared on low-cost substrates such as organic waste, especially those of the fishing industry, and its ability to take advantage of the nitrogen and phosphorus present in such waste, generating a lower environmental impact in this matrix, the meal of the larvae of this fly represents a valid source of protein and fat, with remarkable results mainly in aquaculture (Mancini et al., 2017). In addition to this, the Black Soldier Fly, from the family of the diptera *Stratiomyidae*, can be found in nature in tropical and subtropical areas. It has not been reported as a transmitter of diseases; on the contrary, it acts as a regulating agent of other pathogenic pests such as the common house fly (*Musca domestica*).

**Table 1.** Nutritional requirements of a concentrated feed for Rainbow Trout to ensure good development during the ova to fry stage.

| Macro or Micronutrient | Percentage required in the diet (%) | | |
|---|---|---|---|
| **Crude Protein (1,2)** | ≥ 50 | | |
| **Essential Amino Acids (3)** | | | |
| **Arginine** | ≥1,4 | | |
| **Histidine** | (0,6-1) | | |
| **Isoleucine** | ≥1,0 | | |
| **Leucine** | (1,8-2,5) | | |
| **Lysine** | ≥2,1 | | |
| **Cystine + Methionine** | ≥1,1 | | |
| **Phenylanine** | ≥1,2 | | |
| **Threonine** | ≥1,4 | | |
| **Tryptophan** | ≥0,2 | | |
| **Valine** | ≥1,2 | | |
| **Lipids (1)** | (10-20) | | |
| **If as α-Linolenic Acid** | 20 | | |
| **If as EPA and DHA** | 10 | | |
| **Carbohydrates (3)** | ≤ 20 | | |
| **Minerales (1)** | | | |
| **Calcium (*)** | 0-1 | | |
| **Phosphorus** | 0,7-0,8 (inorganic) | | |
| **Magnesium** | ≥0,006 | | |
| **Iron** | N. D | | |
| **Zinc** | 0,0015-0,003 | | |
| **Iodine** | 0,6-1,1 | | |
| **Selenium** | 0,1-0,35 | | |
| **Vitamins (1,2)** | | | |
| **Vitamin A +** | ≤ 2000 | **Thiamine *** | ≥10 |
| **Vitamin $D_3$ +** | ≤ 3000 | **Riboflavin *** | ≥20 |
| **Vitamin K *** | ≤ 80 | **Niacin *** | 150 |
| **Vitamin E +** | ≥ 30 | **Pantothenic Acid *** | 40 |
| **Antioxidant *** | 200 | **Pyridoxine *** | 10 |
| **Moisture (%)** | 6-10 | **Cobalamin ($B_{12}$) *** | ≥0,02 |
| **Canthaxanthin** | 190-450 | **Folic acid*** | ≥5 |
| **Biotin*** | 1 | **Inositol*** | ≥400 |
| **Ascorbic acid*** | ≥100 | **Colina*** | ≥3000 |

**Label:** +: U.I/Kg food, *: mg/Kg food.
**Sources:** Hilton and Slinger, 1981 (1); Guerrero-Muñoz, 2012 (2); National Research Council, 1993 (3).

On the other hand, although there has been little success in the substitution of fishmeal with raw materials of vegetable origin, Amaranth (*Amaranthus spp.*) as well as Common Buckwheat Flour (*Fagopyrum esculentum*) have a higher crude protein content than that presented by cereals that are also used in fish feed formulations, presenting one of the best amino acid profiles of all protein-rich vegetable feeds, headed mainly by a high content of lysine and methionine which are deficient in most cereals and which are fundamental in the growth of Rainbow Trout. In addition to its protein content, Amaranth and Buckwheat Flours represent a good source of starch, (Venskutonis & Kraujalis, 2013), which makes it suitable for industrial applications in the elaboration of concentrated feed for Trout.

This research, in addition to determining alternative raw materials that reduce the dependence on fishmeal and fish oil, proposes formulations for the nutrition of Rainbow Trout, considering at the same time the limitations that these raw materials have at a nutritional and economic level, through the development of a computer program.

## 2 Background

### *2.1 Black Soldier Fly Larvae Meal in commercial salmonid feed formulas*

Makkar et al. (2014) highlight the potential of rearing Black Soldier Fly larvae as an alternative to reduce by 25-100 % the use of soybean meal and fish meal in fish feed formulation, whose production costs are constantly increasing. In particular, in rainbow trout feed, it was demonstrated that dried pre-pupae of black soldier fly, reared on dairy cattle manure enriched with 25-50 % trout offal, can replace up to 50 % of fishmeal-derived protein in diets for a period of 8 weeks. This replacement had no significant impact on fish growth or sensory quality of trout fillets, although a slight (but not statistically significant) reduction in growth was observed.

During a 78-day period, isonitrogenous, isolipidic and isoenergetic diets were evaluated with increasing levels of partially defatted larval meal of Hermetia illucens (HI) as a substitute for fish meal: 0 % (HI0, control diet), 25 % (HI25) and 50 % (HI50). The results indicated that partially defatted larval meal can be included in trout diets up to 40 % without affecting survival, growth performance, feed conversion factor (kg feed supplied/kg weight gain of trout), somatic indices, physical quality parameters of dorsal fillet and intestinal morphology of the fish.

However, Dumas et al. (2018) investigated the impact of partially defatted black soldier fly larval meal (BSFLM) and mechanically extracted black soldier fly larval oil (BSFLO) on growth, body composition, gut histology and blood plasma biochemistry of rainbow trout during a three-month growth trial. They found that the feed conversion factor increased significantly with each increasing level of BSFLM inclusion ($p < 0.05$) and that there was a negative relationship between blood glu-cosa and BSFLO inclusion level, suggesting a possible antihyperglycemic effect of BSFLO in fasting trout. Based on these results, the authors recommended a maximum inclusion of 13 % for BSFLM in rainbow trout diets, while for BSFLO the inclusion could not exceed 10 %. These limits are associated with a higher digestibility of hydroxyproline in diets containing black soldier fly larvae products, representing potential benefits for trout.

In the study of physicochemical characteristics of rainbow trout fillets, Mancini et al. (2017) and Renna et al. (2017) evaluated three diets with progressive levels of fish meal substitution by *Hermetia illucens* larval meal (Hi): a control diet without Hi inclusion (0 %), Hi25 (25 % Hi inclusion) and Hi50 (50 % Hi inclusion). The results showed an increase in saturated fatty acid content, particularly lauric acid (C12:0), associated with Hi inclusion, at the expense of a decrease in monounsaturated and polyunsaturated fatty acids (including ω3 and ω6). In addition, a reduction in the yellowish coloration of fillets was observed with the Hi50 diet, which negatively impacted the concentration of adenosine monophosphate in fillets. However, no significant differences in the proximal composition or protein profile of the fillet were reported.

Bolton et al. (2021) formulated five diets to evaluate their effects on rainbow trout fillets. These included a basal diet with fish meal (CTRL), two diets with inclusion of 15 % and 30 % black soldier fly (BSF) larvae meal (BSF15 and BSF30, respectively), and two diets with the same levels of BSF but supplemented with a supplemental pro-tease enzyme (BSF15P and BSF30P, respectively), designed to evaluate possible dietary improvement. The results showed an increase in yellow coloration of the muscle as the proportion of BSF in the diet increased ($p = 0.004$). Also, trout fed BSF showed significantly less lipid peroxidation after harvest ($p < 0.001$), with a reduction in malondialdehyde levels proportional to the increase in BSF in the diet, regardless of the addition of protease. In addition, protease treatments increased total protein concentration in trout body content, reflecting potentially improved efficiency of protein utilization. In conclusion, the protease-enriched black soldier fly larvae meal-based diet constitutes a high-energy protein concentrate, viable to replace up to 30% of fish meal in rainbow trout diets, without adverse effects on trout growth.

### *2.2 Amaranth and Buckwheat as part of a concentrated fish feed formulation*

The use of pseudocereals such as amaranth and buckwheat in fish feed formulation presents an underexplored area of study, but with enormous potential for development. Poczyczyński et al. (2014) analyzed the effects of substituting fish oil for amaranth oil in rainbow trout feeds, finding that the highest daily growth rate (3.75%/day) corresponded to the group fed the diet containing the highest level of amaranth oil (7.2% inclusion). Although there is no direct history on the use of amaranth meal as a protein ingredient in rainbow trout diets, Ngugi et al., 2017 evaluated the nutritional properties and feasibility of replacing fish meal with amaranth leaf protein concentrates (ALPC, *Amaranthus hy-bridus*) in the diet of Nile tilapia (*Oreochromis niloticus*). This study compared the effects of substitution in terms of fish growth, nutrient utilization, body composition and apparent digestibility, demonstrating that it is possible to replace up to 80% of fishmeal with ALPC without affecting the performance of *O. niloticus*.

Although at the moment, there is no research linking the use of buckwheat flour in concentrated feed formulations for rainbow trout, it has a crude protein content between 12% and 18.9%, higher than that of cereals that are also commonly used in fish feed formulations (Christa & Soral-Smietana, 2008). More important than the crude protein content, however, is the presence of one of the best amino acid profiles of all protein-rich vegetable feeds, headed mainly by a high lysine content (amounting to about 6 g/100 g of protein) (Bonafaccia et al., 2003), which is fundamental in the growth of Rainbow Trout, and which is deficient in most cereals, which may account for its efficiency in the use of formulations.

## 3 Methodology

### *3.1 Design of a computer program named Truchas.exe*

In order to develop a formulation that meets the nutritional requirements of rainbow trout, it is essential that it provides a composition that satisfies the biological demands necessary for proper development. In this context, the use of programming methods allows the design of diets with an optimal balance of nutrients, resulting in more efficient and profitable fish rearing (National Research Council, 1993). These programs consider several key factors: the specific nutritional needs of rainbow trout, the nutrient content and bioavailable energy in the ingredients, the minimum and maximum restrictions on the concentrations of various components, and, last but not least, the cost of the raw materials used.

Based on the research on the nutritional requirements for optimal growth of rainbow trout, a program was developed and named Trout Formulation.exe. This software, designed on the Microsoft Visual Basic platform and programmed in C++, integrates four main modules that are described below.

*3.1.1 Main Program Window*

The main window appears after logging into the Trout Formulation.exe program (see Figure 1). In this module, the user finds shortcuts to all the functionalities of the software, organized in icons for convenience. In addition, at the bottom of the interface, an information bar shows the name of the active user, as well as the date and time of use.

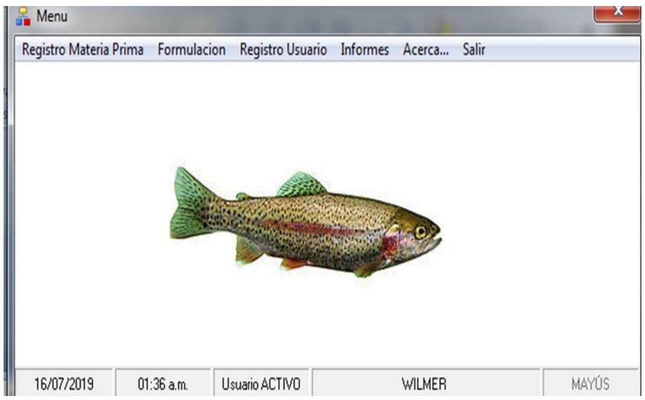



**Figure 1.** Main screen of the program Trout Formulation.exe

*3.1.2 Raw Materials Registration*

By selecting the icon located at the top left of the main window, you access the Raw Material Registration module. This module allows recording the nutritional composition of each raw material used in the feed formulation, covering both macronutrients and micronutrients. The information recorded is stored in an internal database designed in Microsoft Office Access®. The nutritional composition of the raw materials is recorded in specific units per 100 g of the ingredient, as follows: macronutrients and minerals, in percentage by mass (% w/w); vitamins A, D3 and E are quantified in Inter-national Units (I.U.); while the rest of the vitamins are entered in milligrams per kilogram of raw material (mg/kg).

*3.1.3 Formulation*

The Formulation module allows you to perform mass balances to calculate the amounts of macro- and micro-nutrients in the diet, using the units previously described. These calculations are made from the entered proportions of the selected raw materials, applying the following formulas:

$$\%Nutrient = \frac{(M_1 * Nut_{M1} + M_2 * Nut_{M2} .... + M_n * Nut_{Mn})g}{1000g\ of\ formulated\ feed} * 100$$

**Equation 1.** Internal formula of the Program to calculate the nutrients expressed in Percentage (% w/w) where $Nut_{Mn}$: Nutrient contained in the nth raw material used in the formulation.

$$Vitamin = \frac{M_1 * \frac{Nut_{M1}}{100g} + M_2 * \frac{Nut_{M2}}{100g} ... + M_n * \frac{Nut_{Mn}}{100g}}{1000}$$

**Equation 2.** Internal formula of the Program to calculate vitamins expressed in miligrams and International Units

The formulator can access the raw materials stored in the program database and assign the corresponding amount of each to formulate 1000 g of feed (ensuring that the total sum of the ingredients is exactly 1000 g). Once the process is completed, it is possible to assign a unique ID or specific record to save the formulation. The program will automatically calculate the resulting nutritional composition, which will be displayed on the screen when the "Print" button is pressed. Figure 2 illustrates an example of the nutritional composition of a random formulation, expressed in the units previously described.


**Figure 2.** Report shown by the Trout Formulation.exe program, of a random formulation with ID: 4. In blue is denoted crude protein (CP) and essential amino acids, in yellow lipids, in red carbohydrates and in black crude fiber (CF), minerals and other vitamins.

*3.2 Multi-level formulation table*

The feed formulation process, according to the proposed computer program, is characterized by being iterative and generating an unlimited number of alternatives. However, evaluating all these formulations through calculations, field tests and laboratory trials would take considerable time. Therefore, it is essential to establish specific conditions as a preliminary criterion of feasibility and effectiveness, in order to filter and optimize the formulations before entering them into the program. In this context, and based on the literature review, a multilevel table was designed. This tool allows, in a precise way, to identify those combinations of raw materials that, at a theoretical level, meet the nutritional requirements of rainbow trout in their juvenile stage (given that this phase is critical due to its high caloric demand and sensitivity of trout to nutritional imbalances), thus facilitating their subsequent entry into the program to generate the final composition of the formulations. The multi-level table optimizes the process by discarding unviable formulations before their evaluation in the program.

buckwheat flour, whose nutritional composition was documented by Bonafaccia et al. (2003), were included.

To cover lipid requirements and balance ω3/ω6 fatty acids, amaranth oil, based on information from

| Nutritional Component | Fish Meal[1] | | | | Amaranth/ Sarracen Wheat[2] | | Soldier Black Fly Larvae Meal | | |
|---|---|---|---|---|---|---|---|---|---|
| Type | Prime | Super Prime | LT | | Seeds | | Chicken Subs[4] | Fish Subs[5] | |
| Proteins (%) | 67 (min.) | 68 (min.) | 70 (min.) | | 11-21 | | 35 (min.) | 60 (min.) | |
| Lipids (%) | 10 (máx.) | 10 (máx.) | 10 (máx.) | | 10,6 (min) | | 20 (máx.) | 20 (máx.) | |
| Moisture (%) | 10 (máx.) | 10 (máx.) | 10 (máx.) | | 10 (máx.) | | 10 (máx.) | 10 (máx.) | Limitations on Use |
| Lysine (%) | 7,5 | 8-8,5 | 9 | Limitations on Use | 1,16 | Limitations on Use | | | |
| TVN (100mg/100 gr Harina) | 120 | 100 | 85 | | - | | N. D | N. D | |
| Histamine (ppm) | 1000 (máx.) | 500 (máx.) | 100 (máx.) | | - | | N. D | N. D | |
| Antioxidant (ppm) | 150 (máx.) | 150 (máx.) | 150 (máx.) | | N. D | | 150 (máx.) | 150 (máx.) | |
| | Levels of use | | | | Levels of use | | Levels of use | | |

| Levels of use (10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%) | Fish Meal | Amaranth/ Sarracen Wheat | Soldier Black Fly Larvae Meal |
|---|---|---|---|
| Lower levels | None Max. Utility | None: Starch and Protein Utilized | None Max. Utility |
| Intermediate levels | | | Doubts about palatability |
| Higher levels | High price[2] | Excess Carbohydrates[3] | Lipid and Amino Acid Imbalance[4] |

| Level of Inclusion (%) | 5 | 7,5 | 10 | 12,5 | 15 | 17,5 | 20 | >20 |
|---|---|---|---|---|---|---|---|---|
| Fish Oil | Max. utilization profile of ω3 | | | | High-Cost Infeasibility and Blockage Problems[6] | | | |
| Amaranth Oil | Reduction of Fishmeal Dependency[7] | | Lipid imbalance due to excess of ω6[7] | | | | | |

**Sources: (1):** Paucar, 2014; (2): Venskutonis y Kraujalis, 2013, Bonafaccia et al., 2003; (3): Hilton y Slinger, 1981; (4): Spranghers et al., 2017; (5): Barroso et al., 2019; (6): Bortone, 2007 y (7): Conchillo et al., 2006

**Figure 3**. Multilevel table designed based on the composition and limitations in the use of raw materials.

To design the multilevel table, information was collected on the selected raw materials: a) Fish meal: Three types were included from lower to higher quality (Prime, Super Prime and LT - low temperature), based on the nutritional composition and production costs described by Paucar (2014). b) Black soldier fly larvae meal: Nutritional variants of larvae fed with chicken feed and with waste from the fishing industry were considered, according to the findings of Barroso et al. (2019). c) Starch sources: Amaranth flour, obtained from seeds of species such as A. Dubius, A. Caudatus, A. Cruentus and A. hypochondriacus (studied by Venskutonis & Kraujalis, 2013b), and

Poczyczyński et al. (2014), and fish oil, as reported by Conchillo et al. (2006), were incorporated.

In addition, the multilevel table establishes constraints related to the costs of these raw materials. For example, although fishmeal offers an excellent protein and essential amino acid profile, its inclusion as a main ingredient in trout feeds is limited. This is due to the increase in cost, depending on the nutritional quality, which has repercussions on economic formulations. As for amaranth and buckwheat flours, despite their good lysine content, their use is restricted to levels of 20-22% in the formulation, mainly due to their high carbohydrate content, an inherent characteristic of vegetable flours.

When analyzing the limitations of the use of the two types of black soldier fly larvae meal as a pure ingredient, the following is observed: the meal derived from larvae reared on a broiler feed substrate presents a high content of saturated fatty acids, accompanied by a reduced level of ω3 fatty acids, which could lead to growth deficiencies in trout. On the other hand, in the meal obtained from larvae fed with a substrate coming from fishery waste, doubts arise about its palatability from an inclusion level of 50%. Figure 3 illustrates the multilevel formulation table developed.

To complement the multilevel table, the diets proposed by Hilton and Slinger, (1981), have made it possible to determine the appropriate limits for the inclusion of meals, oils, premixes, pigments and antioxidants (see Table 2), with the purpose of reaching the optimum levels of proteins, lipids, carbohydrates, minerals and vitamins necessary for the healthy development of rainbow trout.

**Table 2.** Ideal ratios of raw materials for the formulation of balanced diets for rainbow trout.

| | |
|---|---|
| **Flours (%)** | 84 |
| **Oils (%)** | 10 |
| **Premix Vitamins (%)** | 1 |
| **Premix Minerals (%)** | 1 |
| **Pigmenters (mg/Kg feed)** | 190 |
| **Antioxidants (mg/Kg incorporated flours)** | 200-500 |
| **Water (%)** | 8 |

**Source:** Hilton and Slinger (1981).

## 4 Results and Discussion

After entering various formulations in the *Trout Formulation.exe* program, six theoretical formulations were selected for in situ testing of a concentrated feed for Rainbow Trout in the fry stage. The proportions of raw materials used in these formulations are presented in Table 3.

**Table 3.** Proportion of raw materials in proposed formulations.

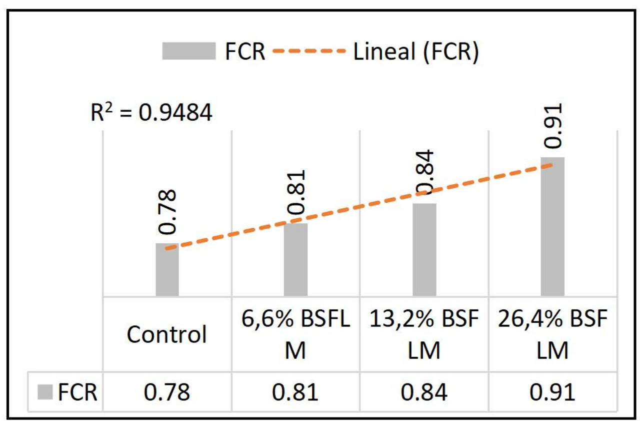
| **Formulation** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| **%** | | | | | | |
| **Fishmeal SP** | 60 | 60 | 57 | 31 | 13 | - |
| **Amaranth Flour** | - | 25 | 22 | 22 | 20 | 21 |
| **Buckwheat Flour** | 25 | - | 6 | - | - | - |
| **Black Soldier Fly Larvae meal +** | - | - | - | 38 | 55 | 67 |
| **Fish Oil** | 10 | 10 | 10 | 4 | 8,5 | 7 |
| **Amaranth Oil** | - | - | - | 2 | - | 1 |
| **Premix Vitamins*** | 1 | 1 | 1 | 1 | 1 | 1 |
| **Premix Minerals*** | 1 | 1 | 1 | 1 | 1 | 1 |
| **Water** | 3 | 3 | 3 | 1 | 1,5 | 2 |

**Label:** SP: Super Prime, +: Black soldier fly larvae meal reared on fish substrate described by Barroso et al. (2019). * With the conditions described by Hilton and Slinger (1981).

On the other hand, tables 4 and 5 show the results of the nutritional composition generated by the program, based on the mass balances of the proposed formulations. Based on these data, the theoretical feasibility of using black soldier fly larvae meal, fed with substrates from fishery waste, as well as amaranth flour and oil and buckwheat flour, was evaluated. These ingredients were considered as possible substitutes for fish meal and fish oil in concentrate feed formulations designed for rainbow trout.

In formulations 1 (which includes up to 60% Super Prime fishmeal) and 3 (with up to 28% vegetable meals), a protein level slightly lower than that reported as optimal for rainbow trout during the fingerling phase (≤45%) was observed (Hilton and Slinger, 1981; Orna, 2010). However, both formulations present a higher balance than required in all essential amino acids for rainbow trout proposed by Halver (1972), highlighting the level of lysine (≥2.1%), which could favor an adequate development of this salmonid.

In formulation 6 (which totally excludes fishmeal), although the protein requirement is met, the lysine level is close to the lower limit necessary for trout feeding. Therefore, the extent to which it is feasible to eliminate the inclusion of fishmeal in optimal formulations should be carefully evaluated.




**Source:** (Dumas et al., 2018).
**Label:** BSFLM: Black soldier fly larvae meal.

**Figure 4.** Increase in Feed Conversion Factor (FCR) with respect to the increase of black soldier fly larvae meal in the formulation.

According to Dumas et al. (2018), an inclusion of up to 26.4% of black soldier fly larvae meal as seen in Figure 4 could increase the Feed Conversion Factor (FCR) (feed administered in g/fish mass gain in g) by 15% in trout, resulting in trout reaching marketable size at least one month later than those fed the control formulation.

However, Renna et al. (2017) reported that a partially defatted black soldier fly larvae meal can be used in rainbow trout diets with inclusion levels up to 40%, without compromising survival, growth, somatic indices, or dorsal fillet quality. Both studies agree on the need for further studies to design feeding strategies and dietary formulations that mitigate the adverse effects of insect meal on the fatty acid profile of trout, which are largely dependent on the substrates used for rearing black soldier fly larvae.

**Table 4.** Theoretical nutritional composition in macronutrients of the formulated diets.

| Nutrient | Formulations | | | | | |
|---|---|---|---|---|---|---|
| | (1) | (2) | (3) | (4) | (5) | (6) |
| **Protein (%)** | 43,81 | 45,25 | 43,29 | 48,48 | 45,48 | 45,14 |
| **Arginine (%)** | 2,50 | 2,87 | 2,73 | 2,58 | 2,48 | 2,06 |
| **Histidine (%)** | 1,05 | 1,07 | 1,02 | 1,15 | 1,12 | 1,08 |
| **Leucine (%)** | 3,27 | 3,34 | 3,20 | 3,06 | 2,71 | 2,41 |
| **Lisina (%)** | 5,00 | 5,10 | 4,87 | 3,78 | 2,25 | 2,07 |
| **Methionine (%)** | 4,09 | 4,13 | 3,78 | 2,48 | 2,36 | 1,78 |
| **Phenylalanine (%)** | 1,73 | 1,86 | 1,77 | 1,68 | 1,48 | 1,32 |
| **Threonine (%)** | 1,64 | 1,76 | 1,68 | 1,64 | 1,52 | 1,33 |
| **Tryptophane (%)** | 0,45 | 0,37 | 0,37 | 0,40 | 0,41 | 0,37 |
| **Valine (%)** | 1,95 | 1,97 | 1,89 | 2,06 | 2,06 | 1,87 |
| **Lipids (%)** | 16,78 | 16,80 | 16,55 | 16,72 | 20,51 | 20,87 |
| **C18:3ω3 (%) *** | 0,38 | 0,38 | 0,37 | 0,34 | 0,40 | 0,39 |
| **C20:5ω3 (%) *** | 5,37 | 5,38 | 5,18 | 4,04 | 6,48 | 6,01 |
| **C22:6ω3 (%) *** | 4,27 | 4,27 | 4,11 | 4,47 | 7,37 | 7,54 |
| **∑ω3 (g/100 g Lip) *** | 54,08 | 54,07 | 53,07 | 33,47 | 46,55 | 40,12 |
| **∑ω6 (g/100 g Lip) *** | 5,056 | 5,86 | 6,09 | 16,29 | 7,79 | 13,15 |
| **ω6/ω3** | 0,09 | 0,11 | 0,11 | 0,486 | 0,17 | 0,33 |
| **Carbohydrates (%)** | 20,05 | 15,42 | 18,34 | 13,57 | 12,34 | 12,96 |
| **Fiber (%)** | 14,44 | 9,07 | 10,09 | 7,29 | 6,39 | 4,86 |

**Label: *:** Balances performed manually

In relation to the level of polyunsaturated fatty acids (HUFA) type ω3, which constitute the main source of energy for the vital functions of Rainbow Trout, formulations 1, 2 and 3, where Amaranth Flour and Buckwheat Flour are used as a partial substitute for Fish Meal, present a balanced content of these fatty acids. This is mainly due to the inclusion of a higher percentage of fish oil, known for its high content of ω3 fatty acids. In spite of having a lower lipid level than other formulations, they reach a value close to 10% w/w in the sum of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This characteristic allows the trout to optimize the use of essential amino acids for the synthesis of “body protein”, while using EPA and DHA in energetic functions, such as homeo-viscous regulation, a process by which fish adjust the composition of the phospholipids of their bio-membranes in response to changes in environmental temperature (National Research Council, 1993).

**Table 5.** Theoretical nutritional composition in micronutrients of the formulated diets.

| Nutrient | Formulations | | | | | |
|---|---|---|---|---|---|---|
| | (1) | (2) | (3) | (4) | (5) | (6) |
| **Phosphorus (%)** | 8,76 | 0,13 | 0,14 | 0,36 | 0,74 | 0,52 |
| **Calcium (%)** | 0,12 | 0,16 | 0,15 | 1,19 | 2,28 | 2,08 |
| **Magnesium (%)** | 3,08 | 0,07 | 0,06 | 0,36 | 0,51 | 0,58 |
| **Iron (%)** | 1,13 | 0,008 | 0,008 | 0,02 | 0,04 | 0,04 |
| **Zinc (%)** | 7,58 | 0,01 | 0,02 | 0,02 | 0,02 | 0,03 |
| **Iodine (%)** | 0 | 0,001 | 0,001 | 0,03 | 0,04 | 0,05 |
| **Selenium (%)** | 0 | 0 | 0 | 0,004 | 0,005 | 0,007 |
| **Thiamine (mg)** | 25,7 | 25,25 | 25,39 | 25,22 | 25,2 | 25,21 |
| **Riboflavin (mg)** | 75,35 | 76,23 | 76,16 | 76,08 | 75,98 | 76,03 |
| **Niacin (mg)** | 142,55 | 127,25 | 131,19 | 126,98 | 126,8 | 126,89 |
| **Pant. Ac (mg)** | 75,00 | 78,75 | 78,30 | 78,30 | 78 | 78,15 |
| **Pyridoxine (mg)** | 15,38 | 16,9 | 16,76 | 16,67 | 16,52 | 16,60 |
| **$B_{12}$ (mg)** | 15 | 15 | 15 | 15 | 15 | 15 |
| **Folic Ac. (mg)** | 5 | 5 | 5 | 5 | 5 | 5 |
| **Biotin (mg)** | 0,25 | 0,25 | 0,25 | 0,25 | 0,51 | 0,25 |
| **Ascorbic Ac. (mg)** | 200 | 218,75 | 216,50 | 78,30 | 215 | 215,75 |
| **Inositol (mg)** | 0 | 1,1 | 0,97 | 0,97 | 0,88 | 0,924 |
| **Choline (mg)** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Vitamin K (mg)** | 13,50 | 13,50 | 13,50 | 13,50 | 13,50 | 13,50 |
| **Vitamin A (U.I)** | 4000 | 4005 | 4004 | 4004 | 4004 | 4004 |
| **Vitamin $D_3$ (U.I)** | 250 | 250 | 250 | 250 | 250 | 250 |
| **Vitamin E (U.I)** | 75 | 75 | 75 | 75 | 75 | 75 |

**Label:** Pant Ac: Pantothenic Acid; Folic Ac: Folic Acid; Ascorbic Ac: Ascorbic Acid

On the other hand, formulations 4, 5 and 6, which integrate Black Soldier Fly larvae meal reared on fishery waste substrates, maintain lipid and protein percentages above the recommended minimum. However, formulation 4 presents a ω6/ω3 ratio higher than the optimum range established for Rainbow Trout feeding (0.08-0.2) (National Research Council, 1993), due to its higher inclusion percentage of Amaranth Oil, rich in ω6 type fatty acids.

Although to a lesser extent, formulation 6 also exhibits such a characteristic; however, it shows a difference of more than 10% in the summation of EPA (C20:5) and DHA (C22:6), which, as mentioned above, are structurally fundamental to the physiological functions of fish. The use of Amaranth Oil as part of the formulation is feasible, as a preliminary study by Poczyczyński et al. (2014) showed that, even with higher levels of Amaranth Oil inclusion than those proposed in these formulations (5.0% and 7.2%), no significant differences were found between the experimental groups and the control group. In addition, the highest specific growth rate (SGR) (3.75%/day) was observed in fish fed with the formulation containing the highest percentage of Amaranth Oil. In this group, there was also a higher content of crude protein and fat in the fish flesh (16.3% and 10.5% of body weight, respectively). Although the fatty acid composition was similar, a higher content of Amaranth Oil in the diet resulted in a marked decrease of EPA and DHA in the lipid profile of the fish.

Formulation 5, which includes only 13% Super Prime Fishmeal, stands out as the most nutritionally adequate formulation to ensure effective and sustainable feeding of Rainbow Trout. This formulation incorporates Black Soldier Fly Larvae Meal, a raw material that can significantly reduce the dependence on fish meal in traditional diets for this salmonid. Modifications in the rearing substrate of this larva further increase the flexibility of its use, since rearing larvae in a medium rich in ω3 fatty acids, as evidenced by Barroso et al. (2019) and Segura-Cazorla (2014), considerably improves their nutritional profile in relation to these compounds.

It is important to note that one factor that could influence the efficacy of substituting fish meal for black soldier fly larvae meal is the presence of chitin in the exoskeleton of the larvae at the prepupal stage. This polysaccharide, which constitutes approximately 87.0 g/kg dry mass (Kroeckel et al., 2012), can affect digestibility and nutrient absorption in Rainbow Trout. Although chitinase activity has been identified in the blood, plasma, and intestinal tract of some fish, it has not been conclusively demonstrated that this enzyme efficiently breaks down chitin in this species. Therefore, diets containing chitin or cellulose may significantly interfere with gastric lysozyme function, as noted by Lindsay (1984). The possible loss of gastric lysozyme could increase the susceptibility of trout to bacterial diseases sensitive to this enzyme, although this hypothesis still requires experimental validation. Since cellulose is a common ingredient in commercial salmonid feeds and is frequently used as a binding agent in experimental diets (starch), this aspect deserves further study.

## 5 Conclusions

Black Soldier fly larvae meal is presented as a promising alternative to replace fish meal in the formulation of concentrated feed for rainbow trout. Its nutritional profile is significantly optimized by using fish waste from the fishing industry as feed substrate. Likewise, the incorporation of Amaranth and Buckwheat flour, thanks to its excellent profile of essential amino acids, would efficiently complement this substitution, enhancing the quality of the formulated feed.

The ratio of ω6/ω3 fatty acids is a determining factor in ensuring that Rainbow Trout meet their energy requirements. To achieve an optimal value (0.08-0.2), the inclusion of fish oil is key. However, validation of the use of amaranth oil as a substitute in formulations requires additional studies to specifically evaluate its impact on the feed conversion ratio (FCR) of rainbow trout.

Based on the analyses carried out, it is concluded that formulation 5, composed of 13% Super Prime fishmeal, 20% Amaranth meal, 55% Black Soldier Fly larvae meal and 8.5% fish oil, stands out as the most adequate from both a nutritional and economic perspective. This formulation satisfies the parameters established in this research, guaranteeing an efficient and sustainable feed for rainbow trout, and constitutes an important step towards the reduction of fishmeal dependence in aquaculture.

The development of a computer program integrated to the application of a multilevel formulation table increased the capacity to calculate and develop 6 formulations for the feeding of Rainbow Trout with high potential for success, which represents a fundamental advance in the optimization of aquaculture, since it is based on rigorous technical and economic criteria for the design of nutritionally balanced formulations that maximize the growth of Trout with a minimized environmental impact.

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