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Improvements in the neurodevelopmental toxicity of heavy metals for *Dicentrarchus labrax* by an algal mixture of *Spirulina platensis* and *Chlorella vulgaris*

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ABSTRACT
In response to the rising demand for aquatic products for human consumption, aquaculture has developed significantly as an agronomic activity worldwide. Proteins and amino acids are significant macromolecules that govern vital metabolic processes and act as building blocks for the creation of compounds with biological significance. Fish play a significant function in human nutrition and are a significant dietary source of high-quality animal proteins and amino acids. The effect of toxic metals (cadmium and lead) on total central amino acids compositions (Essential, Conditionally Essential, and Non-Essential), and Crude protein concentration were examined in the current study for the juvenile European sea *Dicentrarchus labrax*, a stress-sensitive species, and evaluation the protective effect of the dietary *Spirulina platensis* and *Chlorella vulgaris* algal mixture as a supplement. Fish fed the control diet and kept in freshly filtered seawater without the addition of any pollutants (Control). Total metals (lead nitrate and cadmium chloride (0.006 mg/l) in the ratio (1:1) were added to rearing fresh seawater and fish were fed as the following diets in the other groups: control with metal group, 3% algal mixture supplemented diet (D1), 5% algal mixture supplemented diet (D2), and 7% algal mixture supplemented diet (D3) for 5 weeks. The results showed a significant decrease in the total amino acids in the control with the metal group compared to the control and a significant increase in essential amino acids, conditionally essential amino acids, non-essential amino acids, and crude protein concentration in the other 3 diet groups. By concentrating on the contributions towards our understanding of the role these metals have on adverse neurological outcomes in affected populations, this study demonstrated the impact of the mixed mixture of algae *Spirulina platensis* and *Chlorella vulgaris* on the treatment of the effect of cadmium (Cd) and lead (Pb) on neurodevelopment outcomes.

INTRODUCTION

Aquaculture is an important contributor to sustainable food and nutritional stability and is the fastest-growing variable in productivity and profitability. Marine fish are sensitive animals and their survival in captivity is full of challenges. Understanding the relationship between industrial fish farming and fish welfare science is essential to provide optimal survival conditions for fish and management systems. Despite the phenomenal growth of its production, aquaculture is experiencing various problems, and presents many difficulties, particularly when it comes to stress **(Schreck and Tort 2016).**

The European sea bass *Dicentrarchus labrax* is one of the main species of industrial marine fish farming, and the uncontrolled phenomenon of stress can have a major impact on aquaculture producers, leading to increased mortality and economic losses **FSBI (2002).** Instead of using medications to treat sick fish, there is a clear trend toward enhancing fish health and welfare to stop disease outbreaks by using dietary supplements or probiotics **(Reverter *et al*. 2020; El-Saadony *et al*. 2021)**. The antioxidant and immune systems of fish are stimulated by dietary supplements, which play important roles in fish health and welfare **Ahmadifar *et al*. (2021)**. Aquafeed production uses a variety of feed supplements, such as herbal materials, vitamins, minerals, and amino acids that enhance fish welfare and health **Lee *et al.* (2015)**. Amino acids (AAs) play crucial roles in physiological processes, as well as contributing to fish growth as the building blocks of proteins and as metabolic pathway intermediaries. They act as building blocks for the synthesis of numerous biologically significant compounds, such as nucleotides, peptides, hormones, and neurotransmitters. Additionally, (AAs) control the expression of genes and the protein phosphorylation cascade, both of which are crucial for cell signaling **(Wu, 2010; Zhu *et al*., 2013; Webster and Thompson, 2015)**, in addition, the innate and cell-mediated immune responses, as well as the transport and metabolism of nutrients in animal cells **Wang *et al*. *(*2013)**. It is widely recognized that the amount of protein and dietary (AA) an animal consumes significantly impacts their growth, reproduction, health, and feed costs **(Boshra *et al.*, 2006; Watanuki *et al.*, 2009*;* Wu *et al*., 2017)**. Animal AAs are categorized as either Essential (EAAs) or Nonessential (NEAAs) depending on the growth or nitrogen balance or Conditionally Essential Amino Acids (CEAAs) (**Rahimnejad and Lee 2013; Zhu *et al.*, 2013).** For human nutrition, the EAAs are arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, and valine; the CEAAs are glutamine, glutamic acid, glycine, proline, and taurine; and the NEAA are aspartic acid, serine, and alanine. However, the idea of Functional Amino Acids (FAAs) has recently been put forth. FAAs are those that take part in and control important metabolic pathways to enhance an organism's health, survival, growth, development, lactation, and reproduction **Wu (2013)**. The FAAs also show tremendous potential in the prevention and treatment of viral diseases, intrauterine growth restriction, infertility, intestinal and neurological dysfunction, and metabolic diseases (such as obesity, diabetes, and cardiovascular disorders). FAAs in human nutrition include taurine, arginine, cystine, leucine, methionine, tryptophan, tyrosine, aspartate, glutamic acid, glycine, and proline **Wu (2013)**. Determining the dietary AA requirements for various fish species is necessary due to the significant effects of AAs on animal growth, feed costs, and nitrogen pollution (**Rahimnejad and Lee 2013*)***. The dietary requirements of AA depend on animal species, developmental stage, physiological status, intestine microbiota, and environmental factors **Zhu *et al*. (2013)**. The majority of amino acids in the diet come from proteins, and the ratio of essential to non-essential amino acids is used to assess the quality of dietary protein. The dietary essential amino acids (EAA) in high-quality proteins are readily digested and present in amounts that meet human needs **(WHO, 2007).**

Global health concerns include environmental exposure to neurotoxic metals and metalloids like arsenic, cadmium, lead, mercury, or manganese. Environmental metals can affect neurodevelopment, neurobehavioral, and cognition, as well as lead to neurodegeneration, depending on the length of exposure over a lifetime. There is growing evidence that links early-life neurological diseases to environmental exposure to metal contaminants **Carmona *et al*. (2021)**. Harmful heavy metals are those that demonstrate highly toxic qualities when ingested by animals, including humans. Cadmium, lead, and mercury are among the top 10 substances that the World Health Organization considers to be particularly dangerous for human health. Exposure to these metals has also been related to many neurodegenerative problems in humans **Von Stackelberg *et al*. (2015).**

Microalgae have a significant role in various industries around the world, including food production, medicine, biofuels, cosmetics, and agriculture. *Spirulina platensis* and *Chlorella vulgaris* are two of the most well-known microalgae genera, both of which have bioactive substances like protein, vitamins, pigments, long-chain polyunsaturated fatty acids, sterols, and other substances that make them particularly interesting from the perspective of their potential health benefits. Spirulina has been chemically examined and has been demonstrated to be an excellent source of proteins, vitamins, lipids, minerals, nucleic acids, enzymes, and pigments **Andrade *et al*. (2018)**. Spirulina is an important source of protein for human nutrition, with the largest concentrations of leucine, valine, isoleucine, and tryptophan **Demelash (2018)**. Neurodevelopmental disorders (NDDs) are caused by genetic or environmental factors and can include intellectual difficulties, communication problems, traumatic brain injuries, autism spectrum disorders, epilepsies, and motor and coordination problems **Cordeiro *et al*. (2015).** Rodents and fish are model organisms for many of these human illnesses, allowing experiments to clarify the molecular underpinnings of their origins **(Ricceri *et al*., 2007**; **Kisby *et al*., 2013; Green and Planchart 2018).**

This study aimed to illustrate the effect of the mixed mixture of the algal of *Spirulina platensis* and *Chlorella vulgaris* on the treatment of the toxic effect of cadmium (Cd), and lead (Pb) on the brain central amino acids for European sea bass *Dicentrarchus labrax* by specifically focusing on the contributions toward our understanding of the role these metals have on adverse neurological outcomes in affected populations.

MATERIALS AND METHODS

Materials and methods

The experimental diets

Spirulina platensis algae was prepared, according to the technique developed by **Abou El-Kheir *et al*. (2008)** *Spirulina platensis* was grown and harvested at the Hydrobiology Lab of the National Institute of Oceanography and Fisheries in El-Qanater El-Khayria. At the marine hatchery at the National Institute of Oceanography and Fisheries in Alexandria, Egypt, *Chlorella vulgaris* cultures were grown **Hasanein *et al*. (2018)**. The fundamental practical diet was designed to meet the nutritional needs of juvenile European sea bass and contained about 47% crude protein and 12% crude fat. Three concentrations of dry extract were used to create the experimental diets. The basal diet served as the control and a mixture of *Spirulina platensis* and *Chlorella vulgaris* (AM, 1:1) were introduced at 30, 50, and 70g kg-1 diet.

The experimental design

The European sea bass *Dicentrarchus labra* was acquired from the National Institute of Oceanography and Fisheries' marine hatchery in Alexandria and acclimated for a week prior to beginning the experimental circumstances. Following fish acclimation, the initial fish weight of approximately around (IW= 5.0 g), at a stocking density of 15 fish per aquarium, were placed in 15 glass aquariums, each measuring 100 L, and were supplied with filtered natural seawater that was kept at a temperature of 23°C. These were the five experimental groups. The first group was fed a control diet and kept in fresh, filtered seawater without the addition of any pollutants (Control). As a contaminant, lead nitrate and cadmium chloride (0.006 mg/l) in a ratio (1:1) were introduced to the rising fresh filtered seawater and fish were fed as the following diets in the other groups: control with mixed metal, 3% algal mixture supplemented diet (D1), 5% algal mixture supplemented diet (D2), and 7% algal mixture supplemented diet (D3). Three times per day, fish were fed 5% of their body weight. The water was changed daily to avoid waste buildup, and the feces were drained before the first feeding. Filtered seawater was supplied with a salinity of 37.5 ppt and a temperature of 23.4 ±1.2 °C. The oxygen provided by aeration was present during the study at a level of 5.93 mg L-1. For the duration of the trial, water quality parameters were recorded. Total ammonia and nitrite levels were measured throughout the feeding experiment, the PH was 7.6 ±0.31, ammonia levels were 0.05 ± 0.01 and nitrite concentrations were 1.4 mg/l. The natural photoperiod was 10 h light: and 14 h dark. To maintain a steady concentration of heavy metals in each aquarium, freshly prepared stock solutions of heavy metals were supplied daily to rearing water following the residual siphon. Five weeks were spent on the experiment.

Ethical Clearance

The author declares that the sampling of this species under investigation was done following the guidelines of the international conventions for the use of animals in scientific research.

Tissue preparation

The fish was rapidly killed by a sharp knife blow to the head and the entire brain was swiftly removed, fast rinsed in iso-osmolar NaCl salt water to remove blood, and then weighed **Hegazi *et al*. (2010).**

Amino acid compositions:

Three whole brain samples were pooled and homogenized to exactly three times their weight in ice-cold distilled water. Crude homogenates (0.3 g) were deproteinized by the addition of 10 mL g^{-1} sulfosalicylic acid (3.5%). The supernatant was collected after centrifugation at 3500 rpm for 15 min and stored at -16°C for amino acid analysis. The brain's free amino acids were determined using a Beckman amino acid analyzer (model 157 CL).

Estimation of amino-acids:

The determination of amino acids was accomplished according to the method adopted by **Sánchez-Machado *et al*. (2003)**. First, the amino acids were hydrolyzed by hydrochloric acid (HCl) and then derivatized then analyzed on the HPLC.

Derivatization with PITC:

The derivatization procedure was a modification of the method of **González-Castro et al. (1997)**. Amino acid standard solution or sample hydrolysate (30 µL) was placed in a tube and dried in an oven for 20 min at 42^{o}C. Methanol–water–TEA (2:2:1, v/v; 50 µL) was then added to the residue, and the resulting solution was vacuum-dried for 20 min at room temperature (25 ^{o}C). Methanol–water–TEA–PITC (triethylamine(TEA) and phenylisothiocyanate (PITC)) (7:1:1:1, v/v; 50 µL) was then added, and the tubes were vortex for 15 s, then left for 20 min at room temperature. The resulting solution was dried for 100 min at room temperature (25 ^{o}C). After derivatization, Na_2HPO_4 (5 mM containing 5% acetonitrile; 100 µL) was added as the diluent, with vortex mixing for 15 s. The mobile phase was prepared from two solutions, A and B. Solution A was 0.14 M ammonium acetate buffer containing 0.05% (v/v) TEA (pH adjusted to 6.4 with glacial acetic acid). Solution B was acetonitrile-water 60:40 (v/v). The relative abundance of each peak and the comparison to amino acid standards allow for the quantification of each amino acid in the sample.

Statistical analysis

The mean ± SD of each reading parameter is used to represent it. One-way analysis of variance (ANOVA) and multiple comparisons were used to analyze the data by Dunnett's experiment with a computer program (GraphPad 6 In Stat Softeware, Inc.

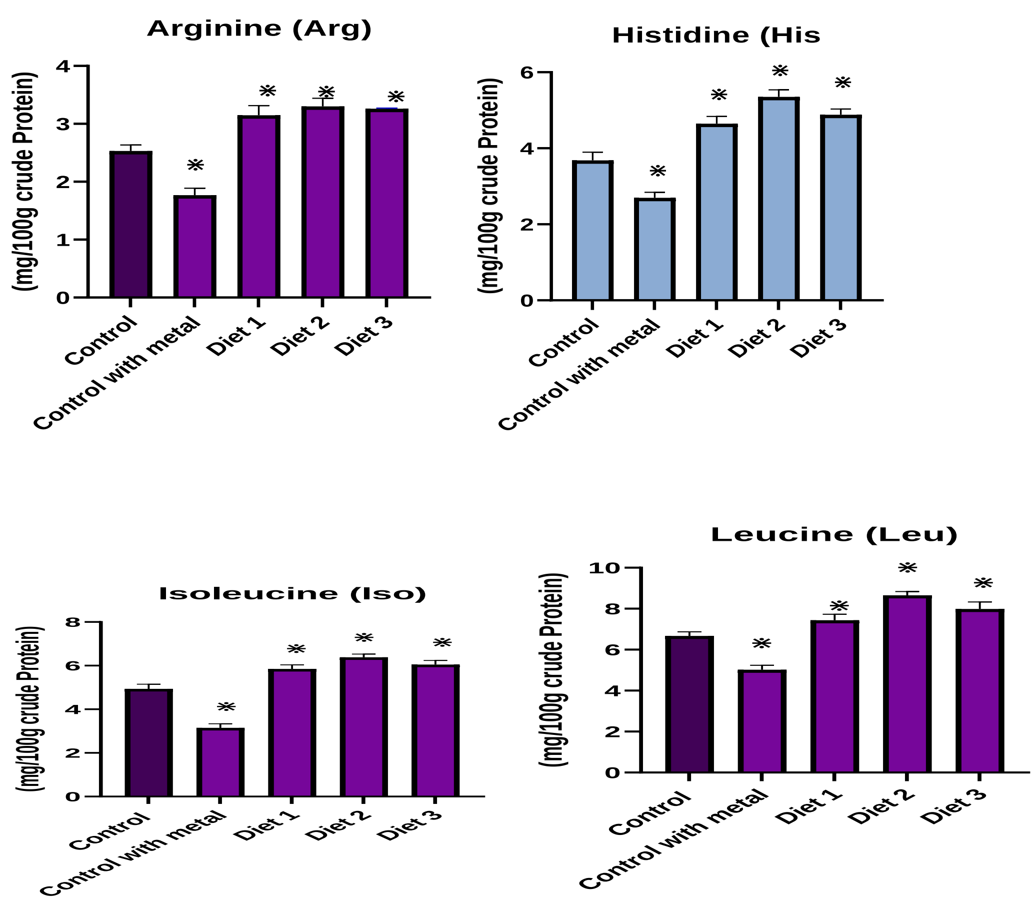
RESULTS

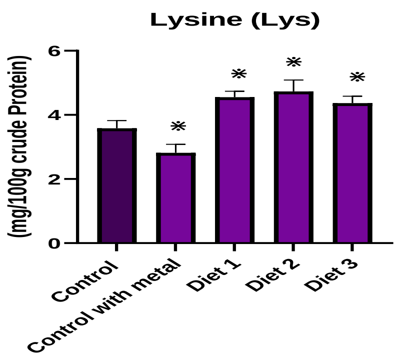
Fish were exposed to mixed of lead nitrate and cadmium chloride (0.006 mg/l) in a ratio (1:1) for 5 weeks. In (Fig. 1), the content of **Essential Amino Acids (EAAs)** in the whole brain tissues arginine(Arg) and histidine (His), showed a significant decrease in them in the control with metal group at ($P < 0.05$) in comparison with the control. Diets with an algal mixture of *Spirulina platensis* and *Chlorella vulgaris* (AM, 1:1) at 3% (D1), 5% (D2), and 7% (D3) showed a significant increase in both amino acids composition between the control and the other 3 diets group. No difference appeared within D1, D2, and D3 in (Arg) but in (His) D2 showed the highest value. In amino acids iso leucine (Iso) and leucine (Leu)There was a significant decrease in the control with metal group at ($P < 0.05$) in comparison with the control and a significant increase between the control and the D1, D2, and D3 groups. There was no significant difference between D1, D2, and D3 in both of them. In amino acids methionine(Met), lysine(Lys), and threonine (Thr) There was a significant decrease in control with the metal group at ($P < 0.05$) in comparison with control and a significant increase between the control and the D1, D2, and D3 groups. There was no significant difference within D1, D2, and D3 in 3 amino acid contents. In phenylalanine (Phe), tyrosine (tyr), Valine (Val), and tryptophan(Try)There was a significant decrease in control with metal group at ($P < 0.05$) in comparison with the control and a significant increase between the control and the D1, D2, and D3 groups. There was no significant difference within D1, D2, and D3 in 3 amino acid contents (Phe), (tyr), and (Val) but in tryptophan (Try) D2 and D3 values were greater than D1. In amino acid cysteine (cyst), there was no value in group control with metal and there was a significant increase between the control group and the D1, D2, and D3 groups. D2 showed the highest value among the 3 diet groups. In general, D1, D2, and D3 showed amino acid content greater than the control and control with metal.

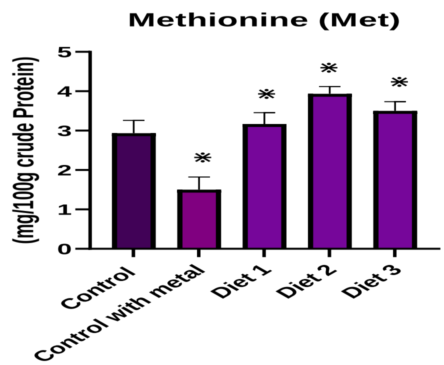
In **Conditionally Essential Amino Acids (CEAA)**, in fig. (2), There was a significant decrease in control with the metal group at ($P < 0.05$) in total values of amino acids glutamic (Glu), glycine(Gly), and proline (Pro) in comparison with the control group and a significant increase between the control group and the groups D1, D2, and D3. There was no significant difference within D1, D2, and D3 in (Glu) and (Gly), amino acid contents but in (Pro) D2 showed the highest value among the 3 diet groups. Three diet groups showed amino acid content greater than the control and control with metal

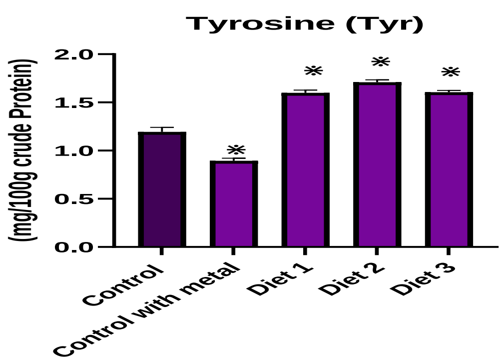
In **Nonessential Amino Acids (NEAAs)** composition in the whole brain tissues (Fig. 3). There was a significant decrease in total amino acid alanine(Ala), aspartic acid(Asp), and serine(Ser) in the control with the metal group at (P < 0.05) in comparison with control and significant increase between the control and the D1, D2, and D3 groups. There was no significant difference within D1, D2, and D3 in (Ala) contents but in (Asp), and (Ser) D2, D3 were greater than D1. The three diet groups showed amino acid content greater than the control and control with metal.

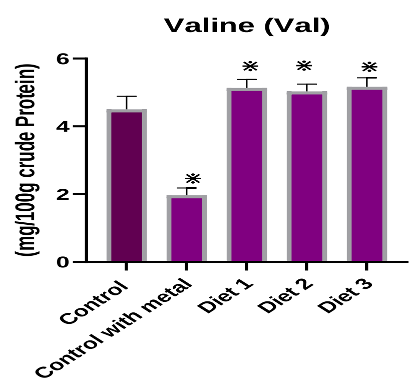
The percentage of crude protein appeared a significant decrease in the control with the metal group at (P < 0.05) in comparison with the control and a significant increase between the control and the D1, D2, and D3 groups in (Fig. 3). There was no significant difference within D1, D2, and D3.




Lysine (Lys)
(mg/100g crude Protein)
0
2
4
6
Control
Control with metal
Diet 1
Diet 2
Diet 3


Methionine (Met)
(mg/100g crude Protein)
0
1
2
3
4
5
Control
Control with metal
Diet 1
Diet 2
Diet 3


Tyrosine (Tyr)
(mg/100g crude Protein)
0.0
0.5
1.0
1.5
2.0
Control
Control with metal
Diet 1
Diet 2
Diet 3


Valine (Val)
(mg/100g crude Protein)
0
2
4
6
Control
Control with metal
Diet 1
Diet 2
Diet 3

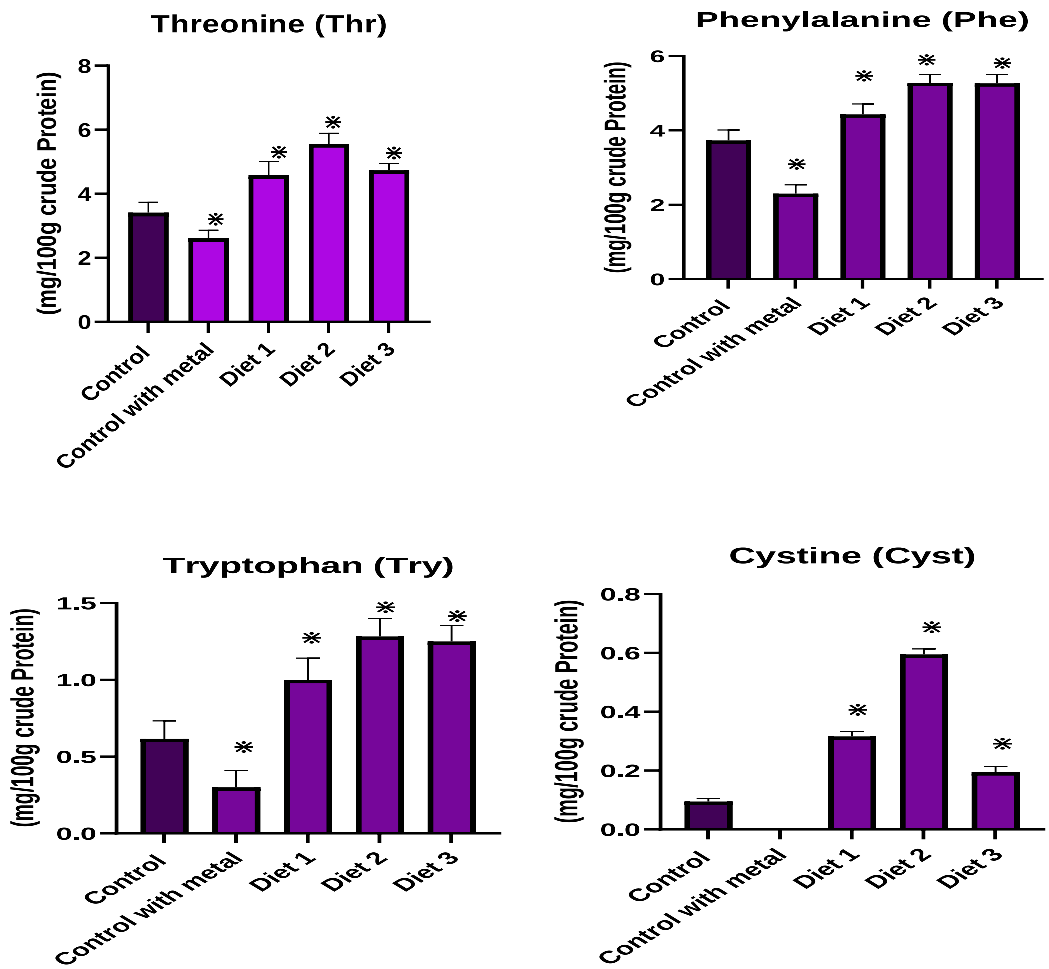


Fig. (1): The effects of an algal mixture of *Spirulina platensis* and *Chlorella vulgaris* on the central Essential Amino Acids (EAAs) composition in the whole brain tissues (mg/100g crude Protein) of *Dicentrarchus labrax* exposed to lead nitrate and cadmium chloride (0.006 mg/l) in the ratio (1:1) for 5week. Each reading represents the Mean± SD of 15 samples. The difference was significant at □$P \leq 0.05$.

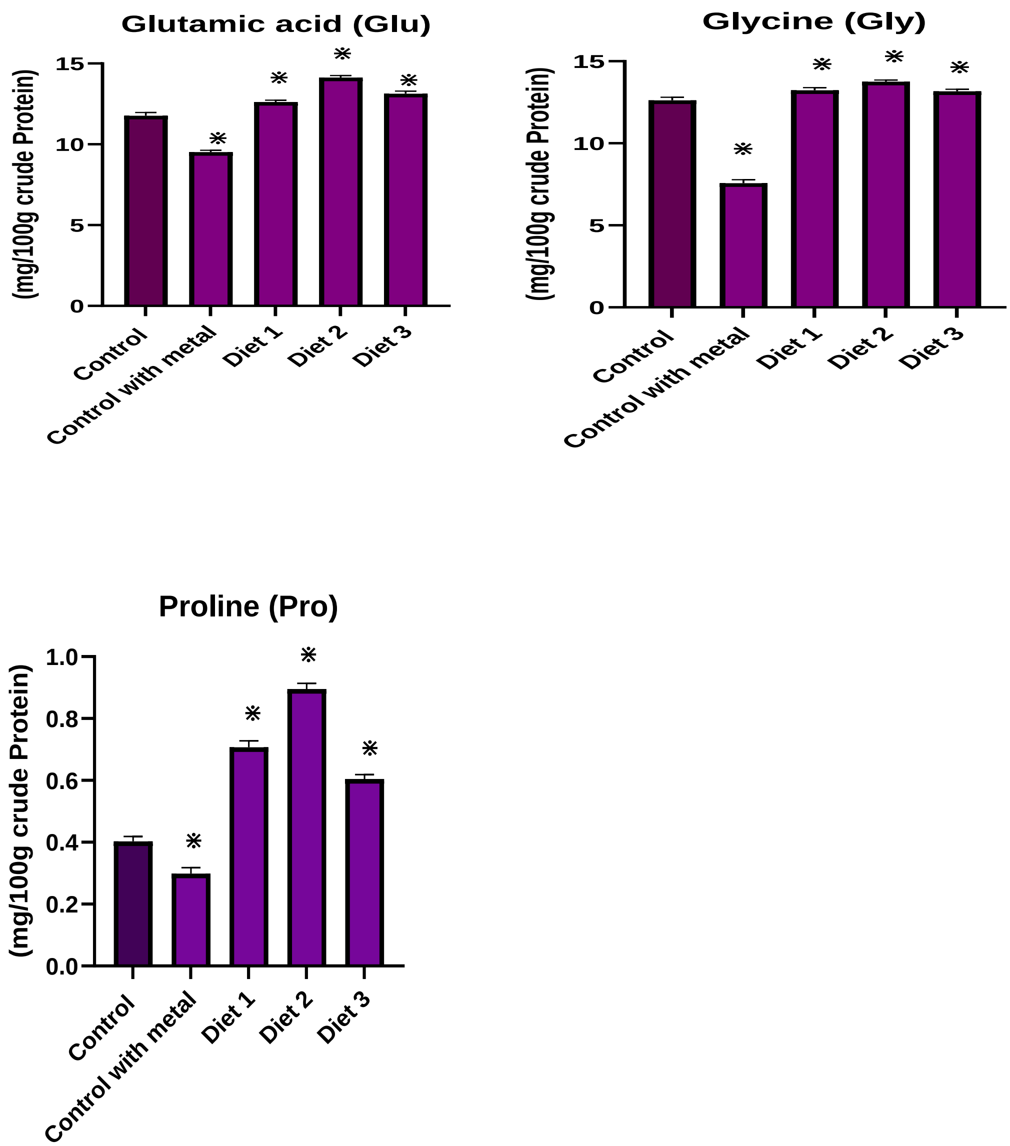


Fig. (2): The effects of an algal mixture of *Spirulina platensis* and *Chlorella vulgaris* on Conditionally Essential Amino Acids (CEAAs) composition in the whole brain tissues (mg/100g crude Protein)of *Dicentrarchus labrax* exposed to lead nitrate and cadmium chloride (0.006 mg/l) in the ratio (1:1) for 5week. Each reading represents the Mean± SD of 15 samples. The difference was significant at P≤0.05.

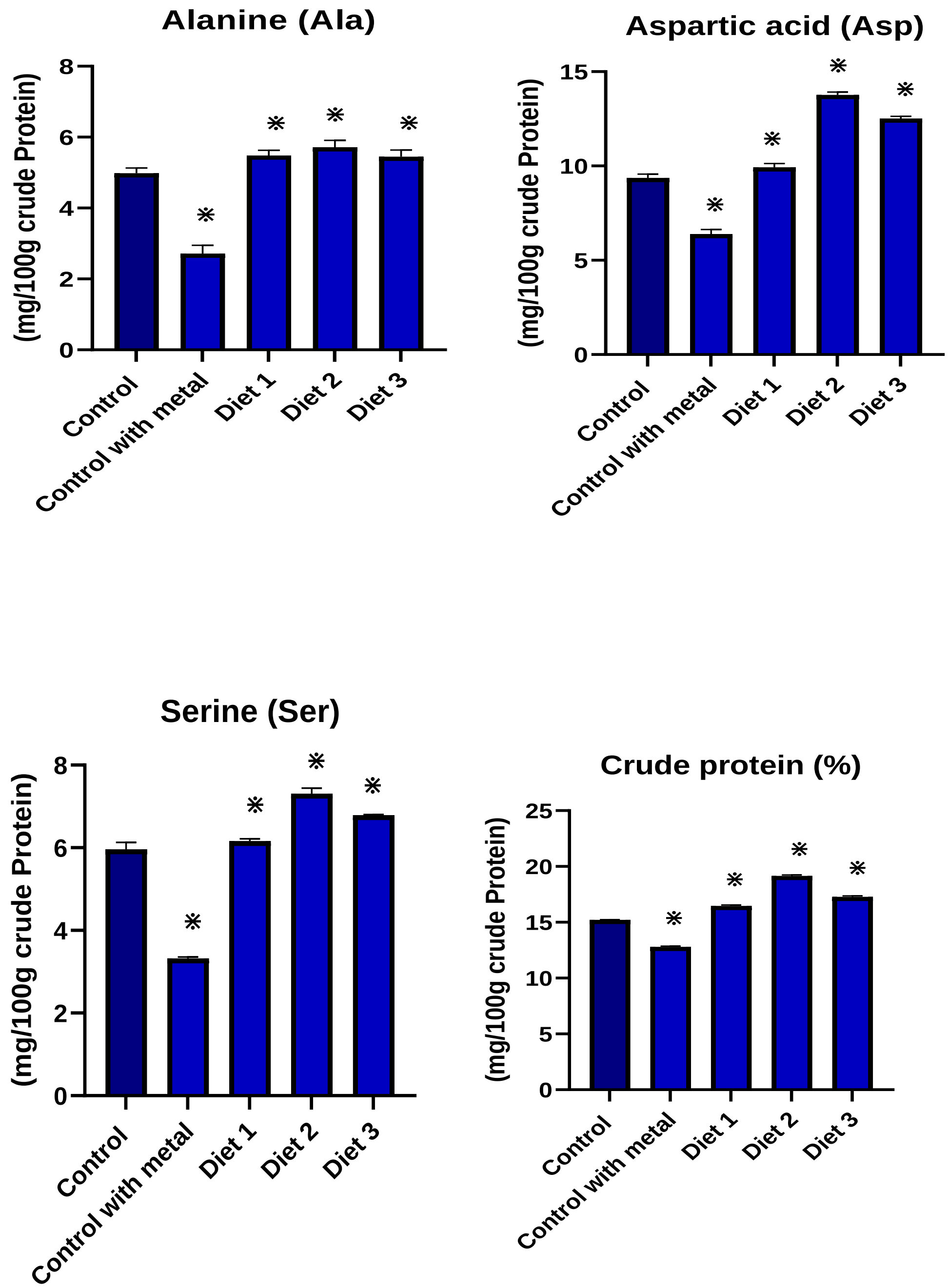


Fig. (3): The effects of an algal mixture of *Spirulina platensis* and *Chlorella vulgaris* on the Nonessential Amino Acids (NEAAs) composition in the whole brain tissues (mg/100g crude Protein) of *Dicentrarchus labrax* exposed to lead nitrate and cadmium chloride (0.006 mg/l) in the ratio (1:1) for 5week and percentage of crude protein. Each reading represents Mean± SD of 15 samples. The difference was significant at □P≤0.05.

DISCUSSION

In the current study, it could be observed that fish in group control with metal showed reduced content of Essential (EAAs), Conditionally Essential Amino Acids (CEAAs), and Nonessential Amino Acids (NEAAs) in the whole brain of *Dicentrarchus labrax.* An algal mixture of *Spirulina platensis* and *Chlorella vulgaris* (AM, 1:1) at 3%, 5%, and 7% as a supplement showed significantly increased values of (EAAs), (CEAA) and (NEAAs) in comparison with control. In this study, the algal mixture showed repairing the harmful effect of toxic metals (lead and cadmium) on the central amino acids and the total crude protein content in the brain tissues of *Dicentrarchus labrax*. These supplemented diets in the three examined ratios can be easily and affordably included in a daily diet for seabass (*Dicentrarchus labrax*) and provide central amino acids that are necessary for improvements and repairing brain toxicity and consequently improving growth. This is illustrated and agreed by our previous studies, **Hasanein *et al.*(2018)** demonstrated the beneficial effects on the growth and feed utilization of *Dicentrarchus labrax* when *Spirulina platensis* and *Chlorella vulgaris* were combined at 3%, 5%, and 7% supplemented diet in the presence of a 0.006 mg/l Cd and Pb concentration, the three supplemented diet reduced the accumulation of cadmium and lead in the white muscles and stimulate ROS scavenging in it. The present results are also suitable and agreed with our previous study (**Hasanein and Helal, 2021)** reported that at concentrations of 3%, 5% and 7%, the dietary supplement of S. platensis and C. vulgaris for seabass (*Dicentrarchus labrax*) has natural chelation properties for the mixture of lead and cadmium in the liver toxicity and gill toxicity that contain high levels of vital metals that have anti-Cd and anti-Pb effects. These dietary supplements increased the antioxidant activities in the liver, which is responsible for scavenging ROS, and repaired the ionic disturbance in the gills (caused by exposure to cadmium and lead). In the present study, the content of brain amino acid **Arginine (Arg)** showed (2.5 control, 1.8 control with metal, 3.1 (D1), 3.3 (D2), and 3.28 (D3) mg/100g crude protein). The three dietary supplements raised arginine content which has enormous properties in repairing physiological conditions. This finding agreed with several studies that explained the role of the addition of AAs as a supplement for fish diets in healthy growth such as **Han *et al*., 2018),** a considerable rise in serum levels of insulin (INS) and insulin-like growth factor-I (IGF-I) has also been linked to dietary arginine. **Wang *et al*. (2015)** reported that arginine promotes the target of the rapamycin (TOR) signaling pathway to encourage protein synthesis and myogenesis. Arginine also activates adenosine 5 -monophosphate (AMP)-activated protein kinase (AMPK) to assist the body in conserving and using the available energy rather than producing lipids. Additionally, arginine has been shown to have a variety of immuno-modulatory effects, including controlling fish's innate and adaptive immune responses, preventing leukocyte apoptosis, and regulating their susceptibility to bacterial infection **Hoseini *et al*. (2020)**. Cell division, wound healing, ammonia elimination, immunological function, and hormone release are all significantly influenced by arginine. Additionally, it serves as a precursor for the biological synthesis of nitric oxide, which is crucial for maintaining blood pressure, clotting, and neurotransmission. Numerous illnesses, including sepsis, preeclampsia, hypertension, erectile dysfunction, anxiety, and others, benefit from its additional treatment in humans. **Mohanty *et al*. (2014)**.

Because mature fish have low activity levels of arginine biosynthesis enzymes such pyrroline-5-carboxylate (P5C) synthase, ornithine transcarboxylase (OTC), and carbamoyl phosphate synthase (CPS) III, arginine is a necessary amino acid for fish **Andersen *et al*. (2016)**. Only N-acetylglutamate synthase (NAGS) in zebrafish *(Danio rerio)* has been thoroughly studied and reported to be an intermediate form from microbial to mammalian NAGS on the evolutionary path. Other urea cycle enzymes such as argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL) have rarely been evaluated in fish **Caldovic *et al*. (2014)**. Although adult fish do not require much adenosine triphosphate (ATP) for the urea cycle because they mostly excrete ammonia through their gills, the urea cycle enzymes are quite active during the early stages of fish development **Wright (2011)**. Numerous physiologically active metabolites, such as nitric oxide (NO), creatine, and polyamines, are produced from arginine in fish **(Han *et al*., 2018; Zheng *et al*., 2019)**. However, depending on the dosage, the fish's health, and the environment, arginine supplementation's effects on fish development and immunological responses may differ **Azeredo *et al*. (2015)**. Reduced fish development rate has been linked to dietary arginine deficiency a lack of dietary arginine has been linked to slower fish growth rates. Appropriate arginine supplementation can enhance fish growth and immunological responses, but an excess of dietary arginine can have the opposite effect **Hoseini *et al*. (2019)**. The amount of arginine needed by fish is not constant and fluctuates depending on the species. Overall, carnivorous species require more arginine in their diets than omnivorous fish **Wang *et al.* (2021)**.

In this study, the content of brain amino acid **Histidine (His)** showed (3.7 control, 2.8 control with metal, 4.68 (D1), 5.37 (D2), and 4.89 (D3) mg/100g crude protein). The algal mixture supplied His which recovered the depletion in group control with metal. This is agreed with **(Liao *et al*., 2013)** who revealed that Histidine aids in the removal of heavy metals from the body's myelin sheath maintaining tissue growth and repairing it. His is a precursor to histamine and has a variety of functions in protein interaction. In addition, **Jiang *et al*.(2016),** reported according to the growth performance of young grass carp fed a histidine-supplemented diet for two weeks the dietary histidine requirement was 7.63 g/kg. In addition, **Michelato *et al*. (2017)** reported the final weight, weight gain, feed conversion ratio, protein efficiency ratio, and net protein utilization in Nile tilapia juveniles fed diets containing graded levels of histidine. Unlike animals and birds, Fish can continually add new muscle fibers to their bodies. Histidine cannot be produced in the body by itself and must be received through nutrition, histidine is an essential amino acid in mammals, fish, and poultry. Histidine deficiency causes a reduction in body weight in some organisms. Additionally, nitrogen balances cannot be kept with a diet lacking histidine. Histidine insufficiency may not impair nitrogen balance if total protein intake is higher than the current standard (0.75 g/kg) according to a study by **Kriengsinyos *et al*. (2002)**. However, **(Moro *et al*. (2020)** illustrated histidine shortage results in a reduction in protein turnover and amino acid oxidation. Given these additional aspects of protein metabolism, histidine is regarded as a necessary amino acid in healthy people. Several studies have also noted histidine's necessity for ruminants. In this study, 5% of the algal mixture showed a higher value of histidine with no significant differential effects between the ratios 5%, 3% and7%.

In this study, the content of brain amino acid **Isoleucine (Iso)** showed (4.9 control, 3.2 control with metal, 5.93 (D1), 6.44 (D2), and 6.11 (D3) mg/100g crude protein). The content of brain amino acid **leucine (Ieu)** showed (6.7 control, 5.17 control with metal, 7.58 (D1), 8.70 (D2), and 7.79 (D3) mg/100g crude protein). The algal mixture supplied Ieu and Iso which are important for protein production and immunity. This agrees with **Wu. *et al*. (2017),** fish require the branched-chain amino acid leucine (Leu) to survive. However, the main focus of the following studies was on how Leu affected growth, protein retention, and immunity as **Deng *et al*. (2014)** on juvenile golden pompano *(Trachinotus ovatus*), **Tan *et al*. (2016)** on rainbow trout (*Oncorhynchus mykiss*), **Choo *et al*. (1991)** on Black carp Juvenile hybrid grouper *Epinephelus fuscoguttatus* ♀×*Epinephelus lanceolatus*♂, **Zhou *et al*.(2019)** on fingerling channel catfish *Ictalurus punctatus,* **Wilson *et al*. (1980)** on blunt snout bream *Megalobrama amblycephala,* **Ren *et al*. (2015) on** *Mylopharyngodon piceus*, Catla catla **Zehra, *et al*. (2015)** on *Heteropneustes fossilis*, and **(Farhat and Khan 2014)** on stinging catfish. Leu promoted proliferation and differentiation, according to studies done recently on primary preterm rat satellite cells and porcine myoblasts **(Jie-Min *et al*., 2015; Zhang *et al*., 2018).** Leu is the only dietary amino acid that may increase the production of muscle protein and it plays a significant therapeutic function in the treatment of stress-related illnesses such as burns, trauma, and sepsis in humans. Leu has been discovered to decrease the breakdown of muscle tissue when taken as a dietary supplement by enhancing the synthesis of muscle proteins. Marine fishes had unusually high levels of leucine **Mohanty *et al*. (2014)**. Leu is one of these EAA and is crucial for healthy growth and appropriate development in both humans and animals **Yamamoto *et al.* (2004).** Although these studies have only been conducted on fish, an ideal dietary leucine level has been shown to increase the secretion of growth hormone and regulate relative gene expression for animal growth **Deng *et al*.(2014).** Leu overload or deficit, however, may impair animal growth and decrease diet conversion **Choo *et al.*(1991)**.

Li, *et al.* (2010), revealed that leu deficiency causes decreased growth rate, low feed efficiency, and poor protein retention according to research on large yellow croaker, *Pseudosciaena crocea*, grass carp *Ctenopharyngodon idellus.* In general, leucine absorption and digestion, which are correlated with the functions of digestive enzymes, determine the rate of fish growth. The capacity of nutrients, such as leucine, to be digested and absorbed is generally correlated with the activity of digestive enzymes **(Wu *et al. (*2016)**. Few studies have been done to look into the effects of dietary leucine on the activities of fish liver enzymes, although many studies have shown that dietary AA plays a significant role in digestive and absorptive enzymes **(Tan *et al*.,2016; Wu *et al.* 2016)**. Leucine and its metabolite, hydroxy-methylbutyrate (HMB), are also crucial for the proper functioning of the immune system in animals. Adding leucine and/or HMB to the diet can increase the quantity of NK cells and liver-associated lymphocytes, which in turn lowers animal mortality (**Giri *et al.*, 2015; Jiang *et al.*, 2015*)***. In the head kidney of *Labeo rohita* fingerlings, appropriate leucine supplementation could improve immunological parameters such as the expression levels of antioxidant enzymes, -microglobulin, and immunoglobulin-M. Additionally, adequate dietary leucine controls the tight junction transcript abundance, intestinal immune status, and immune-related signaling molecules according to **(Wu *et al*., 2017).** Isoleucine is a branched-chain amino acid that is necessary for the development of muscles and healthy growth **(Charlton 2006)**. In this study, the content of amino acid **Valine (Val)** showed (4.7 control, 2.0 control with metal, 5.23 (D1), 5.19 (D2), and 5.48 (D3) mg/100g crude protein. Valine has been identified as an essential amino acid of fish **Nose (1979)**. It is involved in various metabolic pathways and is essential for growth, tissue repair, and maintaining nitrogen balance in the body. Valine deficiency resulted in reduced growth performance in mrigal carp (*Cirrhinus mrigala*) **(Ahmed and Khan, 2006)**. In this study, 3%, 5%, and 7% of the algal mixture showed no different effects between leucine, iso leucine, lysine, tyrosine, and valine.

In this study, the content of brain amino acid **Methionine (Met)** showed (2.9 control, 1.63 control with metal, 3.18 (D1), 3.78 (D2), and 3.69 (D3) mg/100g crude protein. Methionine in mammals is also used to treat depression, alcoholism, allergies, asthma, copper poisoning, radiation side effects, schizophrenia, drug withdrawal, Parkinson's disease, and liver diseases **(Mischoulon and Fava., 2002)**.

In this study, the content of brain amino acid **Threonine (Thr)** showed (3.5 control, 2.7 control with metal, 4.75 (D1), 5.6 (D2), and 4.83 (D3) mg/100g crude protein. Threonine is important for numerous nervous system disorders. In this study fish in the group control with metal showed a reduced amount of threonine in comparison with control. An algal mixture supplement showed significant increases in the values of threonine. The ratios of 3%, 5%, and 7% of the algal mixture showed no different effects between them. So The ratios of 3%, 5%, and 7% of the algal mixture showed a repairing effect for threonine. This is agreed with **(Hyland, 2007)** reported**,** numerous nervous system disorders, such as spinal spasticity, multiple sclerosis, familial spastic paraparesis, and amyotrophic lateral sclerosis, are treated with threonine.

In this study, the content of brain amino acid **Phenylalanine (Phe)** showed (3.8 control, 2.5 control with metal, 4.5 (D1), 5.2 (D2), and 5.1(D3) mg/100g crude protein. The content of brain amino acid **Tyrosine (Tyr)** showed (1.2 control, 0.9 control with metal, 1.6 (D1), 1.7 (D2), and 1.6 (D3) mg/100g crude protein. The content of brain amino acid **Tryptophan (Try)** showed (0.7 control, 0.3 control with metal, 0.9 (D1), 1.3 (D2), and 1.26(D3) mg/100g crude protein. In the three diet groups, the content of brain **Phe**, **Tyr, and Try** reduced the stress response resulting from heavy metal toxicity this agrees with the study of **Salamanca *et al*. (2020)** on gilthead seabream (*Sparus aurata*) and meagre (*Argyrosomus regius*) to study fish welfare in sea farms food supplemented with (phenylalanine and tyrosine) improved the stress response in both species by lowering several stress indicators. (Phe) is an important amino acid, and diets are the only way to consume it. This amino acid is broken down by The two mechanisms that make up the traditional metabolic pathways oxidation to the Tyr and transamination to phenylpyruvate **Shafik *et al*. (2014)**. Tyr affects the synthesis of the thyroid hormones (triiodothyronine and thyroxine) and is a direct precursor of catecholamine hormones the content of plasma thyroid hormones helps identify stress in *Rutilus rutilus caspicus* **Hoseini *et al*. (2014)**. Tyr can also be converted into hydroxyphenyl pyruvate and enter the energy metabolism. A Phe/Tyr-enriched diet in mammals reduces the effects of an acute stressor **Lehnert *et al*. (1984)**. A (Phe) metabolite called hydroxyphenyl pyruvate has been demonstrated by **Cotoia *et al*.(2014)** to enhance cellular viability in mice under stressful circumstances. Both AAs (Phe/Tyr) reduced incidences of bone deformation and death in white seabream larvae (*Diplodus sargus*) **Saavedra *et al*. (2010)** while **(Herrera *et al*. (2016)** confirmed the moderating effect of Phe on stressed cods in (*Gadus morhua*).

Tryptophan promotes brain Serotonin,5HT synthesis, which may stimulate 5HT release and function. It is a natural amino acid precursor in 5HT biosynthesis. The brain's monoaminergic systems, which include serotonin (5-hydroxytryptamine; 5-HT), dopamine DA, and noradrenaline NA as key neurotransmitters, are activated as part of the acute stress response. The neuronal responses in certain regions of the brain, primarily the telencephalon, hypothalamus, and brain stem, are modulated by these monoamines depending on the type of stressor, its duration, and the severity of its occurrence **(Kaslin and Panula, 2001; Barton, 2002; and Martorell-Ribera *et al*., 2020).** Tryptophan should have low toxicity and few negative effects because it is a natural part of the human diet. Due to these benefits, dietary tryptophan supplementation has been utilized with varying degrees of effectiveness in the treatment of neuropsychiatric diseases **(Demelash, 2018)**. The central nervous system's diffuse network of the serotonergic system plays a critical role in the control of mood and behavior cognition. The gut-brain axis is a two-way communication pathway that connects the brain's emotional and cognitive regions with the digestive system's peripheral functions. It is becoming more and more clear that the gut microbiota affects behavior, and this influence has been extended to tryptophan and serotonin, raising the potential that changes in the gut may play a role in the pathophysiology of illnesses of the human central nervous system **(Jenkins *et al*., 2016)**. Depression is frequently accompanied by low levels of certain brain chemicals or neurotransmitters, such as serotonin, dopamine, noradrenaline, and gamma-aminobutyric acid in humans (GABA) **(Van Praag 1983; Diehl and Gershon 1992; Stockmeier 1997; Rush 2007).** The amino acids tryptophan, tyrosine, phenylalanine, and methionine are frequently helpful in treating a variety of mood disorders, including depression, according to several studies **(Petty 1995; Leonard, 1997)**.

In this study, the content of brain amino acid **Glutamic acid (Glu)** showed (11.8 control, 9.6 control with metal, 12.6 (D1), 14.2 (D2), and 13.5 (D3) mg/100g crude protein. In this study, the content of brain amino acid **Glycine (Gly)** showed (12.7control, 7.6 control with metal, 13.2 (D1), 13.8 (D2), and 13.5 (D3) mg/100g crude protein**.** The content of **Proline (Pro)** in the brain showed (0.4 control, 0.3 control with metal, 0.7 (D1), 0.9 (D2), and 0.6 (D3) mg/100g crude protein. The dietary supplementation of the algal mixture increased the content of three (CEAAs) which improves growth performance, antioxidant, and immune responses. Glutamic acid plays a significant role in the metabolism of amino acids, due to its function in transamination reactions and the need to synthesize essential molecules like glutathione, which is needed for the elimination of highly toxic peroxides, and the polyglutamate folate cofactors **Mohanty *et al*. (2014)**. The dietary glycine supplementation improves growth performance, antioxidant, and immune responses in some fish and shellfish species, including Nile tilapia, *Oreochromis niloticus*, largemouth bass, *Micropterus salmoides*, and white-leg shrimp, *Litopenaeus vannamei*. glycine is a non-essential amino acid used in fish nutrition (**Xie *et al*., 2014**). It has been discovered to increase the activity of many antioxidant enzymes and is a part of the antioxidant molecule glutathione **(Xie *et al*., 2016 ; Rossi *et al*., 2021)**. Additionally, supplementing with glutathione has been shown to enhance immune responses in the mitten crab, *Eriocheir sinensis* according to **Liu *et al.* (2019)**, suggesting that dietary glycine supplementation may enhance fish immune systems by promoting glutathione synthesis (**Liu *et al*., 2019)**.

In this study, the content of brain amino acid **Alanine (Ala)** showed (5.2 control, 2.8 control with metal, 5.6 (D1), 5.8 (D2), and 5.45 (D3) mg/100g crude protein. In this study, the content of brain amino acid **Aspartic acid (Asp)** showed (9.4 control, 6.5 control with metal, 10.1 (D1), 13.8 (D2), and 12.7 (D3) mg/100g crude protein. the content of brain amino acid **Serine (Ser)** showed (5.9 control, 3.3 control with metal, 6.2 (D1), 7.4 (D2), and 6.8 (D3) mg/100g crude protein. The dietary supplementation of the algal mixture increased the content of three (NEAAs). NEAA is produced by the body on its own, some of the nutritionally significant NEAA is essential for controlling gene expression, micro-RNA levels, cell signaling, blood flow, nutrient transport, and metabolism in animal cells. They also aid in the development of brown adipose tissue, intestinal microbial growth, anti-oxidative responses, and innate and cell-mediated immune responses. Aspartic acid (FAA) controls the release of critical hormones and is a precursor to AAs methionine, threonine, isoleucine, and lysine. Similar to glycine, cysteine, and tryptophan, serine is the building block for all of these amino acids and is crucial for cell signaling. Schizophrenia can also be treated with serine in humans **(Mohanty *et al*., 2014)**.

CONCLUSION

The algal mixture of of *Spirulina platensis* and *Chlorella vulgaris* at concentrations of 3%, 5% and 7% as a supplement in the diet of ***Dicentrarchus labrax*** revealed improvements in the brain toxicity of cadmium and lead due to increased central amino acids content in the whole brain tissues which consequently improve all physiological functions of the central amino acids.

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