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Luiza Beatriz Hermes

FONTES E NÍVEIS DE FERRO NO CRESCIMENTO E SAÚDE DE TILÁPIA DO NILO (*Oreochromis niloticus*)

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Zootecnia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Mestre em Zootecnia**

Orientador: Prof. Dr. Rafael Lazzari

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RESUMO

TÍTULO: FONTES E NÍVEIS DE FERRO NA NUTRIÇÃO DE TILÁPIAS DO NILO (*Oreochromis niloticus*)

AUTORA: Luiza Beatriz Hermes
ORIENTADOR: Rafael Lazzari

O ferro (Fe) é um micromineral de extrema importância no desenvolvimento dos animais. O presente estudo visou avaliar o desempenho zootécnico, hematologia, imunidade inata, parâmetros bioquímicos e oxidativos de juvenis de tilápia (*Oreochromis niloticus*) submetidas a dietas contendo níveis (60 e 85 mg/kg) e fontes (orgânica e inorgânica) de ferro. Os peixes (16,57 ± 2,25 g; n= 400) foram distribuídos em 20 tanques (240 L, 20 peixes cada) e alimentados três vezes ao dia, durante sete semanas. O experimento foi composto por cinco tratamentos, em delineamento inteiramente casualizado: dieta basal (DB), sem inclusão de Fe na mistura mineral, 60 inorgânico, 60 orgânico, 85 inorgânico e 85 orgânico. O aumento da fonte orgânica de Fe na dieta melhora o desempenho de juvenis de tilápia. Fe orgânico aumenta o ganho de peso relativo (261,34%), peso final (61,73g) e biomassa (1254,55%). O índice digestivo somático e o índice hepatossomático foram mais altos em peixes alimentados com 85 Fe inorgânico. A glicose e a amônia do fígado das tilápias aumentam em peixes alimentados com Fe orgânico. O Fe orgânico melhora o hematócrito e a hemoglobina corpuscular média. Os valores de lisozima (LZ) diminuíram em peixes alimentados com Fe orgânico, mas a atividade hemolítica do sistema complemento (HACS) e o teste NBT aumentam com Fe orgânico. Peixes alimentados com dieta Fe 85 orgânico apresentaram melhor resposta antioxidante. Concluiu-se que a inclusão de fonte orgânica de Fe e nível de 85mg kg é mais eficiente para juvenis de tilápia do Nilo.

Palavras-chave: Fe, Inorgânico, Piscicultura, Orgânico.

ABSTRACT

TITLE: LEVELS AND SOURCES OF IRON IN DIETARY OF NILE TILAPIA (*Oreochromis niloticus*)

AUTHOR: Luiza Beatriz Hermes
ADVISOR: Rafael Lazzari

Iron (Fe) is an extremely important micromineral in the development of animals. The present study aimed to evaluate the zootechnical performance, hematology, innate immunity, biochemical and oxidative parameters of tilapia juveniles (*Oreochromis niloticus*) fed diets containing levels (60 and 85 mg/kg) and sources (organic and inorganic) of iron. Fish (16.57 ± 2.25 g) were fed three times a day for 7 weeks, fish (400) were distributed in 20 tanks (240 L, 20 fish each). The experiment consisted of 5 treatments, in a completely randomized design: basal diet (DB), without inclusion of Fe in the mineral mixture, 60 inorganic, 60 organic, 85 inorganic and 85 organic. Inclusion of the organic source of Fe in the diet improves the performance of tilapia juveniles. Organic Fe improves relative weight gain (261.34%), final weight (61.73g) and biomass (1254.55%). Somatic digestive index and hepatosomatic index were higher in fish fed with inorganic Fe 85. Tilapia liver glucose and ammonia increase in fish fed organic Fe. Organic Fe improves hematocrit and mean corpuscular hemoglobin. Lysozyme (LZ) values decreased in fish fed with organic Fe, but the hemolytic activity of the complement system (HACS) and the NBT test increased with organic Fe. Fish fed an organic Fe 85 diet showed better antioxidant responses. It was concluded that the inclusion of an organic source of Fe at the 85 mg/kg level is more efficient for Nile tilapia juveniles.

Key-words: Fe, Fish-farming, Inorganic, Organic.

Sumário

1. INTRODUÇÃO

Em 2018 a aquicultura teve produção de 51,3 milhões de toneladas (FAO,2020). Vários fatores influenciam o crescimento desta cadeia, como tecnologia na produção e distribuição do pescado, incentivo do consumo de uma proteína mais saudável, aumento da disponibilidade do produto para os consumidores, entre outros fatores (FURUYA, 2013; PEIXE BR, 2021).

As tecnologias de produção vêm aumentando para atender os consumidores cada vez mais exigentes quanto à qualidade do pescado. A nutrição do pescado tem extrema importância quando se trata de qualidade, pois o alimento dos peixes influencia diretamente a qualidade e desenvolvimento do pescado (DURIGON, 2021; FAO, 2016)

Os minerais são importantes nutrientes nas dietas dos peixes, responsáveis por contribuir em diversas funções metabólicas, como capacidade antioxidante, equilíbrio ácido-basico, trocas gasosas etc. Cada mineral é exigido em determinada quantidade e estes são classificados conforme a necessidade exigida. Os macrominerais são minerais exigidos em quantidades maiores (expresso em gramas por kg ou porcentagem) e os microminerais são exigidos em menores quantidades (miligramas ou microgramas por kg) (PIERRI., 2020; WEBSTER; LIM, 2015).

Vários fatores influenciam a necessidade mineral dos peixes, pois os mesmos possuem a capacidade de absorção de nutrientes do ambiente via branquial e cutânea, o que dificulta o conhecimento exato da quantidade necessária à cada espécie e cada fase de produção (LAZZARI & RADUNZ NETO, 2014). As formas orgânicas possuem maior biodisponibilidade em comparação às inorgânicas.

O ferro (Fe) é um micromineral exigido em quantidades pequenas, o que acarreta sobredose ou deficiência muito facilmente. É necessário para diversas funções metabólicas, sendo a principal delas o transporte de oxigênio ligado ao Fe contido na hemoglobina presente nas hemácias. Além de atuar com cofator antioxidante, doses acima da necessidade fisiológica dos peixes pode ocasionar aumento da atividade oxidante das células (LALL & KAUSHIK., 2021).

A tilápia é um peixe onívoro da família dos ciclídeos e apresenta extrema importância econômica no panorama da aquicultura, pois a espécie ocupa o terceiro lugar na produção mundial e o primeiro na produção brasileira (FAO, 2020; PEIXE BR, 2021). Ocupa estas posições devido às características como ausência de espinhas musculares, rápido desenvolvimento, resistência a manejo e oscilações de temperatura, além da aceitação dos consumidores (DOS SANTOS *et al*. 2011).

2. OBJETIVO

2.1. GERAL

Testar uma forma orgânica e outra inorgânica de ferro e duas concentrações na dieta para juvenis de tilápia.

2.2. ESPECÍFICOS

Avaliar o crescimento e desempenho de tilápias alimentadas com dois níveis de ferro.
Verificar alterações na saúde de tilápias com fontes orgânicas e inorgânicas de ferro.
Identificar alterações no sistema imune dos peixes alimentados com as dietas.

3. REVISÃO BIBLIOGRÁFICA

3.1. MINERAIS

Minerais são nutrientes importantes na fisiologia animal, são responsáveis por inúmeros processos metabólicos, como, por exemplo, manutenção e desenvolvimento do esqueleto, manutenção dos sistemas coloidais, regulação do equilíbrio ácido-basico, além de participar como constituintes imprescindíveis de algumas células, hormônios e enzimas (LALL, 2002).

A disponibilidade dos minerais é influenciada por diversos fatores como a qualidade da água de cultivo, a forma química, a interação com outros componentes da dieta (fibra, fitatos, oxalatos), o estado fisiológico e patológico dos animais, o tamanho e a espécie consumidora (PRABHU; SCHRAMA; KAUSHIK, 2016).

Além de questões de disponibilidade e forma na qual os minerais são ofertados, existem diversas interações mineral/mineral e mineral/vitamina que influenciam diretamente no sucesso do cultivo (HILTON, 1989). Relações antagônicas acontecem quando elementos com configuração eletrônica semelhante competem por sítios de ligação. Relações sinérgicas ocorrem quando um elemento melhora a utilização de outro.

Um exemplo de sinergismo entre minerais é cobre e o ferro. O cobre influencia no metabolismo do ferro durante a hematopoese e na sua absorção, sendo a ceruloplasmina, um complexo cobre-proteína, que oxida o ferro para a forma férrica (Fe^{+3}), viabilizando seu transporte para outros tecidos (LOVELL, 1998). Outro exemplo, mas com vitamina e mineral, é a capacidade da vitamina C de alterar a valência do Fe, tornando-o mais disponível (HILTON, 1989).

O ferro também possui interação com substâncias antinutricionais. Segundo El-Saidy&Gabbe (2005) dietas formuladas contendo farelo de algodão e sulfato de ferro, diminuem a toxicidade do gossipol que capta a lisina, tornando assim indisponível ao animal. Este é um aminoácido de extrema importância na deposição muscular, influenciandodiretamente no crescimento animal.

Já o zinco é um mineral que compete com o ferro por sítios de ligação das proteínas transportadoras, tornando assim a absorção de ferro prejudicada (SIGNOR et al., 2010)

Os peixes são capazes de absorver minerais do ambiente seja pela via cutânea ou branquial. Em sistemas intensivos esta absorção é insuficiente, necessitando a suplementação, uma das várias dificuldades na determinação real da exigência de minerais dos peixes (LAZZARI &RADÜNZ NETO, 2014).

Os minerais podem ser classificados em macronutriente ou micronutriente e como orgânico ou inorgânico.

Compreende-se como macrominerais os exigidos em escala de gramas: fósforo, enxofre, cálcio, magnésio, sódio, potássio e cloro. Já os microminerais são exigidos em escalas de microgramas ou miligramas: cobalto, cromo, cobre, iodo, manganês, ferro, selênio e zinco (LALL & KAUSHIK, 2021).

Os orgânicos e inorgânicos são caraterizados conforme a sua estrutura e composição molecular. Os orgânicos compreendem os minerais quelatados ou ligados a moléculas orgânicas como proteínas, carboidratos ou aminoácidos. Devido à estas ligações, são mais estáveis, evitando que o mineral seja digerido no trato digestório, podendo ser melhor absorvido e utilizado pelo animal (NGUYEN *et al*., 2019).

Já os inorgânicos são minerais ligados a íons como sulfatos e carbonatos, entre outros.Estes possuem baixa disponibilidade quando comparados aos orgânicos(NGUYEN *et al.,* 2019).

Alguns estudos relatam as vantagens da utilização de compostos orgânicos na nutrição de peixes, sendo os principais benefícios da sua utilização a diminuição da inclusão destes nas dietas e diretamente menor gasto devido à melhor assimilação dos minerais pelos organismos, menor excreção de compostos ao meio tendo em vista à qualidade da água de cultivo, melhor desempenho dos animais, entre outros (NGUYEN *et al*., 2019; LIN & SHIAU 2009).

3.2. FERRO

O ferro é um mineral extremamente abundante no ambiente, encontra-se principalmente na forma férrica (Fe^{+3}). Esta forma é de baixa biodisponibilidade aos seres vivos, já a forma ferrosa (Fe^{+2}) é mais biodisponível, e estável em pH alcalino, mas é rapidamente oxidada à forma férrica na presença de oxigênio, tornando-se insolúvel (RESTREPO, DÍAZ, RONDÓ 2020).

Elemento essencial na respiração celular pela atividade de redução oxidativa e transferência de elétrons, o ferro é encontrado no corpo principalmente na forma complexada à proteínas, como compostos heme (hemoglobina e mioglobina), enzimas heme (citocromos, catalase, peroxidase, entre outras) e compostos não-heme (tranferrina, ferritina e ferro contendo flavoproteínas) (LALL & KAUSHIK, 2021).

A alimentação é considerada a maior fonte de ferro para os peixes, pois, usualmente, as águas naturalmente contêm baixa quantidade deste elemento de forma solúvel. Peixes podem

absorver ferro solúvel da água pelas brânquias, pois a adição de sulfato ferroso à água aumenta o crescimento e o nível de hemoglobina em robalo espada (ROEDER & ROEDER, 1966; BURY & GROSELL, 2003).

A deficiência nutricional de ferro resulta em redução da hemoglobina, hematócrito, volume globular médio e concentração de hemoglobina globular média, resultando em anemia microcítica e hipocrômica. A exigência nutricional mínima em ferro para manutenção da eritropoiese da tilápia é de 60,0 mg Fe kg^{-1} (FURUYA, 2013).O Nutrient Requirements of Fish and Shrimp (NRC), (2011) considera a exigência de 85,0 mg Fe kg^{-1}. Estas dosagens não estão caracterizadas pela sua fonte ou disponibilidade, o que dificulta a certeza da dosagem.

O excesso de ferro nos peixes pode ocasionar alterações na peroxidação lipídica, já que o ferro em excesso participa como agente oxidante sequestrando elétrons das moléculas do organismo, o que acarreta danos aos tecidos. Este excesso pode causar acúmulo de ferro nas brânquias, ocasionando disfunção respiratória. (SINGH *et al*, 2019)

Sabe-se que a deficiência de ferro limita a produção de hemoglobina, causa anemia, compromete a respiração celular e o desempenho produtivo. (DA SILVA *et al.,* 2012)

3.3. TILÁPIA-DO-NILO

Na piscicultura destaca-se a tilápia do Nilo, *Oreochromis niloticus*, que apresenta bom desempenho produtivo com carne saborosa de ótima qualidade, baixo teor de gordura e calorias, alto rendimento de filé e inexistência de espinhas intramusculares (DOS SANTOS *et al*., 2011), característica que a torna apropriada para industrialização, possuindo elevado valor comercial no mercado consumidor regional e internacional (PEIXE BR., 2020). Esta pertencente à família dos ciclídeos e é distribuída nas regiões tropicais e subtropicais, apresentando viabilidade para a produção intensiva, em virtude dos altos índices de produtividade e adaptação às condições de cativeiro. Pode ser cultivada em diferentes latitudes, sob os mais diversos sistemas de produção, abrangendo baixos ou sofisticados níveis tecnológicos, em ambientes abertos ou fechados (LIM & WEBSTER, 2006).

Ao se intensificar a produção devemos levar em consideração que o meio não “sustenta” altas densidades. Este ambiente não conseguirá manter a quantidade de animais e desenvolvê-los de maneira satisfatória, por isso a necessidade de alimentação artificial, no intuito de atender às exigências dos animais, atendendo o mercado com a qualidade do produto que o consumidor exige.

A espécie tem hábitos onívoros e, no Brasil, as dietas são fabricadas primordialmente com produtos de origem animal, farinha de subprodutos de abatedouro avícola, farinha de carne e ossos e farinha de pescado, produtos e subprodutos de origem vegetal que variam conforme a região e a sazonalidade local, principalmente derivados da soja, do algodão, do milho, do arroz e do trigo e fontes alternativas de disponibilidade específica regional, como leveduras. As dietas são suplementadas com calcários, fosfatos, farinha de ossos e suplementos minerais para atingir os níveis de exigência para cada sistema ou fase de produção.

Frequentemente são publicados artigos sobre a exigência nutricional da espécie e mesmo assim são encontradas lacunas, como em Lall & Kaushik. (2021), onde os autores apresentam exigências minerais a diversas espécies. Porém, quando se trata do ferro para tilápias, não apresentam nenhuma informação. A espécie necessita dos mesmos minerais considerados essenciais em outras espécies (LALL, 2002; LIM & WEBSTER, 2006).

Além da necessidade de se encontrar a exigência de ferro para tilápias, há ainda lacunas como a exigência cada fase de cultivo em diferentes sistemas e a linhagem além da composição do mineral utilizado.

A exigência da tilápia híbrida foi estimada por Shiau & Su (2003), baseada nos valores obtidos pelo ganho de peso, concentração de ferro no fígado e série vermelha do sangue, utilizando duas fontes diferentes de ferro: o citrato férrico e o sulfato de ferro. Foram encontradas a necessidade de se suplementar 150 a 160 mg de Fe/kg como citrato de ferro e 85 mg de Fe/kg como sulfato de ferro, mostrando que a fonte inorgânica tem maior biodisponibilidade para a tilápia híbrida (SHIAU & SU, 2003).

4. MANUSCRITO

4.1.Artigo 1

Dietary iron affect growth, digestive index and biochemical parameters of juvenile tilapia

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Abstract

This study aimed to evaluate the effects of dietary iron (Fe) on growth performance, digestive indices and biochemical parameters of tilapia juveniles fed with two doses (60 and 85 mg/kg) and two forms of iron (organic and inorganic). Fish (16.57 ± 2.25 g) were feed three times a day, during 7 weeks, when fish (400) were distributed in 20 tanks (240 L, 20 fish each). The experiment was composed by 5 treatments, in a completely randomized design: basal diet (BD), without Fe inclusion in mineral mixture, 60 Inorganic, 60 Organic, 85 Inorganic and 85 Organic. Increase in dietary Fe organic source improve growth and biochemical parameters of tilapia. The Fe organic improv relative weight gain (261.34%), final weight (61.73g) and biomass (1254.55%). Fe organic increase somatic digestive index but hepatosomatic index was highest in fish feed with 85 Fe inorganic. The glucose and ammonia of liver from tilapias increase in fish feed with Fe organic. This concluded that inclusion of Fe organic source and level of 85mg/kg is more efficient for juveniles of Nile tilapia.

Key words: Inorganic, Fe, nutrition, minerals, organic

CRediT authorship contribution statement

Luiza Beatriz Hermes: Conceptualization, investigation, methodology, formal analysis, project administration, roles/writing - original draft, writing - review and editing

Nilce Coelho Peixoto: Formal analysis, investigation, review and editing

Emerson Giuliani Durigon: Conceptualization, investigation, formal analysis and editing;

Joziane Soares de Lima: Conceptualization, investigation, methodology, writing - review and editing

Rafael Lazzari: Supervision roles/writing - original draft, writing - review and editing

Declaration of conflict interest:

None

1. INTRODUCTION

Minerals are required by fish in macro and micro portions (LALL & KAUSHIK, 2021). Microminerals are vital to fish as they are responsible for several functions such as growth, metabolism and health. They are needed in low concentrations, which facilitates their deficiency, which may cause several losses to production. (PIERRI *et al.,* 2021; WATANABE et al., 1997). Mineral nutrition is difficult, as fish are able to absorb the minerals contained in the water via gill and skin, thus making it difficult to know exactly the amount needed by them. (BALDISSEROTTO, 2018).

Iron (Fe) is an extremely important micromineral for the physiological activities of fish, such as O_2 transport and antioxidant activities. The minimum nutritional requirement in Fe for maintenance of tilapia erythropoiesis is 60 mg Fe kg^{-1} (Furuya, 2013); the Nutrient Requirements of Fish and Shrimp (NRC) (2011) considers the requirement of 85 mg Fe kg^{-1}. These requirements are unclear as to the source of mineral used. Some authors emphasize the lack of information about the amount and source of Fe used in tilapia nutrition (Lazzari & Radunz Neto., 2014; Lall & Kaushik, 2021). This lack of information is due to the various forms of cultivation, ingredients used in nutrition and growth stages tested.

To avoid mineral deficiencies in fish diets, a higher levels of minerals is generally used, especially for microminerals in inorganic forms, due to low bioavailability, antinutritional factors and competition for binding sites. These overdoses may cause intoxication and overload the environment with an excess of excreted minerals (Nguyen et al., 2019; Pierri et al., 2021).

In fish production, a juvenile stage is extremely important to obtain an excellent product, as it is in the crucial stage of body development and any deficiency results in low development, muscle atrophy, mortality, among other factors.

Tilapia production represents 8.3% of total fish production, being the third most produced species in the world (FAO, 2021). This position is a result of the characteristics of the

species, such as easy reproduction, rapid growth, resistance to high stocking densities and different temperatures, in addition to consumer demand (Sanchez *et al.,* 2021). Thus, this study aimed evaluated sources and levels of Fe in tilapia nutrition.

2. MATERIALS AND METHODS

The feeding trial was conducted in the fish farming laboratory of the Department of Animal Science and Biological Sciences of the Universidade Federal de Santa Maria (UFSM), Palmeira das Missões (27°55'16.9" S, 53°19'05.7" W, and altitude 639 m). The Ethics Committee on the Use of Animals of UFSM approved all experimental procedures (protocol number: 4892180321).

2.1. EXPERIMENTAL DIETS

The diets (treatments) used are described according:

-BD: No Iron added to the mineral mixture (Basal Diet);

-85 Fe Org: 85 mg/kg of Organic Fe;

-85 Fe Inorg: 85 mg/kg of Inorganic Fe;

-60 Fe Org: 60 mg/kg of Organic Fe;

-60 Fe Inorg: 60 mg/kg of Inorganic Fe.

The diets were formulated according to Abdel-Tawwab et al., (2010). Two levels and two sources of iron added to the premix and a diet without the addition of iron to the premix were used. The sources used were organic from (Fe-amino acid Availa-Fe®) and inorganic (Fe^{2+} sulfate or Synth®) with levels 85 mg/kg and 60 mg/kg, as found in the NRC (2011) and Furuya (2013), respectively.

The ingredients were mixed in a horizontal mixer (Monte Castelo-Mod: AML 07). During this process, the vitamin and mineral premix, oil and distilled water were added to the ingredients. The mass of all treatments was subjected to pelletization and then to drying at 55°C for 24 h. After drying, the diets were stored in closed containers at a temperature of -15°C. The formulation of diets and composition for each treatment are shown in Table 1.

2.2. ANIMALS, EXPERIMENTAL CONDITIONS AND WATER QUALITY

The fish underwent a period of 14 days to adapt to the system. 400 animals with an initial weight of 16 ± 2.25 g were used, distributed in 20 tanks with a useful volume of 240 L. The treatments were randomly distributed, 20 animals were used per tank, fed 3 times a day (8:00, 13:00 and 18:00 h).

The recirculation aquaculture system contained a total of 8.300 L, had two biofilters, a decanter, a reservoir and 20 tanks (240 L) with individual water and oxygen supply. The water temperature was maintained with the aid of a thermostat and oxygenation with a blower. The dirt from the tanks was removed by siphoning twice a day (10:00 and 16:00 h).

The temperature and oxygen of the water were checked daily, with the aid of a digital oximeter, as well as the total ammonia and nitrite, by an Alfakit® colorimetric kit, weekly, the evaluated parameters are described below.

The pH with pHmeter model MPA – 210 P, the total alkalinity (mg $CaCO_3$/L) by the neutralization titration method and the total hardness (mg $CaCO_3$/L) by the complexation titration method. The water parameters remained in the ideal ranges for the species according to El-Sayed & Kawanna., (2008). Temperature 25.65 °C ± 2.32, dissolved oxygen 7.50 mg/L ± 1.20, pH 7.30 ± 0.32, total ammonia 0.25 ± 0.30 mg/L, nitrite 0. 01 ± 0.01 mg/L, alkalinity 50 mg $CaCO_3$ / L, hardness 50 mg/L $CaCO_3$.

2.3. SAMPLE COLLECTION

At the end of 7 weeks, the fish were fasted for approximately 12 h and then the zootechnical parameters were measured. Two fish were removed per tank (8 per treatment), being anesthetized and euthanized by spinal section, subsequently. Among these animals, data were collected for somatic and tissue indices for analysis of biochemical parameters and proximate composition.

2.4. SAMPLE ANALYSIS

2.4.1. Body composition

The initial body composition analysis was performed using a sample of 20 fish and one animal per tank (4 per treatment) was used at the end of the experimental period. The composition of whole fish was analyzed, and such parameters were evaluated: moisture, ash and crude protein, following methodologies recommended by the AOAC (1995), lipids extracted and quantified by the method of Bligh and Dyer (1959).

2.4.2. Growth performance

In biometrics, the fish were measured and weighed, being evaluated:

- Weight (g);
- Total length (cm);

From these data, the following were calculated:

- Daily weight gain (g) (weight gain/days of experimentation);

- Total weight gain (g) (final weight - initial weight);

- Specific growth rate expressed in %/day [TCE=100 x (ln final average weight ln initial average weight)/time (day)];

- Condition factor (average weight/standard length^3);

- Relative weight gain (final weight - initial weight / initial weight x 100);

- Total biomass (g) (Final weight x number of fish).

2.4.3. Somatic index parameters

- Carcass yield (%): RC = [(gutted weight with head and gills)/(whole weight)]*100;

- Digestive-somatic index (%): IDS = (tract weight/whole weight)*100;

- Hepatosomatic index (%): IHS = (liver weight/whole weight)*100;

2.4.4. Biochemical parameters

For the analysis of total proteins (Bradford, 1976), liver (100 mg) and muscle (400 mg) tissue samples were heated to 100°C with KOH 6N and then centrifuged at 3500 rpm for 10 min. For the determination of glycogen (Krismaw, 1962), ethyl alcohol was added for the hydrolysis and precipitation of the polysaccharide. For the quantification of free amino acids (Moore & Steinethyl alcohol was added for the hydrolysis and precipitation of the polysaccharide. For the quantification of free amino acids (MOORE and STEIN, 1948), 250 mg liver and 1 g muscle samples were homogenized in buffer (20 mM TFK, pH 7.5) and centrifuged at 3500 rpm for 10 min. Alanine-aminotransferase and aspartate-aminotransferase levels were quantified using commercial colorimetric kits, by the methodology of Reitman & Frankel, (1957). For the quantification of glucose and ammonia, samples of muscle (200 mg), liver (50 mg) and gills (100 mg) were homogenized in 10% TCA. For the measurement of

glucose, a commercial kit by enzymatic methodology was used, while for the determination of total ammonia, the salicylate technique was used according to Verdouw *et al*., (1978).

2.4.5. Statistical analysis

The data obtained were submitted to the Shapiro-Wilk normality test, followed by the Two-Way ANOVA, where the differences between levels and sources were compared by the Student's "t" test. In addition, when significant interaction occurred, data were comparedd separately between sources ("t" test, $p<0.05$).

3. RESULTS

Tilapia fed diets containing Fe Org had higher weight compared to those fed Fe Inorg diets (Table-2). Fish fed with 85 Fe inclusion showed values equal to the BD diets, being superior to the others in all zootechnical performance parameters, except for the condition factor. Fish fed with Fe Inorg presented lower development in all parameters evaluated when compared to the others, except for Fe 60 where the values were statistically equal (Fig. 1). The biomass of fish fed with 60 Fe Inorg was the lowest among the other diets tested, while tilapia fed with Fe Org had the highest biomass (Fig 1 A).

There was no effect on tilapia protein composition (Table-3), but lipids and dry matter of fish fed 85 Fe Org diets (Fig 2A, B) were higher. However, mineral matter was higher in fish submitted to 60 Fe and BD diets. The carcass yield of fish did not differ between the tested diets. The somatic digestive index of fish showed similar behavior to the lipids and dry matter of fish submitted to Fe Org, 85 Fe and BD diets, exhibiting values higher than those of animals submitted to the other diets. Fish fed with 85 Fe Inorg (Fig 2C) had a higher hepatosomatic index than animals from the other diets tested.

Higher liver ammonia values were observed in fish fed 85 Fe Inorg (Fig 3A), but AST was equal in fish fed BD, 60 Fe and Fe Org. The amino acid parameters showed lower values in Fe Org (Fig 3C). But there was no difference in the other diets tested. Glucose from the liver of fish fed Fe Inorg was lower, while glycogen was higher (Fig 3B).

Biochemical parameters are shown in Table-4. AST in fish liver did not show difference between the evaluated diets, whereas ALT in the same tissue was higher in Fe Org and BD. On the other hand, 60 Fe did not differ from the others, which presented lower values in fish fed with 85 Fe and Fe Inorg.

Muscle glucose was not detected in any of the treatment groups (values not shown), but the glycogen (Fig 3D) of fish fed with BD was higher when compared to the others, the same occurs with amino acids. Ammonia from fish muscles did not differ between the treatments tested. The tilapia muscle AST and ALT showed higher values in animals submitted to diets containing Fe Org.

The ammonia in the gills of fish fed with 85 Fe Inorg and 60 Fe Org was lower than the others diets tested (Fig 3E).

4. DISCUSSION

The results obtained show the positive effect of using the organic source of iron at the level (60 mg/kg) on the performance of tilapia. Furthermore, when using an inorganic source, probably due to the availability of the mineral, it is necessary to use a higher level (85mg/kg).

The growth of fish fed with 60 Fe Inorg was lower when compared to the others. Fe can accelerate the catabolism of amino acids, which may result in a deficiency of them, thus causing less growth in fish due to the low availability of amino acids. In some studies, low development

was verified in Atlantic Salmon and Rohu carp fed with inorganic sources of Fe (Andersen *et al*., 1998; Behera *et al*., 2014.),

The AST and ALT enzymes are important for the metabolism of carbohydrates and proteins and for the use of amino acids for oxidation or gluconeogenesis. Such enzymes may be indicative of cell destruction (Afshari *et al*., 2021; Kumar *et al*., 2019).

Tilapia liver the AST differed statistically in fish fed BD and Fe Inorg diets. Fish fed with BD showed higher values of ammonia in the liver, indicating that there was a possible deamination of AA, which may have caused an increase in ammonia in the liver of these animals. Tilapia liver AST was higher in fish fed Fe Inorg diets, but ALT from the same tissue was lower. Corroborating the present study, Afshari *et al*., (2021) found similar enzymatic activities using inorganic sources and Fe and copper (Cu) nanoparticles.

The amino acid parameters of tilapia fed with Fe Inorg showed lower values. In fish fed the other diets there was no significant difference, indicating the possibility of an efficient absorption of amino acids in the intestinal portion and supplying the animals' needs, as they presented biomass values higher than 60 Fe Inorg (Fig 1E). The same result was found by Uczay *et al*., (2019) in Silver catfish juveniles supplemented with protein hydrolysates.

Tilapia liver glucose was lower in the Fe Inorg diet, while liver glycogen was higher, showing a possible gluconeogenesis to meet the animal's glucose needs. Fish fed the other treatments did not show such response. This demonstrates a better use of glucose without the need for gluconeogenesis and deposition of excess glucose in the form of lipids, since animals fed with these diets had higher amounts of body lipids than those fed with Fe Inorg (Nelson & Cox., 2018; Gressler *et al*., 2015).

The muscle glycogen of fish submitted to BD diet was higher when compared to the other diets tested, with the same occurring with amino acids. Glycogen may have been produced

from the breakdown of amino acids, to meet a possible energy demand of animals (Nelson & Cox., 2018). Walker *et al*. (2020) found an increase in glycogen in tilapia exposed to Cr_2O_3.

The body dry matter of fish submitted to BD, 85 Fe Org diets showed higher values than the other diets tested but did not differ from the values found by Pierri *et al*., (2019) and Ernzen Pessini *et al*., (2020). The mineral matter of fish fed with 60 Fe and BD were superior to the other diets tested. This may be associated with the absorptive capacity of minerals contained in water (Watanabe *et al*., 1997), thus increasing mineral deposition.

5. CONCLUSION

For Nile tilapia juveniles the use of iron in organic and the level 60 mg/kg proved to be enough from provide a better growth and health.

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6. REFERENCES

Abdel-Tawwab, M., Ahmad, M.H., Khattab, Y.A.E., Shalaby, A.M.E., 2010. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus (L.).* Aquaculture 298, 267–274. https://doi.org/10.1016/j.aquaculture.2009.10.027

Afshari, A., Sourinejad, I., Gharaei, A., Johari, S.A., Ghasemi, Z., 2021. The effects of diet supplementation with inorganic and nanoparticulate iron and copper on growth performance, blood biochemical parameters, antioxidant response and immune function of snow trout *Schizothorax zarudnyi* (Nikolskii, 1897). Aquaculture 539. https://doi.org/10.1016/j.aquaculture.2021.736638

Andersen, F., Lygren, B., Maage, A., & Waagbø, R. 1998. Interaction between two dietary levels of iron and two forms of ascorbic acid and the effect on growth, antioxidant status and some non-specific immune parameters in Atlantic salmon (*Salmo salar*) smolts. Aquaculture, 161(1-4), 437-451. https://doi.org/10.1016/S0044-8486(97)00291-3

AOAC, Official Methods of Analysis: Changes in Official Methods of Analysis Made at the Annual Meeting. Supplement, Association of Official Analytical Chemists, 1990.

Behera, T., Swain, P., Rangacharulu, P. v., Samanta, M., 2014. Nano-Fe as feed additive improves the hematological and immunological parameters of fish, *Labeo rohita H*. Applied Nanoscience. 4, 687–694. https://doi.org/10.1007/s13204-013-0251-8

Bligh, E. G., & Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology, 37(8), 911-917. https://doi.org/10.1139/o59-099

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

El-Sayed,A.F.M., Kawanna. M., 2008. Effects of dietary protein and energy levels on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock in a recycling system. Aquaculture, 280, 179–184. https://doi.org/10.1016/j.aquaculture.2008.04.030

Ernzen Pessini, J., Daitx de Oliveira, V., Souza de Sa, L., Pedreira Mourino, J. L., Pettigrew, J., & Machado Fracalossi, D. 2021. Dietary supplementation of Viligen™ to Nile tilapia improves growth and gut morphology. Aquaculture Nutrition 00:1–12 https://doi.org/10.1111/anu.13247

FAO, 2021. The state of world fisheries and aquaculture. Rome. 2021 https://doi.org/10.4060/ca9229en

Gressler, L. T., Sutili, F. J., Da Costa, S. T., Parodi, T. V., da Silva Pês, T., Koakoski, G., Baldisserotto, B. 2015. Hematological, morphological, biochemical and hydromineral responses in *Rhamdia quelen* sedated with propofol. Fish Physiology and Biochemistry, 41, 463-472. https://doi.org/10.1007/s10695-014-9997-5

Jobling, M. 1983. A short review and critique of methodology used in fish growth and nutrition studies. Journal of Fish Biology, 23, 685-703. https://doi.org/10.1111/j.1095-8649.1983.tb02946.x

Krisman, C. R. 1962. A method for the colorimetric estimation of glycogen with lodine. Analytical biochemistry, 4, 17-23. https://doi.org/10.1016/0003-2697(62)90014-3

Kumar, N., Gupta, S.K., Bhushan, S., Singh, N.P., 2019. Impacts of acute toxicity of arsenic (III) alone and with high temperature on stress biomarkers, immunological status and cellular metabolism in fish. Aquatic Toxicology 214. https://doi.org/10.1016/j.aquatox.2019.105233

Lall, S.P., Kaushik, S.J., 2021. Nutrition and metabolism of minerals in fish. Animals. https://doi.org/10.3390/ani11092711

Moore, S., Stein, W.H., 1948. Photometric ninhydrin method for use in the chromatography of amino acids. The Journal of biological chemistry 176, 367–388. https://doi.org/10.1016/s0021-9258(18)51034-6

Nelson, David L.; Cox, Michael M. Princípios de Bioquímica de Lehninger-7. Artmed Editora, 2018.

Nguyen, L., Kubitza, F., Salem, S.M.R., Hanson, T.R., Allen Davis, D., 2019. Comparison of organic and inorganic microminerals in all plant diets for Nile tilapia *Oreochromis niloticus*. Aquaculture 498, 297–304. https://doi.org/10.1016/j.aquaculture.2018.08.034

PEIXE BR. Associação Brasileira da Piscicultura. Anuário Brasileiro da Piscicultura, São Paulo, Brasil. 2021.

Pierri, B. da S., Silva, A.D., Cadorin, D.I., Ferreira, T.H., Mouriño, J.L.P., Filer, K., Pettigrew, J.E., Fracalossi, D.M., 2021. Different levels of organic trace minerals in diets for Nile tilapia juveniles alter gut characteristics and body composition, but not growth. Aquaculture Nutrition 27, 176–186. https://doi.org/10.1111/anu.13175

Reitman, S., & Frankel, S. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology, 28, 56-63. https://doi.org/10.1093/ajcp/28.1.56.

Rostagno, H.S., Albino,L.F.T., Donzele,J.L., Gomes,P.C., de Oliveira, R.F., Lopes, D.C., et al, Composição e valor nutritivo dos alimentos. In: Horacio Santiago Rostagno (4ed.). Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais (2017) 488

Sanchez, M.S. dos S., Lins-Rodrigues, M., Pessini, J.E., Barcellos, L.J.G., Bittencourt, F., Boscolo, W.R., Signor, A., 2021. Dietary supplementation of betaine improves growth performance and reduces lipid peroxidation in Nile tilapia. Aquaculture Nutrition 27, 1861–1870. https://doi.org/10.1111/anu.13323

Uczay, J., Battisti, E.K., Lazzari, R., Pessatti, M.L., Schneider, T.L., Hermes, L.B., Peixoto, N.C., Fabregat, T.E.H.P., 2019. Tilapia protein hydrolyzate improves growth performance, protein absorption and antioxidant status in Silver catfish (*Rhamdia quelen*). Aquaculture Research 50, 3192–3201. https://doi.org/10.1111/are.14273

Verdouw, H., Van Echteld, C. J. A., & Dekkers, E. M. J. 1978. Ammonia determination based on indophenol formation with sodium salicylate. Water Research, 12, 399-402. https://doi.org/10.1016/0043-1354(78)90107-0

Walker, A. M., Copatti, C. E., Melo, F. V., & Melo, J. F. 2020. Metabolic and physiological responses to intraperitoneal injection of chromium oxide in hyperglycaemic Nile tilapia juveniles. Aquaculture, 517, 734821. https://doi.org/10.1016/j.aquaculture.2019.734821

Watanabe, T., Kiron, V., Satoh, S., 1997. Aquaculture Trace minerals in fish nutrition, Aquaculture. https://doi.org/10.1016/S0044-8486(96)01503-7

Figure captions

Fig.1 (1A) Final weight (FW) of tilapia fed diets containing iron (Fe Inorg) (1B) Total length (TL) of tilapia fed diets containing iron (Fe Inorg) (1C) Specific growth rate (SGR) of tilapia fed diets containing iron (Fe Inorg) (1D) Relative weight gain (RWG) of tilapia fed diets containing iron (Fe Inorg) (1E) Biomass (B) of tilapia fed diets containing iron (Fe Inorg). Values expressed as mean ± standard deviation. Different letters indicate differences according to the Student's t test ($P < 0.05$).

Fig.2 (2A) Lipids (LP) of whole body of tilapia fed diets containing iron (Fe Inorg) (2B) Dry Matter (DM) of whole body of tilapia fed diets containing iron (Fe Inorg) (2C) Hepatosomatic Index (HI) of tilapia fed diets containing iron (Fe Inorg). Values expressed as mean ± standard deviation. Different letters indicate differences according to the Student's t test ($P < 0.05$).

Fig.3 (3A) Ammonia (AM) tilapia liver fed diets containing iron (Fe Org) (3B) Glycogen (GLY) tilapia liver fed diets containing iron (Fe Org) (3C) Amino acid free (FAA) tilapia liver fed diets containing iron (Fe Inorg) (3D) Glucose (GLU) tilapia muscle fed diets containing iron (Fe Inorg) (3E) Ammonia (AM) tilapia gill fed diets containing two levels and two sources of iron. Values expressed as mean ± standard deviation. Different letters indicate differences according to the Student's t test ($P < 0.05$).

Table 1. Ingredients and approximate composition of five experimental diets

Ingredients (g/kg)	Diets				
	BD	85 Fe Org	85 Fe Inorg	60 Fe Org	60 Fe Inorg
Tilapia meal[a]	13.53	13.53	13.53	13.53	13.53
Soybean meal	50.00	50.00	50.00	50.00	50.00
Corn	20.00	20.00	20.00	20.00	20.00
Wheat meal	10.67	10.07	9.82	10.37	10.24
Soybean oil	2.00	2.00	2.00	2.00	2.00
Vitamin and mineral mixture [b]	2.00	2.00	2.00	2.00	2.00
Vitamin C	1.00	1.00	1.00	1.00	1.00
Salt	0.80	0.80	0.80	0.80	0.80
Fe Org (mg/kg)	-	0.60	0.85	-	-
Fe Inorg (mg/kg)	-	-	-	0.30	0.43
Approximate composition (%)					
Crude protein	34.86	33.07	33.04	33.19	34.65
Lipids	6.74	7.11	6.02	6.66	7.38
Dry matter	94.64	95.36	94.63	95.30	95.70
Ash	10.23	9.69	9.25	9.73	9.33
DE (MJ/kg) [c]	13.40	13.34	13.31	13.37	13.35
Calcium [d]	4.11	4.11	4.11	4.11	4.11
Phosphorus[d]	2.35	2.35	2.35	2.35	2.35
Iron [d]	145.38	204.15	228.63	204.76	229.49

[a] Tilapia residue meal; [b] vitamin and mineral pre-mix without iron prepared by Puro Trato®. Composition: Vitamin B9 (2.400 mg); Vitamin B3 (48 g); Vitamin B5 (24 g); Vitamin B7 (96 mg); 200 mg; Vitamin A (2.400.000 IU); Vitamin D3 (400.000 IU); Vitamin E (24.000 IU); Vitamin B1 (9.600 mg); Vitamin B2 (9.600 mg); Vitamin B6 (9.600 mg); Vitamin B12 (9.600 mg); Vitamin C (96 g); Vitamin K3 (4,800 mg); Iodine (200 mg); Manganese (40 g); Selenium (200 mg) Zinc (6.000 mg); Cobalt (20 mg); Copper (1.250 mg); [c] Digestible energy = [(Crude protein × 23.61 MJ/kg × 0.9) + (Lipids × 39.82 MJ/kg × 0.85) + (carboihydrates × 17.21 MJ/kg × 0.50)] (Jobling., 1983); [d] Calculated according to the Brazilian Poultry and Swine Table (Rostagno., 2017)

Table - 2 Growth performance of tilapia fed diets containing sources and levels of iron

		Levels		Sources		P			
	BD	60 Fe	85 Fe	Fe Org	Fe Inorg	Levels	Sources	Inter	SEM
FW	58.37	41.80^{b*}	59.74^{a}	61.73^{a*}	39.81^{b*}	<0.0001	<0.0001	<0.0001	2.54
TL	14.92	12.66^{b*}	14.68	14.57^{a}	12.76^{b*}	<0.0001	<0.0001	<0.0001	0.21
SGR	2.57	1.61^{b*}	2.62^{a}	2.69^{a*}	1.55^{b*}	<0.0001	<0.0001	<0.0001	0.08
CF	1.76	1.87^{*}	1.89^{*}	2.00^{a*}	1.77^{b}	0.04	<0.0001	0.39	0.08
RWG	251.97	152.23^{b*}	261.34^{a}	273.37^{a*}	140.19^{b*}	<0.0001	<0.0001	<0.0001	15.12
B	1167.37	836.02^{b*}	1194.76^{a}	1254.55^{a*}	796.23^{b*}	<0.0001	<0.0001	<0.0001	15.12

Means with different letters, between the factors differ by the Student's t test. Means marked with * differ from diets without added iron. BD: basal diet, no addition of iron (Fe) in the vitamin and mineral pre-mix; Fe Org: Organic: Fe Inorg: Inorganic; SEM: Standard error of means; Inter: Interaction; FW: Final weight (g); TL: Total length (cm); SGR: Specific growth rate (% day^{-1}); CF: Condition factor; RWG: Relative weight gain (%); B: Biomass (%)

Tabel-3 Whole body composition and hepatosomatic and somatic digestive index of tilapia fed diets containing sources and levels of iron

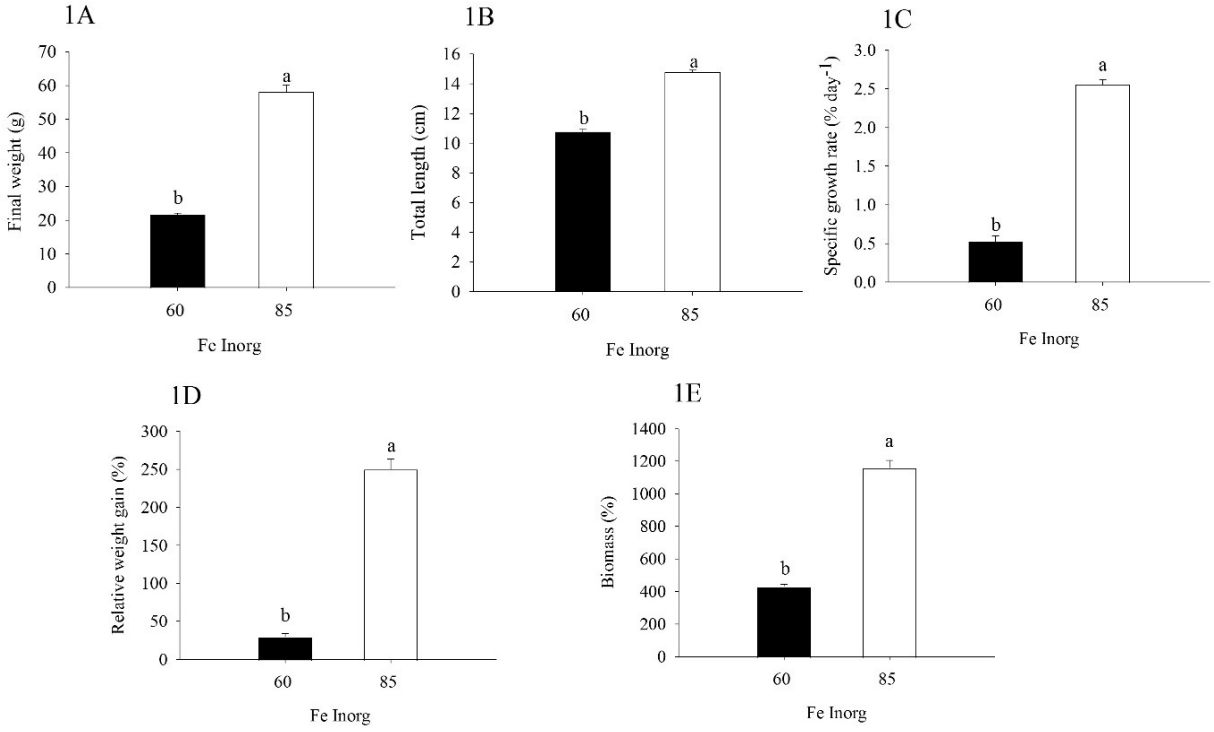
		Levels		Sources		P-value			
	BD	60 Fe	85 Fe	Fe Org	Fe Inorg	Levels	Sources	Inter	SEM
CP	11.21	11.32	11.49	11.98	10.83	0.91	0.06	0.55	1.10
LP	4.27	3.55^{b*}	4.61^{a}	4.66 ^{a}	3.50^{b*}	0.004	0.0007	0.0005	0.41
DM	23.36	21.91^{b*}	23.69^{a}	23.81^{a}	21.79^{b*}	0.002	0.0003	<0.0001	0.64
MM	4.91	4.90^{a}	4.52^{b*}	4.61	4.81	0.04	0.18	0.48	0.24
CY	87.44	86.69	87.67	87.04	87.36	0.50	0.73	0.35	2.39
HI	7.97	6.98*	6.57*	6.48^{b*}	7.07^{a}	0.03	0.15	0.008	1.13
SDI	1.64	1.38^{b*}	1.52^{a}	1.56^{a}	1.34^{b*}	0.12	0.03	0.06	0.27

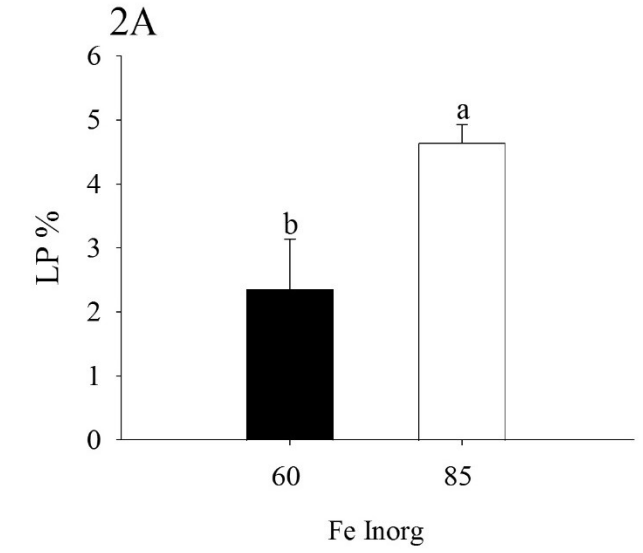
Means with different letters, between the factors differ by the student's t test. Means marked with * differ from diets without added iron. BD: basal diet, no addition of iron (Fe) in the vitamin and mineral pre-mix; Fe Org: Organic: Fe Inorg: Inorganic; SEM: Standard error of means; Inter: Interaction; CP: Crude Protein (%); LP: Lipids (%); DM: Dry Matter (%); MM: Mineral Matter (%); CY: Carcass Yield (%); SDI: Somatic Digestive Index (%); HI: Hepatosomatic Index (%)

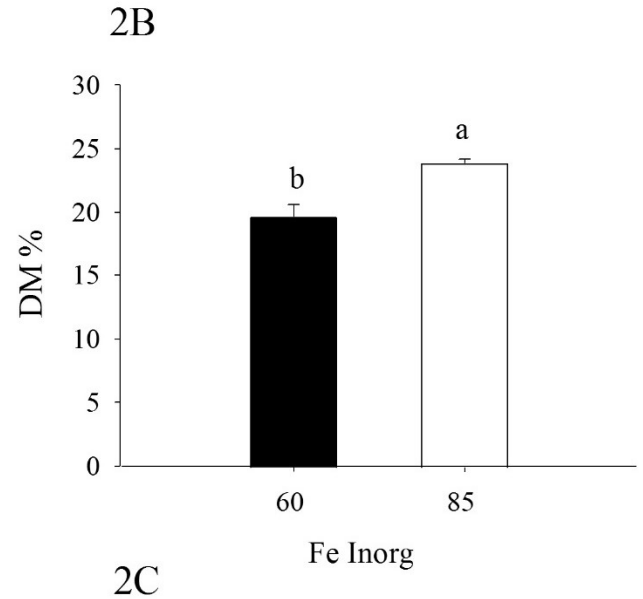
Tabel-4 Biochemical parameters of tilapia fed diets containing sources and levels of iron

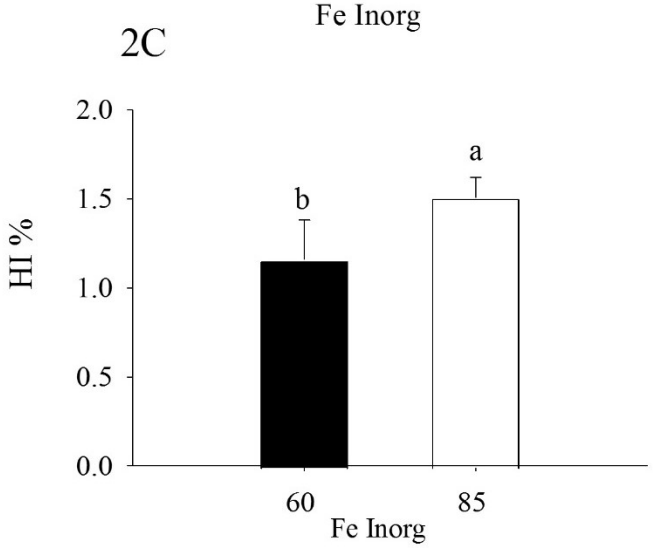
		Levels		Sources		P-value			
	BD	60 Fe	85 Fe	Fe Org	Fe Inorg	Levels	Source	Inter	SEM
Liver									
AM	0.057	0.050*	0.053*	0.052*	0.051*	0.0008	0.13	<0.0001	0.003
PT	33.06	29.36*	29.76*	26.37b*	34.42a	0.07	<0.001	0.63	3.64
GLY	2.41	5.11*	4.60	3.62b	6.45a*	0.08	0.008	0.004	2.08
FAA	79.80	59.87	72.84	75.77a	42.00b*	0.14	0.001	0.03	18.86
GLU	6.02	6.55	7.59	9.30a*	3.08b	0.31	0.001	0.16	2.51
AST	4347.7	4495.8	4645.5	4495.1	4696.4*	0.18	0.21	0.009	372.70
ALT	4971.4	4015.0	3782.30*	4334.8a	3278.4b*	0.08	0.03	0.94	1198.41
Muscle									
AM	0.01	0.009	0.009	0.001	0.009	0.20	0.52	0.15	0.001
PT	0.55	0.94a*	0.33b*	0.64a*	0.56b	<0.001	0.90	0.86	0.05
GLU	0.80	0.53*	0.56*	0.56*	0.52*	0.03	0.59	0.009	0.21
FAA	75.21	54.41b*	69.19a	64.49	58.65*	0.0008	0.06	0.07	10.75
AST	1713.37	1795.22a	1697.56b	1794.91a	1675.39b	0.03	0.002	0.17	109.57
ALT	733.08	808.24	664.09	884.73a*	559.55b*	0.08	0.0001	0.49	159.79
Gill									
AM	0.018	0.017	0.017	0.017	0.017*	0.18	0.57	0.001	0.001

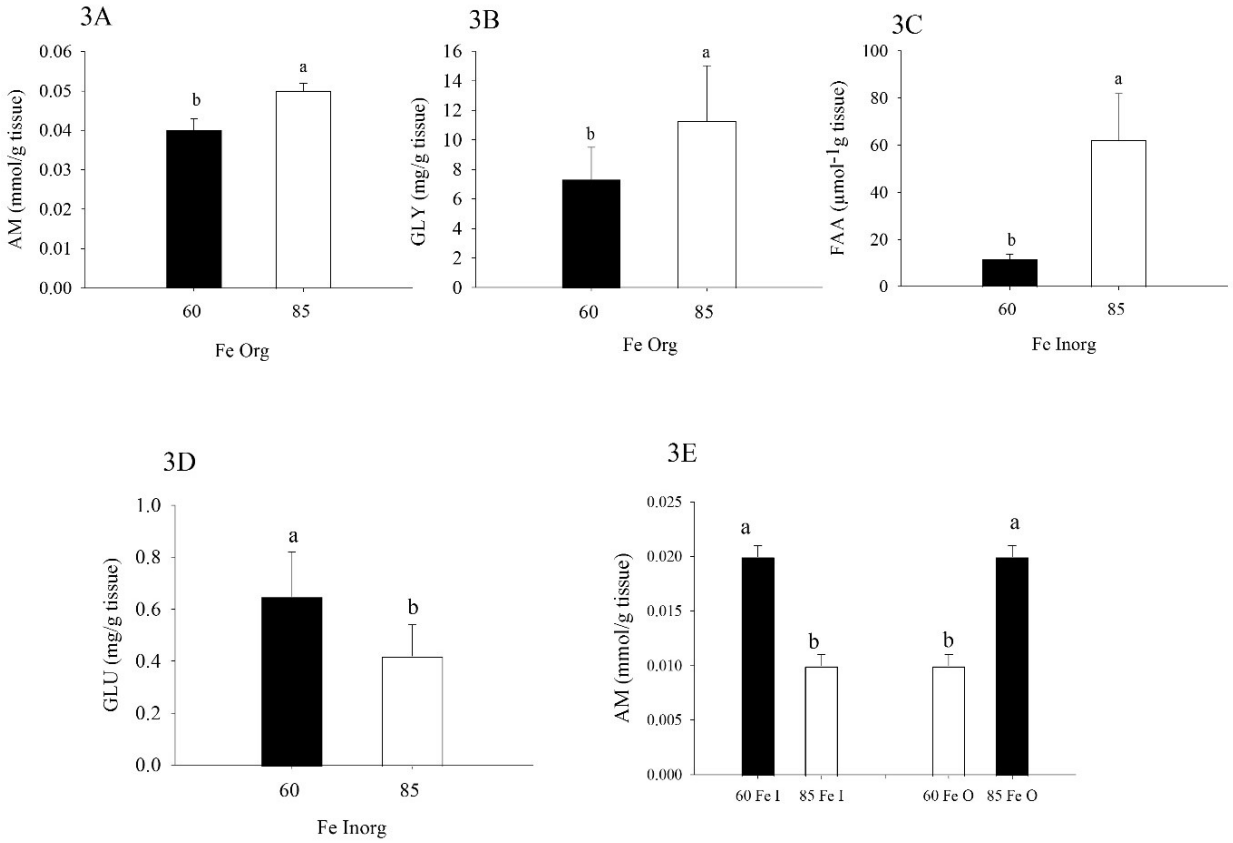
Means with different letters, between the factors differ by the Student's t test. Means marked with * differ from diets without added iron. BD: basal diet, no addition of iron (Fe) in the vitamin and mineral pre-mix; Fe Org: Organic: Fe Inorg: Inorganic; SEM: Standard error of means; Inter: Interaction; FAA: Aminoacid free ($\mu mol^{-1}g$ tissue); AST: Aspartate aminotransferase (U/g tissue); ALT: Alanine aminotransferase (U/g tissue); AM: Ammonia (mmol/g tissue); PT: Protein (mg/g tissue); GLY: Glycogen (mg/g tissue); GLU: Glucose (mg/g tissue).

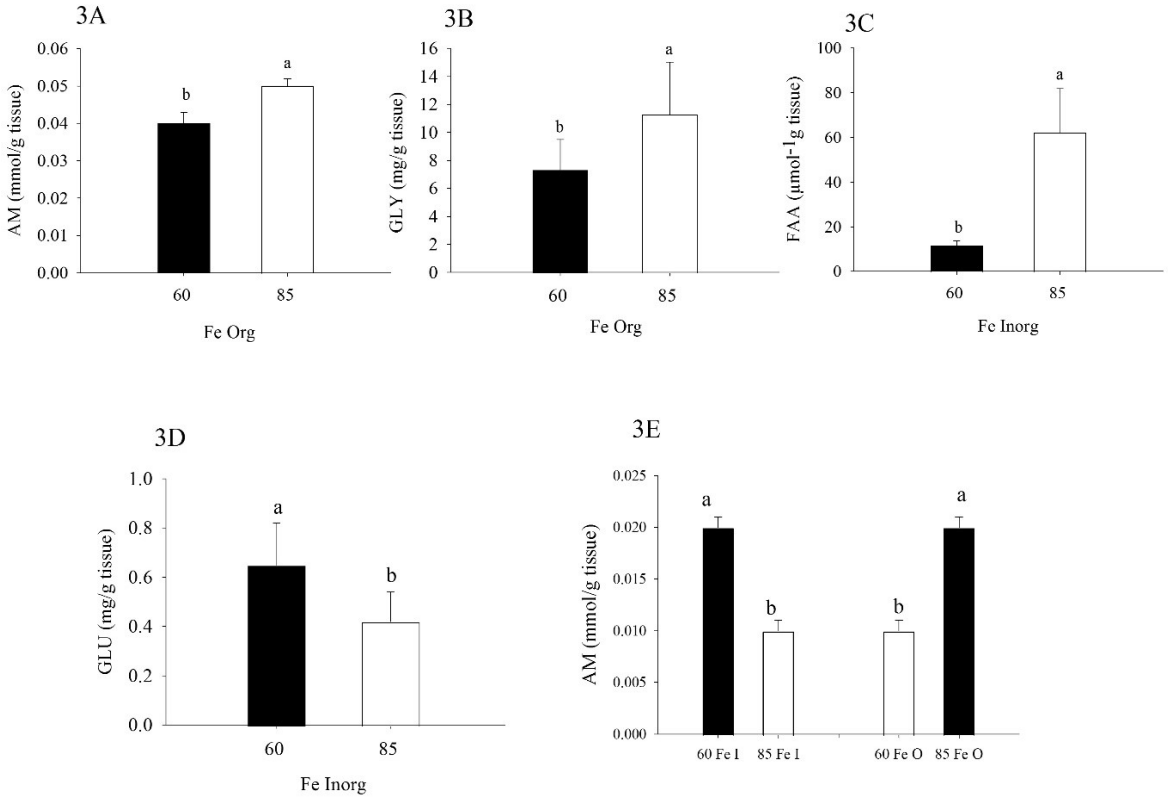

1A
Final weight (g)
70
60
50
40
30
20
10
0
a
b
60
85
Fe Inorg
1B
Total length (cm)
16
14
12
10
8
6
4
2
0
a
b
60
85
Fe Inorg
1C
Specific growth rate (% day-1)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
b
60
85
Fe Inorg
1D
Relative weight gain (%)
300
250
200
150
100
50
0
a
b
60
85
Fe Inorg
1E
Biomass (%)
1400
1200
1000
800
600
400
200
0
a
b
60
85
Fe Inorg


2A
LP %
6
5
4
3
2
1
0
a
b
60
85
Fe Inorg


2B
DM %
30
25
20
15
10
5
0
a
b
60
85
Fe Inorg


2C
HI %
2.0
1.5
1.0
0.5
0.0
a
b
60
85
Fe Inorg


3A
AM (mmol/g tissue)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
b
a
60
85
Fe Org
3B
GLY (mg/g tissue)
16
14
12
10
8
6
4
2
0
b
a
60
85
Fe Org
3C
FAA (µmol-1g tissue)
100
80
60
40
20
0
b
a
60
85
Fe Inorg
3D
GLU (mg/g tissue)
1.0
0.8
0.6
0.4
0.2
0.0
a
b
60
85
Fe Inorg
3E
AM (mmol/g tissue)
0.025
0.020
0.015
0.010
0.005
0.000
a
b
b
a
60 Fe I
85 Fe I
60 Fe O
85 Fe O


3A
AM (mmol/g tissue)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
b
a
60
85
Fe Org
3B
GLY (mg/g tissue)
16
14
12
10
8
6
4
2
0
b
a
60
85
Fe Org
3C
FAA (µmol-1g tissue)
100
80
60
40
20
0
b
a
60
85
Fe Inorg
3D
GLU (mg/g tissue)
1.0
0.8
0.6
0.4
0.2
0.0
a
b
60
85
Fe Inorg
3E
AM (mmol/g tissue)
0.025
0.020
0.015
0.010
0.005
0.000
a
b
b
a
60 Fe I
85 Fe I
60 Fe O
85 Fe O

4.2. Artigo 2

Dietary iron affect hematological and oxidative responses and innate immunity in Nile tilapia (*Oreochromis niloticus*)

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Abstract

Iron is an important mineral for fish development. In this way, we investigate the action of two sources (Fe-organic and Fe-inorganic) and two levels (60 and 85 mg/kg Fe diet) of iron in innate immunity, antioxidant and hematologic responses of Nile tilapia. Fish (16.57 ± 2.25 g) were feed three times a day, during 7 weeks, when fish (400) were distributed in 20 tanks (240 L, 20 fish each). The experiment was composed by 5 treatments, in a completely randomized design: basal diet (BD), without Fe inclusion in mineral mixture, 60 Inor, 60 Org, 85 Inorg and 85 Org. The results showed than Fe organic improve hematocrit and mean corpuscular hemoglobin. The values of lysozyme (LZ) decrease in fish fed with Fe Org but hemolytic activity of the complement system (HACS) and NBT test increase with Fe Org. Fish fed diet 85 Org showed better antioxidant responses. This concluded that inclusion of Fe organic source and level of 85mg/kg is more efficient for juveniles of Nile tilapia.

Keywords: Inorganic, Fe, nutrition, minerals, organic

CRediT authorship contribution statement

Luiza Beatriz Hermes: Conceptualization, investigation, methodology, formal analysis, project administration, roles/writing - original draft, writing - review and editing

Nilce Coelho Peixoto: Formal analysis, investigation, review and editing

Eduardo Kelm Battisti: Formal analysis and editing;

Thamara Luisa Staudt Schneider: Conceptualization, investigation, methodology, writing - review and editing

Rafael Lazzari: Supervision roles/writing - original draft, writing - review and editing

Declaration of conflict interest:

None.

1. Introduction

Fish are an important source of proteins, vitamins and minerals intake for human consumption, resulting in a world consumption of 20.5 kg per capita in 2018. This consumption is being supplied by aquaculture mainly in terrestrial production, since extractive fishing is stagnant. Tilapia is very significant in aquaculture, this specie is cultivated in different systems and that require exogenous food (FAO, 2020). The composition of those diet considers the most representative macronutrients, such as amino acids and fatty acids. However, the microelements, as vitamins and minerals, are also extremely important (Raghunath *et al.,* 2009).

Minerals are extremely important nutrients for the development, growth and reproduction of fish (Lall & Kaushiki., 2021). Microminerals are necessary for several vital functions of animals, being use in small quantity which can cause deficiency and it can also cause an overdose tendency by trying to meet the amount demanded by the animals. The iron (Fe) is classified as a micromineral and has vital functions as hemoglobin conformation, gene regulation and electron transfer reaction. Some enzymes contain Fe such as catalase are responsible for antioxidant activities and they have an important role in the health of animals and humans (Lin *et al*., 2021).

Although widely used in fish diets, inorganic sources have low bioavailability as they contain anti-nutritional factors and are linked to inorganic molecules such as oxides, sulfates, chlorides, carbonates and phosphates, which make absorption difficult and may compete for binding sites with other substances (Pierri *et al*., 2021). Chelated or organic minerals are used in animal feed to improve the absorption of trace minerals, due to their greater bioavailability, they are less sensitive to antinutritional factors such as phytate (Nguyen *et al*., 2019).

In addition, minerals are known to interact with other nutrients due to their lability and tendency to form chemical bonds. The interrelationships between mineral elements may cause physiological or biochemical consequences. Such interactions can be classified as positive or

synergistic, negative or antagonistic. For example, the interaction between copper (Cu) and Fe for the formation of hemoglobin is a possible interaction (Lall & Kaushiki., 2021; O'Dell & Sunde., 1997). Zinc (Zn), on the other hand, competes with Fe at the binding sites, in other words, the excess of Zn can impede the absorption of Fe (Kondaiah*et al.,* 2019).

The optimum Fe level for some species is consolidated, however, for tilapia there is still a gap. Lall and Kaushik (2021), indicated reference values for some species, but not for tilapia. The previous values 85 mg/kg and 60 mg/kg respectively (NRC - National Research Council, Nutrient Requirements of Fish (NRC., 2011) and FURUYA (2013)) do not specify the source to be used, whether organic or inorganic.

Thus, the objective of this work was to test these two reference levels with organic and inorganic iron in hematological parameters, innate immunity and oxidative responses in Nile tilapia (*Oreochromis niloticus*).

2. Material and methods

The feeding trial was conducted in the Fish Farming Laboratory, Department of Biological and Animal Sciences of the UFSM, *campus* Palmeira das Missões (27°55'16.9" S, 53°19'05.7" W, and altitude 639 m). The Ethics Committee on the Use of Animals of UFSM approved all procedures (protocol number: 4892180321).

2.1. Experimental design, diets and feeding

The trial was designed as a 2 X 2 factorial arrangement composed of two Fe - levels (60 and 85 mg/kg) and two Fe -sources organic (Fe-amino acid Availa-Fe®) and inorganic (Fe^{2+} sulfate or Synth®), plus a feel-free basal diet in mineral mixture diet. Diets isonitrogenous (crude protein 35%) and isoenergetics (digestive energy 13 MJ/kg) were formulated based on Abdel-Tawwab *et al* (2010) . Five experimental diets (4 replications) were used: basal diet

(BD), 85 Org Fe (85 mg/kg organic Fe); 85 Fe Inorg (85 mg/kg inorganic Fe); 60 Fe Org (60 mg/kg organic Fe); 60 Fe Inorg (60 mg/kg inorganic Fe) (Table 1).

The ingredients used in feed manufacture were mixed in a horizontal mixer (Monte Castelo-Mod: AML 07). During this process were added: the premix, Fe sources, oil and distilled water. The prepared mass was subjected to pelleting and dried at 55°C for 24 h. After drying, the diets were stored at a temperature of -15°C. The approximate compositions of diets were determined following the methodologies described by the Association of Official Analytical Chemists (AOAC., 1990).

Fish were fed three times a day: 8:00 am, 1:00 and 6:00 pm until they reach apparent satiation, for seven weeks. The residues of feces were removed by siphoning twice a day: 10:00 am and 3:00 pm.

2.2. Fish and experimental conditions

The experiment was completely randomized with 20 polypropylene tanks (240 L). Twenty fish were stored per tank (initial weight=16.57 ± 2.25 g), totalizing 400 juveniles. For two weeks, the fish were adapted to the system. At the beginning and at the end of trial, all fish were individually weighed and measured. Before measurements, the fish were deprived of food for 14 h and anesthetized with eugenol at 50 mL/L (Vidal *et al.,* 2008).

The trial was carried out in a recirculation aquaculture system (RAS) (capacity 8.300 L), contained two biofilters, a solid decanter and a reservoir. The system had temperature control and oxygen injection. The pH, oxygen and temperature were monitored daily. Ammonia, nitrite, alkalinity and hardness were monitored weekly. The water quality parameters were maintained according to the needs of the species (El- Sayed & Kawanna., 2008), and are listed as follows: temperature 25.65 ± 2.32°C, dissolved oxygen 7.50 ± 1.20 mg/L, pH 7.30 ± 0.32, ammonia

0.10 ± 0.03 mg NH_3/L, nitrite 0.02 ± 0.005 mg NO_2^-/L, alkalinity 46 ± 4.20 mg $CaCO_3$/L and hardness 55 ± 2 mg $CaCO_3$/L.

2.3. Sample collection and analysis

At the end of the experiment, blood samples from eight fish per treatment were collected puncturing the caudal vein, with syringes (3 ml) soaked in heparin. Blood samples were used for analyses of the erythrocyte series and immunity. For the oxidative analyses, eight fish per treatment were sacrificed by spinal cord section for tissue collection (liver, gills and muscle).

2.4. Hematological parameters

The erythrocyte series were determined total number of erythrocytes (Neubauer chamber), hematocrit (Ht) (microhematocrit method) and hemoglobin (Hb) (spectrophotometry). Subsequently, the following erythrocyte indexes were calculated using the formulas:

Mean cell volume (MCV) = (Ht×10)/number of erythrocytes;

Mean corpuscular hemoglobin (MCH) = (Hb×10)/number of erythrocytes;

Mean concentration of hemoglobin corpuscular (MCHC) = (Hb×100)/Ht.

2.5. Immune parameters

2.5.1. Plasma lysozyme activity

Lysozyme (LZ) activity was determined by aa turbidimetric assay as described by Jørgensen *et al*. (1993). Briefly, a suspension (200 µL) of Micrococcus lysodeikticus in phosphate-buffered saline solution (0.2 g/L) at pH 5.2, previously determined to be optimum for Silver catfish LZ activity, was mixed with plasma, (10 µL) in a flat bottomed 96 well plates.

Plates were read with a microplate reader (Biochrom, EZ Read 2000) at 450 nm. LZ activity (units/mL) was calculated using the following formula: [(Δabsorbance(4–1 min)/3)/0.001] × 100. A LZ activity unit was defined as the amount of enzyme producing a decrease in absorbance of 0.001/min. Hen egg-white LZ was used as positive control.

2.5.2. Superoxide anion production (NBT test)

Superoxide anion ($O2^-$) production was determined by the reduction of nitroblue tetrazoliun (NBT) (Siwicki *et al*., 1994). Heparinized blood from each fish was mixed with an NBT solution and incubated at 25ºC for 30 min. Thereafter, an aliquot of 50 µL was added to 1 mL of N, N-dimethylformamide and spun at 6000 × g for 5 min. The supernatant was separated, and its optical density reading was taken at 540 nm. Absorbance was then converted to mg NBT diformazan/mL blood as in Li & Gatlin (2003).

2.5.3. Hemolytic activity of the complement system

Hemolytic activity of the complement system (HACS) was measured as reported by Sutili *et al.* (2016) with modifications. An 80% diluted sample (80 µL of plasma + 20 µL of sterile sodium phosphate buffer - PBS, 0.01 M, pH 7.2) was added to 100 µL of 2% washed sheep red blood cells (SRBC) and incubated at 25ºC for 60 min. Next, the mixture was centrifuged at 2500 × g for 5 min. An aliquot of 100 µL was then dispensed into a 96-well plate and measured at 450 nm in a plate reader (Biochrom, EZ Read 2000). Hemolysis percent was obtained by comparing total hemolysis (100%: SRBC + DI water) and no-hemolysis (0%: SRBC + PBS) controls: % hemolysis = [(A540 sample–A540 no-hemolysis)/(A540 total hemolysis–A540 no-hemolysis)]×100.

2.6. Oxidative stress parameters

To determine the stress parameters, the gills, liver and muscle tissues were analyzed.

2.6.1. Thiobarbituric acid reactive substances assay

For thiobarbituric acid reactive substances assay (TBARS) according to Buege& Aust (1978), was added to incubation medium 0.67% thiobarbituric acid, 10% trichloroacetic acid, and sample. The reaction time was of 30 min, at 100°C, for get the malondialdehyde (MDA) TBA pink complex. Next, the tubes were cooled in ice, centrifuged at 4000 rpm for 15 min, and read at 535 nm. The absorbances were compared to standard curve of MDA.

2.6.2. Non-protein sulfhydryl

One aliquot of homogenized tissue was mixed with 10% trichloroacetic acid (1:1, vol:vol), and centrifuged at 3000 rpm/10 min. The supernatant was used for quantifying the non-protein sulfhydryl (NPSH). These molecules were analyzed after adding 10 mM 5,5′-dithiobis (2-nitrobenzoic acid) solution prepared in 20 mM potassium phosphate buffer, pH 7.5, to sample. The absorbance of resultant chemical compound, of yellow color, was immediately determined at 412 nm and compared to standard curve containing reduced glutathione (Ellman., 1959).

2.6.3. Catalase

The homogenized tissue supernatant was added to 50 mM potassium phosphate buffer, pH 7.0. The enzymatic reaction of catalase (CAT) was initiated by adding of substrate (0.79 M hydrogen peroxide) and the resultant absorbances were monitored during 2 min (10/10 s), at 240 nm in quartz cuvette. The spectrophotometer was resented to zero absorbance with a blank

solution, reaction medium without substrate. The CAT activity was measured using the molar absorption coefficient of 43.6 for hydrogen peroxide (Aebi, 1984).

2.6.4. Superoxide dismutase

The superoxide dismutase (SOD) activity was quantified according to Misra &Fridovich (1972). For determination of activity was utilized the system of autoxidation of epinephrine with concomitant formation of adrenochrome (pink). To an aliquot of 50 mM glycine-HCl buffer, pH 10.5, was added to different volumes of sample. The assay was initiated by adding of 60 mM epinephrine bitartrate and the absorbance was monitored (λ = 480 nm) during 1 min (10/10 s). The reading interval (the min) was determined by assay without sample where the absorbance increase is more pronounced than others. The enzymatic activity is expressed as unit of SOD (U SOD), volume of sample that inhibits the formation of product in 50%.

2.7. Statistical analysis

The data obtained were submitted to the Shapiro-Wilk Normality test, followed by a Two-Way ANOVA, where the differences between levels and sources were compared by Student's "t" test. Furthermore, when significant interaction occurred, data were compared separately between sources ($P<0.05$).

3. Results

The relative weight gain (RWG) was higher in fish feeding with Fe Org (273.37%) and 85 Fe (261.34%), not differing from the group fed with BD (251.97%). The fish fed with 60 Fe showed RWG (152.23%) was statistically similar to fish fed diets containing the source of Fe Inorg (140.19%). (Data not shown).

3.1. Hematological parameters

Fish fed diets containing Fe Org showed higher values of Ht, Hb and MCH (Table 2). The Ht of fish fed diets with Fe Inorg was lower compared to fish fed diets containing Fe Org, but both did not differ from the group fed BD. On the other hand, fish fed diets containing Fe Org had higher Hb level compared to fish fed diets containing Fe Inorg and BD (Fig.1A).

3.2. Immune Parameters

The innate immunity values of fish show in table 3. LZ activity was lower in fish fed diets containing Fe Org compared to Fe Inorg and BD. Hemolytic activity of the complement system of fish who was feed with a diet contains Fe Inorg showed lower HACS in comparison of Fe Org and BD.

Fish fed diets containing Fe Inorg and at the 60 Fe level resulted in lower superoxide anion production (NBT) in relation to the other groups (Table 3). However, NBT level from fish fed diets with two levels of Fe Org exhibited greater response at the 85 Fe level than at the 60 Fe level (Fig.1B).

3.3. Oxidative parameters

The oxidative stress parameters are shown in Table 4. CAT was higher (Fig 2A) in fish fed 85 Fe Inorg. In fish muscle the diet with Fe Org influenced the CAT activity, the highest values were observed in fish that received diets containing Fe Org as compared to the Fe Inorg group, which did not differ from the BD group. CAT was lower in the gills of fish fed with BD.

Superoxide dismutase (SOD) in fish liver was lower when fed diets containing 60 Fe compared to the 85 Fe (Fig 2D). In muscles, it was the diet with Fe Org that resulted in lower SOD activity, while the diet with Fe Inorg and 60 Fe showed higher SOD activity (Fig 3A). In tilapia gills fed with these same treatments, SOD enzymatic activity was no detectable.

TBARS was higher in fish fed 60 Fe Inorg (Fig 2B). In fish muscles and gills, TBARS levels did not differ among experimental groups.

Fish that received the diets containing 85 Fe and Fe Org had a higher concentration of NPSH in the liver compared to the 60 Fe and Fe Inorg groups, but they did not differ from the BD (Fig 2C) The NPSH in tilapia muscles was higher at the 85 Fe among the levels tested but did not differ from the Fe Org group (Fig 3B). In fish gills, the BD presented the lowest value from in the NPHS, the highest value is 85 Fe Inorg and 60 Fe Org (Fig 3C).

4. Discussion

4.1. Hematological parameters

Ht and Hb values found is this study is like observed by El-Saidy& Gaber (2004) for Nile tilapia fed diets containing different Fe-levels. Furthermore, the blood values found are as already found for the species (Abozeid *et al*., 2021).

Hb values were also higher in fish fed Fe Org, compared to Fe Inorg and BD. Fe is the main component of Hb and may influence the increase in its amount of Hb in RBC, as observed in other studies (Abozeid *et al.,* 2021; Afshari *et al*., 2021; Qiao *et al*., 2013; Behera *et al*., 2014). MCH represents the size of the hemoglobin contained in RBC (El Basuini *et al.,* 2020). In the present study, it was observed that, in addition to larger amounts, the level of Hb was greater in fish fed diets containing Fe Org.

Shiau & Su (2002) described that the tilapia may suffer from hypochromic microcytic anemia, characterized by a decrease in Hb, Ht, MCV and MCHC, using a diet below 149 mg/kg of Fe. The authors supplemented the diets with inorganic sources of Fe (ferric citrate and ferrous sulfate) and observed that the bioavailability of the sources influences the mineral absorption

by the species. In this study symptoms of deficiency of Fe were not observed, once the fish showed growth and good health.

4.2. Immune parameters

Immune parameters are important because to demonstrate the defense capability and health status of fish by the of LZ, HACS, and antibody responses (Sutili *et al*., 2016). In this study, fish fed with organic Fe source had lower LZ activity and higher HACS and NBT.

Lysozyme is an antibacterial enzyme secreted by leukocytes, plays an important role in the innate immune system of fish (Sun *et al*., 2013; Liu *et al*., 2006). Fish fed inorganic minerals showed increased LZ along with increased phagocytic activity (NBT) and superoxide anion production by leukocytes (Afshari *et al*., 2021; Behera *et al*., 2014; Hossian *et al*., 2020; El Basuini *et al.,* 2016). In the present study, the lowest levels of LZ were recorded in the group fed Fe Org in the diet. Apparently, the group fed with organic source showed better health condition.

Complement activity (HACS) of fish plasma was higher in Fe Org, which suggest improving fish immunity. Hossian *et al* (2020) find improvement in complement activity by feeding Rainbow trout (*Oncorhynchus mykiss*) with dietary inorganic monophosphates. Sotoudeh *et al*., (2020) have find had elevated of complement activity in the feeding of Yellow seabream with organic acid salts, but Andersen *et al*., (1998) detect the complement activities of Atlantic salmon (*Salmo salar*) were not influenced used dietary iron sources (both organic and inorganic form).

4.3. Oxidative stress parameters

The lipoperoxidation measured by TBARS is the degradation of lipids, the result of this degradation is oxidative damage, and this is a useful analysis to observe the oxidative stress

(Hossian *et al*., 2020). YU *et al.,* (2021) find for Coho salmon (*Oncorhynchus kisutch*), when the diet content is Fe Inorg the TBARS level is increased. Corroborating in the current study with 60 Fe Inorg, indicating that lower levels of Fe Inorg may increase lipidic peroxidase.

Furthermore, 85 Fe Inorg has lower TBARS, corroborating with YU *et al*., (2021) about the lower Fe content not being sufficient to reduce lipoperoxidase. This may occur because Fe may have antioxidant activity (Zhang *et al*., 2016) but in fish liver fed with Fe Org they showed lower TBARS, in both levels demonstrating that Fe Org can protect the liver from lipidic peroxidase (Afshari *et al*., 2021).

CAT and SOD are important enzymes to protect tissues from reactive oxygen species (ROS) which may cause oxidative damage (Yu *et al*., 2021; Dawood *et al*., 2020). The present study found the CAT was reduced in the liver of fish fed diets containing Fe Org and 60 Fe, which may mean that fish do not need to protect themselves from oxidative damage, unlike diets containing 85 Fe Inorg with higher CAT than in others. In fish muscles, the Fe Org diet influenced the CAT, the fish may be protecting them themselves for ROS which are caused by normal biological activities. The Fe Inorg group, but did not differ from the BD group, which did not have Fe addition in the mineral mixture, as it is an important cofactor of CAT, an activity that (Yu *et al.,* 2021) being insufficient in protection against ROS. CAT was lower in the gills of fish fed with BD due to the low amount of Fe available in BD.

SOD is an important enzyme because it can stimulate the dissociation of two superoxide radicals in H_2O_2 (Kishawy *et al*., 2020). In this study, SOD in fish liver was lower when fed diets containing 60 Fe Inorg compared to 85 Fe Inorg. The liver is the most metabolic organ in the system and stores iron in the form of ferritin, which can influence the increase in SOD, since Fe can be dissociated into the ionic form, making it able to participate in the generation of inhibited free radicals by SOD (Welch *et al*., 2002). In tilapia muscle, the diet with Fe Org resulted in lower SOD activity of this enzyme is lower in this tissue. In tilapia gills fed with

these same treatments, SOD does not showed results. Fe can play an important role in antioxidant system, functioning as scavenger, being part of the antioxidant metalloenzyme SOD (Fe/Mn-SOD)(de Oliveira *et al*., 2018).

Fish that received the diets containing 85 Fe Inorg had a higher concentration of NPSH in the liver compared to the other groups. NPSH indicates the ability of fish to prevent the oxidation of protein tissues (Marasca *et al*., 2020). In tilapia muscle, the diet with 85 Fe NPSH levels were higher than other groups. In fish gills, the 85 Fe Inorg and 60 Fe were highest in NPSH results.

5. Conclusion

The fish fed with Fe Org in the diet show better growth and health status, including improves antioxidant capacity and prevents tissue lipid peroxidation. It is recommended to use the level 85 mg/kg diet.

Acknowledgment

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6. References

Abdel-Tawwab, M., Ahmad, M. H., Khattab, Y. A., & Shalaby, A. M. 2010. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus (L.).* Aquaculture, 298, 267-274.

Abozeid.A.M., Abdel-Rahim.M.M, Abouelenien.F, Elkaradawy.A, Mohamed.R.A., 2021 *Quillaja saponaria* and/or *Yucca schidigera* ameliorate water quality, growth performance, blood health, intestine and gills histomorphology of Nile tilapia, *Oreochromis niloticus*. Aquaculture Research, 52, 6117-6131.

Aebi, H. 1984. Catalase in vitro. Methods in enzymology, 105, 121-126.

Afshari.A, Sourinejad.I, Gharaei.A, Johari.S.A, Ghasemi.Z, 2021. The effects of diet supplementation with inorganic and nanoparticulate iron and copper on growth performance, blood biochemical parameters, antioxidant response and immune function of snow trout *Schizothorax zarudnyi* (Nikolskii, 1897). Aquaculture, 539, 736638.

Agriculture Organization of the United Nations. Fisheries Department. 2020. The State of World Fisheries and Aquaculture, Food & Agriculture Org.

Andersen, F., Lygren, B., Maage, A., & Waagbø, R. 1998. Interaction between two dietary levels of iron and two forms of ascorbic acid and the effect on growth, antioxidant status and some non-specific immune parameters in Atlantic salmon (*Salmo salar*) smolts. Aquaculture, 161, 437-451.

Association of Official Analytical Chemists (AOAC), 2000. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Behera, T., Swain, P., Rangacharulu, P. V., Samanta, M. 2014. Nano-Fe as feed additive improves the hematological and immunological parameters of fish, *Labeo rohita H*. Applied Nanoscience, 4, 687-694.

Buege, J. A., & Aust, S. D. 1978. Microsomal lipid peroxidation. In Methods in enzymology 52, 302-310. Academic press.

Dawood, M. A., Zommara, M., Eweedah, N. M., Helal, A. I., & Aboel-Darag, M. A. 2020. The potential role of nano-selenium and vitamin C on the performances of Nile tilapia (*Oreochromis niloticus*). Environmental Science and Pollution Research, 1-10.

de Oliveira, L. F., Santos, C., Risso, W. E., & dos Reis Martinez, C. B. 2018. Triple-mixture of Zn, Mn, and Fe increases bioaccumulation and causes oxidative stress in freshwater neotropical fish. Environmental toxicology and chemistry, 37, 1749-1756.

El Basuini, M. F., El-Hais, A. M., Dawood, M. A., Abou-Zeid, A. E. S., EL-Damrawy, S. Z., Khalafalla, M. M. E. S., Dossou, S. 2016. Effect of different levels of dietary copper nanoparticles and copper sulfate on growth performance, blood biochemical profiles, antioxidant status and immune response of red sea bream (*Pagrus major*). Aquaculture, 455, 32-40

El Basuini, M. F., Teiba, I. I., Zaki, M. A., Alabssawy, A. N., El-Hais, A. M., Gabr, A. A., ... & Dossou, S. 2020. Assessing the effectiveness of CoQ10 dietary supplementation on growth performance, digestive enzymes, blood health, immune response, and oxidative-related genes expression of Nile tilapia (*Oreochromis niloticus*). Fish & shellfish immunology, 98, 420-428.

Ellman, G. L. 1959. Tissue sulfhydryl groups. Archives of biochemistry and biophysics, 82, 70-77.

El-Saidy, D. M., & Gaber, M. M. 2004. Use of cottonseed meal supplemented with iron for detoxification of gossypol as a total replacement of fish meal in Nile tilapia, *Oreochromis niloticus (L.)* diets. Aquaculture Research, 35, 859-865.

El-Sayed.A.F.M, Kawanna,M, 2008. Effects of dietary protein and energy levels on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock in a recycling system. Aquaculture, 280, 179–184.

Furuya, W. M. (2017). Nutrição de tilápias no Brasil. Varia Scientia Agrárias, 3, 133-150.

Hossain, M. S., Chance, A. B., El Kertaoui, N., Wattiez, X., Houndji, A., Mandiki, S. N., & Kestemont, P. 2020. Dietary inorganic monophosphates in high plant ingredient-based diets influence nutrient digestibility, postprandial macro-mineral status and immune functions of juvenile rainbow trout, *Oncorhynchus mykiss*. Aquaculture Nutrition, 26, 2178-2194.

Jobling, M. 1983. A short review and critique of methodology used in fish growth and nutrition studies. Journal of Fish Biology, 23, 685-703.

Jørgensen, J. B., Sharp, G. J., Secombes, C. J., & Robertsen, B. 1993. Effect of a yeast-cell-wall glucan on the bactericidal activity of rainbow trout macrophages. Fish & Shellfish Immunology, 3, 267-277.

Kishawy.A.T., RoushdyE.M., Hassan.F.A, Mohammed.H.A, Abdelhakim.T.M, 2020. Comparing the effect of diet supplementation with different zinc sources and levels on growth performance, immune response and antioxidant activity of tilapia, *Oreochromis niloticus.* Aquaculture Nutrition, 26, 1926-1942.

Kondaiah, P., Yaduvanshi, P. S., Sharp, P. A., Pullakhandam, R. 2019. Iron and zinc homeostasis and interactions: does enteric zinc excretion cross-talk with intestinal iron absorption?. Nutrients, 11, 1885.

Lall, S. P., & Kaushik, S. J. 2021. Nutrition and Metabolism of Minerals in Fish. Animals, 11, 2711.

Li, P., & Gatlin III, D. M. 2003. Evaluation of brewers yeast (*Saccharomyces cerevisiae*) as a feed supplement for hybrid striped bass (*Morone chrysops*× *M. saxatilis*). Aquaculture, 219, 681-692.

Lin, S., Hu, X., Li, L., Yang, X., Chen, S., Wu, Y., & Yang, S. 2021. Preparation, purification and identification of iron-chelating peptides derived from tilapia (*Oreochromis niloticus*) skin collagen and characterization of the peptide-iron complexes. LWT, 111796.

Liu, H., Zheng, F., Cao, Q., Ren, B., Zhu, L., Striker, G., & Vlassara, H. 2006. Amelioration of oxidant stress by the defensin lysozyme. American Journal of Physiology-Endocrinology and Metabolism, 290, E824-E832.

Marasca, S., Battisti, E. K., Durigon, E. G., Peixoto, N. C., Uczay, J., Baldisserotto, B., Lazzari, R. 2020. Growth, hematology, metabolism, and oxidative parameters of silver catfish (*Rhamdia quelen*) fed diets containing *Lippia alba* leaf. Aquaculture, 529, 735730.

Misra, H. P., & Fridovich, I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological chemistry, 247, 3170-3175.

Nguyen, L., Kubitza, F., Salem, S. M., Hanson, T. R., & Davis, D. A. 2019. Comparison of organic and inorganic microminerals in all plant diets for Nile tilapia *Oreochromis niloticus.* Aquaculture, 498, 297-304.

NRC, National Research Council, Nutrient Requirements of Fish. 2011.Committee on Animal Nutrition. Board on Agriculture. National Research Council. National Academy Press,Washington DC., USA. ,

O'Dell, B. L., & Sunde, R. A. (Eds.). 1997. Handbook of nutritionally essential mineral elements. CRC Press.

Pierri, B. D. S., Silva, A. D., Cadorin, D. I., Ferreira, T. H., Mouriño, J. L. P., Filer, K., Fracalossi, D. M. 2021. Different levels of organic trace minerals in diets for Nile tilapia juveniles alter gut characteristics and body composition, but not growth. Aquaculture Nutrition, 27, 176-186.

Qiao, Y. G., Tan, B. P., Mai, K. S., Ai, Q. H., Zhang, W. B., & Xu, W. 2013. Evaluation of iron methionine and iron sulphate as dietary iron sources for juvenile cobia (*Rachycentron canadum).* Aquaculture Nutrition, 19, 721-730.

Raghunath, M., Venu, L., Padmavathi, I., Kishore, Y. D., Ganeshan, M., Kumar, K. A., Rao, K. R. 2009. Modulation of macronutrient metabolism in the offspring by maternal micronutrient deficiency in experimental animals. Indian J Med Res, 130, 655-665.

Rostagno, H.S., Albino,L.F.T., Donzele,J.L., Gomes,P.C., de Oliveira, R.F., Lopes, D.C., et al, Composição e valor nutritivo dos alimentos. In: Horacio Santiago Rostagno (4ed.). Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais (2017) 488

Shiau, S. Y., & Su, L. W. 2003. Ferric citrate is half as effective as ferrous sulfate in meeting the iron requirement of juvenile tilapia, *Oreochromis niloticus*× *O. aureus*. The Journal of nutrition, 133, 483-488.

Siwicki.A.K., Anderson.D.P, Rumsey.G.L, 1994. Dietary intake of immunostimulants by rainbow trout affects nonspecific immunity and protection against furunculosis. Veterinary immunology and immunopathology, 41, 125-139.

Sotoudeh, E., Sangari, M., Bagheri, D., Morammazi, S., & Torfi Mozanzadeh, M. 2020. Dietary organic acid salts mitigate plant protein induced inflammatory response and improve humoral immunity, antioxidative status and digestive enzyme activities in yellowfin seabream, *Acanthopagrus latus*. Aquaculture Nutrition, 26, 1669-1680.

Sun, R., Yue, F., Qiu, L., Zhang, Y., Wang, L., Zhou, Z., Song, L. 2013. The CpG ODNs enriched diets enhance the immuno-protection efficiency and growth rate of Chinese mitten crab, *Eriocheir sinensis*. Fish & shellfish immunology, 35, 154-160.

Sutili, F. J., Gatlin III, D. M., Rossi Jr, W., Heinzmann, B. M., & Baldisserotto, B. 2016. In vitro effects of plant essential oils on non-specific immune parameters of red drum, Sciaenops ocellatus L. Journal of animal physiology and animal nutrition, 100, 1113-1120.

Vidal, L. V. O., Albinati, R. C. B., Albinati, A. C. L., Lira, A. D. D., Almeida, T. R. D., & Santos, G. B. 2008. Eugenol como anestésico para a tilápia-do-nilo. Pesquisa Agropecuária Brasileira, 43, 1069-1074

Welch, K. D., Davis, T. Z., Van Eden, M. E., & Aust, S. D. 2002. Deleterious iron-mediated oxidation of biomolecules. Free radical biology and medicine, 32, 577-583

Yu, H., Chen, B., Li, L., Zhang, Q., Liu, D., Wang, Z., & Shan, L. 2021. Dietary iron (Fe) requirement of coho salmon (*Oncorhynchus kisutch*) alevins assessed using growth, whole body and hepatic Fe concentrations and hepatic antioxidant enzymes activities. Aquaculture Research.

Zhang, L., Feng, L., Jiang, W. D., Liu, Y., Jiang, J., Li, S. H., ... & Zhou, X. Q. 2016. The impaired flesh quality by iron deficiency and excess is associated with increasing oxidative damage and decreasing antioxidant capacity in the muscle of young grass carp (*Ctenopharyngodon idelus*). Aquaculture Nutrition, 22 191-201

Figure captions

Fig.1 (1A) Hematocrit (Ht) of tilapia fed diets containing iron (Fe Inorg) (1B) Superoxide anion production (NBT test) in the blood of tilapia fed diets containing two levels of organic iron (Fe Org). Values expressed as mean ± standard deviation. Different letters indicate differences according to the Student's t test ($P < 0.05$).

Fig. 2 Antioxidant status in the liver of tilapia fed diets containing two levels of Fe Inorg. (2A, 2B, 2C, 2D). Values expressed as mean ± standard deviation. Different letters indicate differences according to the Student's test ($P < 0.05$).

Fig.3 Antioxidant status in the muscle (3A, 3B) and Gllis (3C) of tilapia fed diets containing two levels of Fe Org and Fe Inorg. Values expressed as mean ± standard deviation. Different letters indicate differences according to the Student's test ($P < 0.05$).

Table 1. Ingredients and approximate composition of five experimental diets

Ingredients (g/kg)	Diets				
	BD	85 Fe Org	85 Fe Inorg	60 Fe Org	60 Fe Inorg
Tilapia meal [a]	13.53	13.53	13.53	13.53	13.53
Soybean meal	50.00	50.00	50.00	50.00	50.00
Corn	20.00	20.00	20.00	20.00	20.00
Wheat meal	10.67	10.07	9.82	10.37	10.24
Soybean oil	2.00	2.00	2.00	2.00	2.00
Vitamin and mineral mixture [b]	2.00	2.00	2.00	2.00	2.00
Vitamin c	1.00	1.00	1.00	1.00	1.00
Salt	0.80	0.80	0.80	0.80	0.80
Fe Org (mg/kg)	-	0.60	0.85	-	-
Fe Inorg (mg/kg)	-	-	-	0.30	0.43
Approximate composition (%)					
Crude protein	34.86	33.07	33.04	33.19	34.65
Lipids	6.74	7.11	6.02	6.66	7.38
Dry matter	94.64	95.36	94.63	95.30	95.70
Ash	10.23	9.69	9.25	9.73	9.33
ED (mj/kg) [c]	13.40	13.34	13.31	13.37	13.35
Calcium [d]	4.11	4.11	4.11	4.11	4.11
Phosphorus [d]	2.35	2.35	2.35	2.35	2.35
Iron [d]	145.38	204.15	228.63	204.76	229.49

[a] Tilapia residue meal; b vitamin and mineral pre-mix without iron prepared by Puro Trato®. [b]Composition: Vitamin B9 (2.400 mg); Vitamin B3 (48 g); Vitamin B5 (24 g); Vitamin B7 (96 mg); 200 mg; Vitamin A (2.400.000 IU); Vitamin D3 (400.000 IU); Vitamin E (24.000 IU); Vitamin B1 (9.600 mg); Vitamin B2 (9.600 mg); Vitamin B6 (9.600 mg); Vitamin B12 (9.600 mg); Vitamin C (96 g); Vitamin K3 (4,800 mg); Iodine (200 mg); Manganese (40 g); Selenium (200 mg) Zinc (6.000 mg); Cobalt (20 mg); Copper (1.250 mg);

[c] Digestible energy = [(Crude protein × 23.61 MJ/kg × 0.9) + (Lipids × 39.82 MJ/kg × 0.85) + (Carboihydrates × 17.21 MJ/kg × 0.50)] (Jobling., 1983);

[d] Calculated according to the Brazilian Poultry and Swine Table (Rostagno *et al*., 2017). BD: basal diet: no addition of iron (Fe) in the vitamin and mineral pre-mix; Fe Org: organic; Fe Inorg: inorganic.

Table 2. Hematological parameters of tilapia fed diets containing sources and levels of iron

		Levels		Sources		*P-value*			
	BD	60 Fe	85 Fe	Fe Org	Fe Inorg	Levels	Sources	Inter	Sem
HT	29.56	28.52	30.33	30.78[a]	28.22[b]	0.26	0.0008	0.0004	2.40
HB	5.86	6.40	6.36	6.90[a*]	5.76[b]	0.39	0.003	0.31	0.82
RBC	1.72	1.72	1.76	1.85	1.62	0.94	0.07	0.16	0.29
MVC	176.18	192.60	169.04	189.08	165.09	0.34	0.24	0.22	33.08
MCH	32.48	36.13	35.94	39.95[a*]	31.67[b]	0.54	0.008	0.08	6.33
MCHC	20.30	22.44	22.06	22.94	21.26	0.31	0.15	0.55	2.52

Means with different letters, between the factors differ by the Student's t test. Means marked with * differ from diets without added iron. BD: basal diet, no addition of iron (Fe) in the vitamin and mineral pre-mix; Fe Org: organic; Fe Inorg: inorganic; SEM: Standard error of means; Inter: Interaction; Ht: hematocrit (%); Hb: hemoglobin (g/dL); RBC: erythrocyte (10^6/µL); MVC: mean cell volume (fL); MCH: mean corpuscular hemoglobin (pg); MCHC: mean corpuscular hemoglobin concentration (g/dL);

Table 3. Immune parameters of tilapia fed diets containing sources and levels of iron

		Levels			Sources			*P-value*	
	BD	60 Fe	85 Fe	Fe Org	Fe Inorg	Levels	Sources	Inter	Sem
LZ	250.00	227.78	197.22	172.22[b]*	252.78[a]	0.33	0.01	0.64	73.63
HACS	73.25	66.56	70.22	69.93[a]	66.86[b]*	0.14	0.21	0.06	5.91
NBT	0.34	0.28[b]*	0.33[a]	0.34[a]	0.27[b]*	<0.0001	<0.0001	<0.0001	5.91

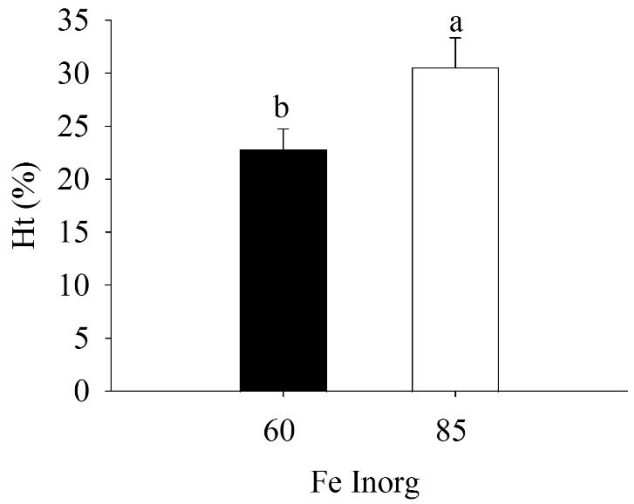
Means with different letters, between the factors, differ by the "t" and Student tests. Means marked with * differ from basal diet. (BD) Basal diet: no addition of Iron (Fe) in the vitamin and mineral pre-mix; Fe Org: organic; Fe Inorg: inorganic; SEM: Standard error of means; Inter: Interaction; LZ: Lysozyme activity (units/mL); HACS: Hemolytic activity of the complement system (%); NBT: superoxide anion production (OD 540 nm).

Table 4. Oxidative stress parameters of tilapia fed with two sources and two levels of iron and a diet without iron

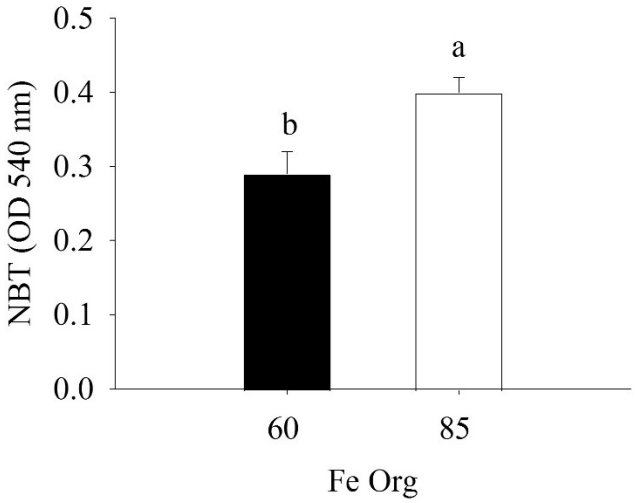
		Levels		Sources		p-value			
	BD	60 Fe	85 Fe	Fe Org	Fe Inorg	Levels	Sources	Inter	Sem
Liver									
CAT	11.01	8.43^{b}	13.09^{a}	9.80^{b}	12.80^{a}	0.001	0.06	0.01	3.03
SOD	1.31	0.84^{b*}	1.64^{a*}	1.22	1.26	<0.0001	0.69	0.0002	0.29
TBARS	26.96	93.07^{a*}	18.95^{b}	20.63^{b}	84.95^{a*}	<0.0001	<0.0001	<0.0001	16.79
NPSH	116.95	111.07^{b}	120.77^{a}	120.39^{a}	111.58^{b}	0.05	0.009	0.001	10.01
Muscle									
CAT	0.06	0.07	0.06	0.08^{a*}	0.05^{b}	0.06	0.002	0.12	0.02
SOD	8.57	10.43^{a*}	7.76^{b}	5.58^{b*}	12.60^{a*}	<0.0001	<0.0001	<0.0001	1.12
TBARS	9.45	9.72	8.14	8.82	8.31	0.65	0.85	0.50	3.99
NPSH	56.24	53.62^{b}	62.64^{a*}	60.26*	57.08	<0.0001	0.002	0.008	4.20
Gill									
CAT	0.39	0.56*	0.56*	0.56*	0.57*	0.02	0.83	0.44	0.14
TBARS	133.05	115.08	118.97	113.46	122.43	0.27	0.38	0.75	24.81
NPSH	30.43	41.64*	40.76*	42.01*	40.51*	<0.0001	0.39	0.0008	5.18

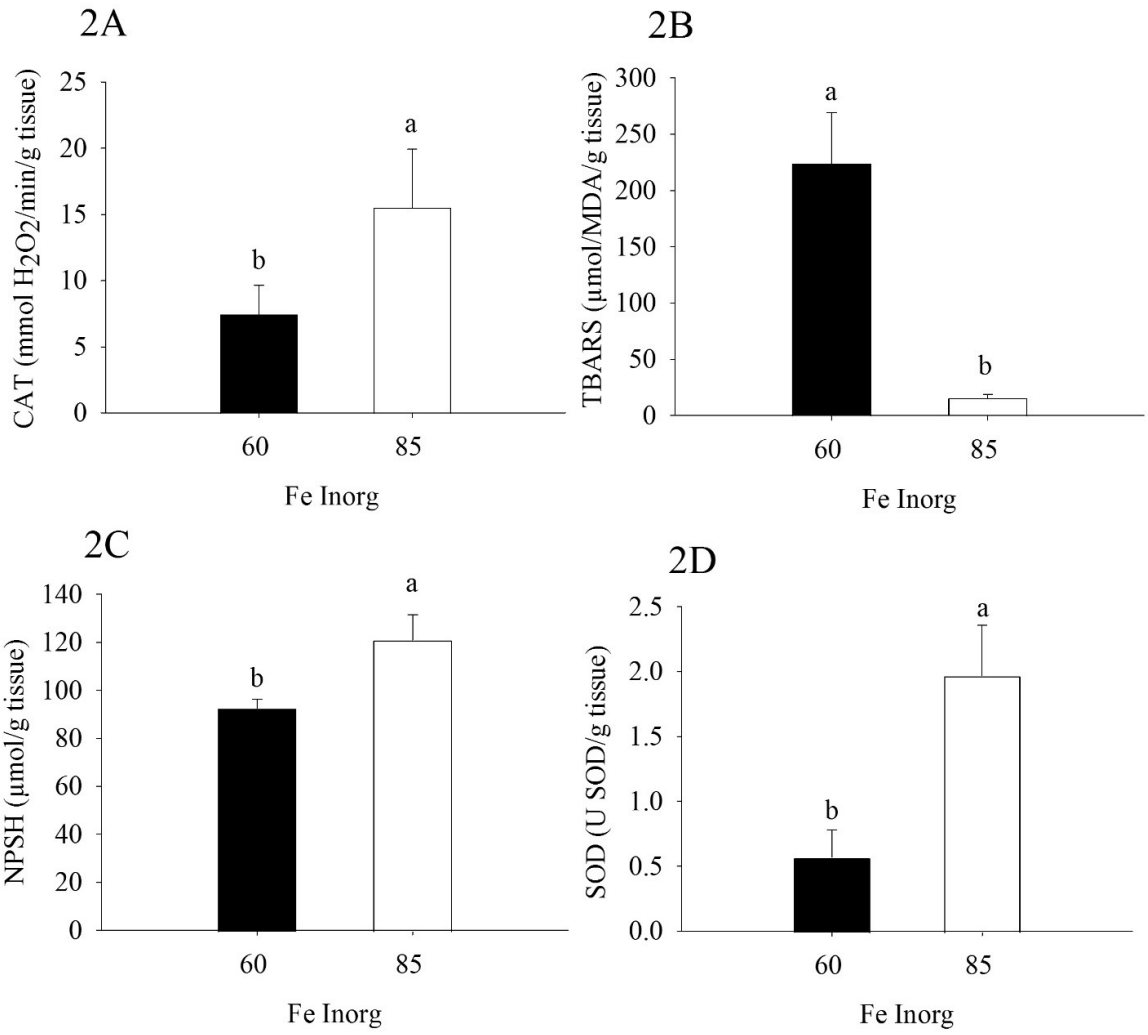
Means with different letters, between the factors, differ by the "t" and Student tests. Means marked with * differ from basal diets. (BD): Basal diet: no addition of Iron (Fe) in the vitamin and mineral pre-mix; Fe Org: organic; Fe Inorg: inorganic; Inter: Interaction; CAT: catalase (mmol H_2O_2/min/g tissue); SOD: superoxide dismutase (U SOD/g tissue); TBARS: thiobarbituric acid reactive substances (µmol/MDA/g tissue); NPSH: non-protein thiols (µmol/g tissue). SEM: Standard error of means

1A

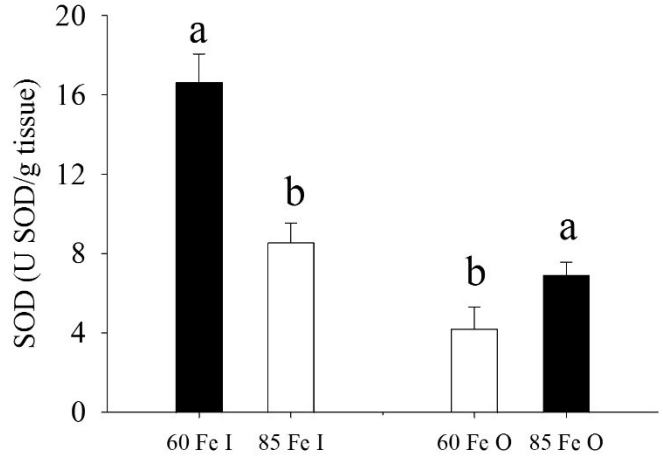

35
30
25
20
15
10
5
0
Ht (%)
a
b
60
85
Fe Inorg

1B

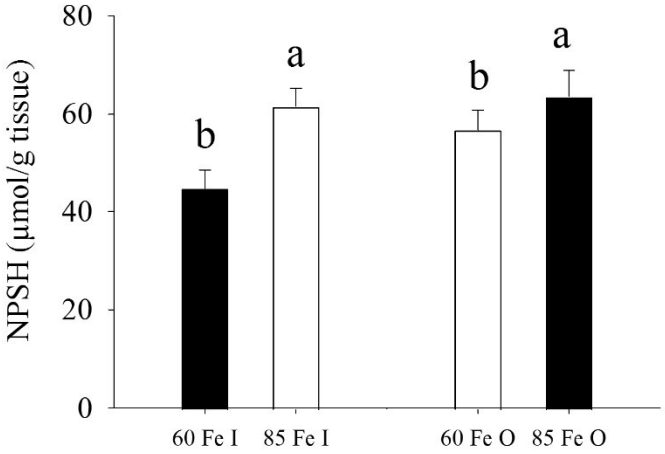

0.5
0.4
0.3
0.2
0.1
0.0
NBT (OD 540 nm)
a
b
60
85
Fe Org


2A
CAT (mmol H2O2/min/g tissue)
25
20
15
10
5
0
a
b
60
85
Fe Inorg
2B
TBARS (µmol/MDA/g tissue)
300
250
200
150
100
50
0
a
b
60
85
Fe Inorg
2C
NPSH (µmol/g tissue)
140
120
100
80
60
40
20
0
a
b
60
85
Fe Inorg
2D
SOD (U SOD/g tissue)
2.5
2.0
1.5
1.0
0.5
0.0
a
b
60
85
Fe Inorg

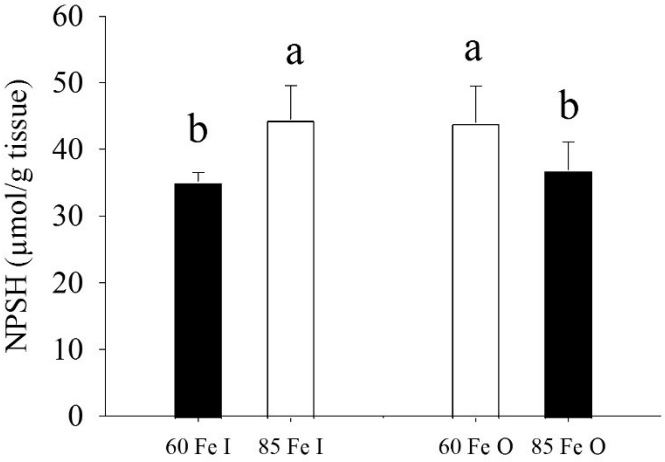
3A


SOD (U SOD/g tissue)
20
16
12
8
4
0
a
b
b
a
60 Fe I
85 Fe I
60 Fe O
85 Fe O

3B


NPSH (µmol/g tissue)
80
60
40
20
0
b
a
b
a
60 Fe I
85 Fe I
60 Fe O
85 Fe O

3C


NPSH (µmol/g tissue)
60
50
40
30
20
10
0
b
a
a
b
60 Fe I
85 Fe I
60 Fe O
85 Fe O

5. CONCLUSÕES E CONSIDERAÇÕES FINAIS

A inclusão de ferro orgânico nas dietas de juvenis de tilápia do Nilo expuseram resultados significativamente melhores quando comparados as dietas com ferro inorgânico e sem a adição de ferro no premix. Os principais resultados zootécnicos demonstram um melhor desempenho dos peixes alimentados com os níveis 60 e 85 mg/kg de Fe orgânico, não diferindo entre os níveis utilizados.

A biodisponibilidade de minerais quelatados é consolidada na nutrição animal e vem demonstrando cada vez mais potencial de utilização, não apenas no ótimo desenvolvimento dos animais, mas também quanto a parte ambiental do cultivo, devido aos mesmos facilitarem a absorção e diminuírem a excreção de determinados contaminantes ao ambiente.

O que ainda não se encontra consolidado na nutrição animal e principalmente em peixes são as interações entre minerais e minerais, vitaminas e minerais e minerais e outras substâncias. Além da capacidade que os peixes possuem de absorver minerais presentes na água, o que influencia diretamente no desenvolvimento dos animas e foi demonstrado pelo presente estudo onde as tilápias que não receberam adição de Fe no premix não apresentaram nenhum sintoma de deficiência de Fe como anemia ou baixo desenvolvimento, devido a sua necessideade mineral ter sido suprida com o ferro contido nos ingredientes utilizados na ração.

A mesma eficiência do Fe orgânico é apresentada pelo presente estudo nos parâmetros oxidativos, bioquímicos e a capacidade imune das tilápias demonstrando a importância da utilização de um nutriente eficiente na nutrição de tilápias para não apenas um excelente desenvolvimento em visão de produto, mas também em relação a qualidade deste, que tem como finalidade a nutrição humana, cada vez é mais exigente com a qualidade e bioseguridade dos produtos consumidos.

Como já exposto anteriormente a nutrição animal é de extrema importância no desenvolvimento da cadeia do pescado e ainda hoje encontramos diversas lacunas,tanto no que rege quantidades e formas de produtos utilizados, como também a utilização em total pelo animal, como rotas alternativas, animais sob condições adversas, excessos de outros minerais, competições de sítios de ligação entre as mais diversas capacidades animais. Demonstrando assim a importância de novos estudos sobre minerais e suas formas e diversas possíveis interações. Além disso, os efeitos dos minerais de acordo com a velocidade de crescimento dos peixes (capacidade genética) e os sistemas de produção devem ser considerados em estudos futuros.

6. REFERÊNCIAS

ANTONY JESU PRABHU, P.; SCHRAMA, J. W.; KAUSHIK, S. J. Mineral requirements of fish: A systematic review. **Reviews in Aquaculture,** v. 8, n. 2, p. 172–219, 1 jun. 2016.

BEZERRA, S. et al. Criação de tilápia em tanques. **Sebrae**, p. 1–28, 2014.

BURY, N; GROSELL, M. Iron acquisition by teleost fish. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, v. 135, n. 2, p. 97-105, 2003.

DA SILVA, R, A. et al. Relação zinco e ferro em dietas para larvas de tilápia (*Oreochromis niloticus*). **Acta Iguazu**, v. 1, n. 3, p. 88-97, 2012.

DOS SANTOS, L. D. et al. Dietary conjugated linoleic acid (CLA) for finishing Nile tilapia. **Aquaculture Nutrition**, v. 17, n. 2, p. e70-e81, 2011.

DURIGON, G. E et al. Exigências nutricionais para bagres nativos do Brasil: uma revisão. **Research, Society and Development,** v. 10, n. 13,2021.

EL-SAIDY, D. M. S. D.; GABER, M. M. A. Effect of dietary protein levels and feeding rates on growth performance, production traits and body composition of Nile tilapia, *Oreochromis niloticus* (L.) cultured in concrete tanks. **Aquaculture Research**, v. 36, n. 2, p. 163–171, 2005.

FAO. The State of World Fisheries and Aquaculture 2016: Contributing to food security and nutrition for all. Rome, Italy: 2016.

FAO. 2020. The State of Food and Agriculture 2020. Overcoming water challenges in agriculture. Rome. https://doi.org/10.4060/cb1447en.

FURUYA, M,W. Nutrição de tilápias no Brasil. **Varia Scientia Agrárias**, v. 3, n. 1, p. 133-150, 2013.

HILTON, J. W. The interaction of vitamins, minerals and diet composition in the diet of fish. **Aquaculture**, v. 79, n. 1-4, p. 223-244, 1989.

LALL, S. P. The Minerals. Fish Nutrition. Elsevier **Academic Press,** San Diego, CA, USA, p. 259-308, 2002.

LALL, S. P.; KAUSHIK, S. J. Nutrition and metabolism of minerals in fish. **Animals**, v.11, n. 9, p 2711, 2021.

LAZZARI, R.; RADÜNZ NETO, J. Exigências de vitaminas e minerais para peixes. In: Sakomura, N.K.; Silva, J.H.V.; Costa, F.G.P.; Fernandes, J.B.K.; Hauschild, L. (Org.). Nutrição de Não Ruminantes - ISBN 9788578051327. 1ed.Jaboticabal, SP: FUNEP, 2014, v. 1, p. 447-458.

LIN, -Y; SHIAU, S.-Y. Mutual sparing of dietary requirements for alpha-tocopherol and selenium in grouper, *Epinephelus malabaricus*. **Aquaculture**, v. 294, p. 242-245, 2009.

LIN, Y.-H. et al. Dietary copper requirement reevaluation for juvenile grouper, *Epinephelus malabaricus*, with an organic copper source. **Aquaculture**, v. 310, p.173-177, 2010

NGUYEN, L. et al. Comparison of organic and inorganic microminerals in all plant diets for Nile tilapia *Oreochromis niloticus*. **Aquaculture,** v. 498, p. 297–304, 1 jan. 2019.

PEIXE BR. Associação Brasileira da Piscicultura. Anuário Brasileiro da Piscicultura, São Paulo, Brasil. 2020.

PEIXE BR. Associação Brasileira da Piscicultura. Anuário Brasileiro da Piscicultura, São Paulo, Brasil. 2021.

PIERRI et al. Different levels of organic trace minerals in diets for Nile tilapia juveniles alter gut characteristics and body composition, but not growth. **Aquaculture Nutrition**. v.00 p.1–11. 2020.

RESTREPO, G. M; DÍAZ, L.E.; RONDÓ, P.H. C. Classic and emergent indicators for the assessment of human iron status. **Critical Reviews in Food Science and Nutrition**, p. 1-14, 2020.

ROEDER, M; ROEDER, R. H. Effect of iron on the growth rate of fishes. **The Journal of nutrition**, v. 90, n. 1, p. 86-90, 1966.

SHIAU, S.-Y.; SU, L.-W. Ferric Citrate is Half as Effective as Ferrous Sulfate in Meeting the Iron Requirement of Juvenile Tilapia, *Oreochromis niloticus* × *O. aureus*. **The Journal of Nutrition**, v. 133, n. 2, p. 483–488, 2003.

SIGNOR, A. et al. Parâmetros hematológicos da tilápia-do-Nilo: efeito da dieta suplementada com levedura e zinco e do estímulo pelo frio. **Ciência Animal Brasileira**, v. 11, n. 3, 2 out. 2010.

SINGH, M et al. Iron mediated hematological, oxidative and histological alterations in freshwater fish *Labeo rohita***. Ecotoxicology and environmental safety**, v. 170, p. 87-97, 2019.

WEBSTER, C. D.; LIM, C. Minerals Dietary Nutrients, Additives, and Fish Health: Wiley Online Books., 20 November 2021. Disponível em: <https://doi.org/10.1002/9781119005568.ch9>

ANEXO A - CERTIFICADO DE APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS CEUA – UFSM

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que a proposta intitulada "FONTES E NÍVEIS DE FERRO NO CRESCIMENTO E SAÚDE DE TILÁPIA (Oreochromis niloticus)", protocolada sob o CEUA nº 4892180321 (ID 003362), sob a responsabilidade de **Rafael Lazzari** *e equipe; Emerson Giuliani Durigon; Thamara Luisa Staudt Schneider; Bernardo Baldisserotto; Luiza Hermes; Nagiezi de Menezes Lovatto; Nilce Coelho Peixoto; Juliano Uczay* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 11/05/2021.

We certify that the proposal "SOURCES AND IRON LEVELS IN THE GROWTH AND HEALTH OF TILAPIA (Oreochromis niloticus) ", utilizing 400 Fishes (400 males), protocol number CEUA 4892180321 (ID 003362), under the responsibility of **Rafael Lazzari** *and team; Emerson Giuliani Durigon; Thamara Luisa Staudt Schneider; Bernardo Baldisserotto; Luiza Hermes; Nagiezi de Menezes Lovatto; Nilce Coelho Peixoto; Juliano Uczay* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 05/11/2021.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 08/2021 a 11/2021 Área: Departamento de Zootecnia E Ciências Biológicas Ufsm/pm

Origem:	Não aplicável biotério						
Espécie:	Peixes	sexo:	Machos	Idade:	90 a 100 dias	N:	400
Linhagem:	Tilápia/ Gift			Peso:	13 a 18 g		

Local do experimento: Biotério de Piscicultura/Campus Palmeira das Missões. Responsável Técnico: Médica Veterinária Luciana Fagundes Christofari

Santa Maria, 29 de dezembro de 2021

Dra. Patricia Bräunig
Presidente da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Profa. Dra. Vania Lucia Loro
Vice-Presidente da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

ANEXO B – ARTIGO 1 - NORMAS DE SUBMISSÃO DA REVISTA ANIMAL FEED SCIENCE AND TECHNOLOGY

ANIMAL FEED SCIENCE AND TECHNOLOGY

An International Scientific Journal Covering Research on Animal Nutrition, Feeding and Technology

AUTHOR INFORMATION PACK

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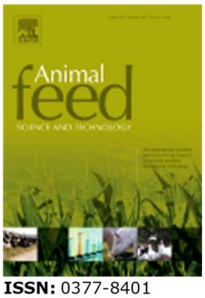

ISSN: 0377-8401

DESCRIPTION

Animal Feed Science and Technology is a unique journal publishing scientific papers of international interest focusing on **animal feeds** and their **feeding**.

Papers describing research on feed for ruminants and non-ruminants, including **poultry**, **horses**, **companion animals** and **aquatic animals**, are welcome.

The journal covers the following areas:
Nutritive value of feeds (e.g., assessment, improvement) Methods of **conserving** and **processing** feeds that affect their nutritional value **Agronomic** and **climatic** factors influencing the nutritive value of feeds **Utilization** of feeds and the improvement of such Metabolic, production, reproduction and **health responses**, as well as potential environmental impacts, of diet inputs and feed technologies (e.g., feeds, feed additives, feed components, mycotoxins) Mathematical models relating directly to **animal-feed interactions** Analytical and experimental methods for **feed evaluation** Environmental impacts of feed technologies in animal production

The journal does not encourage papers with emphasis on animal products, molecular biology, genetics or management, or the regulatory or legal aspects of feeds as well as animal production studies with a focus on animal nutrition that do not have a direct link to a feed or feed technology.

Manuscripts must be prepared in accordance with the journal's Guide for Authors.
Before preparing their manuscript, it is suggested that authors examine the following editorials by the Editors-in-Chief:

Editorial on terminology and analytical methods (Anim. Feed Sci. Technol. 118 (2005) 181-186)
Editorial on experimental design and statistical criteria (Anim. Feed Sci. Technol. 129 (2006) 1-11)
Editorial on general suggestions and guidelines (Anim. Feed Sci. Technol. 134 (2007) 181-188)
Editors comments on plagiarism (Anim. Feed Sci. Technol. 154 (2009) 292-293)
Editorial on review techniques and responding on editorial comments (Anim. Feed Sci. Technol. 155 (2010) 81-85)
Editorial on use of replicates in statistical analyses in papers submitted for publication in Animal Feed Science and Technology (Anim. Feed Sci. Technol. 171 (2012) 1-5)

For an example of a sample manuscript click here.

GUIDE FOR AUTHORS

INTRODUCTION

Types of article

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2. Review Articles
3. Short Communications
4. Book Reviews

Original Research Papers should report the results of original research. The material should not have been previously published elsewhere, except in a preliminary form.

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Professor G. Flachowsky
Federal Research Centre of Agriculture
Institute of Animal Nutrition
Bundesallee 50
D-38116 Braunschweig
Germany

Manuscripts describing the use of commercial feed products are welcome, but should include the following information: major components, contents of active ingredients (for example enzyme activities). Independent verification, as opposed to a manufacturers guarantee, is always desirable and often avoids difficulties in the review process, especially where there are no, or few, treatment impacts. The Editors reserve the right to reject any manuscript employing such products, wherein this information is not disclosed.

Submissions concerning feedstuff composition are welcome when published and/or accepted analytical procedures have been employed. However, unusual feedstuffs and/or a wide range of data are pre-requisites.
Submissions concerning NIRS may be suitable when more accurate, precise or robust equations are presented. Mathematical, technical and statistical advancement, may constitute the foundation for acceptance. For more details see the editorial in Vol. 118/3-4.

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AUDIENCE

Animal Scientists, Crop Scientists, Feed Manufacturers, Feed Additive Producers.

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State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

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The following definitions should be used, as appropriate:

a. aNDFom-NDF assayed with a heat stable amylase and expressed exclusive of residual ash.
b. NDFom-NDF not assayed with a heat stable amylase and expressed exclusive of residual ash.
c. aNDF-NDF assayed with a heat stable amylase and expressed inclusive of residual ash.
d. NDF-NDF assayed without a heat stable amylase and expressed inclusive of residual ash.
e. ADFom-ADF expressed exclusive of residual ash.
f. ADF-ADF expressed inclusive of residual ash.
g. Lignin (sa)-Lignin determined by solubilization of cellulose with sulphuric acid.
h. Lignin (pm)-Lignin determined by oxidation of lignin with permanganate.

While expressions of NDF and ADF inclusive of residual ash will continue to be acceptable (i.e., the terms aNDF, NDF and ADF above), the Editors-in-Chief highly recommend reporting all fibre values, including digestibilities, on an OM basis. Silica is partially soluble in ND, is quantitatively recovered in AD, and so may contribute to the 'fibre' values and to subsequent digestibility coefficients.

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Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

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This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Follow internationally accepted rules and conventions: use the international system of units (SI). If other quantities are mentioned, give their equivalent in SI. You are urged to consult IUB: Biochemical Nomenclature and Related Documents for further information.

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SI or SI-derived units should be used throughout (e.g. MJ and not Kcal for energy concentrations). Concentrations should be expressed on a 'per kg' basis (w/w); however, w/v, v/v, mol/mol or M may be accepted depending on the circumstances. In addition, 'units' and 'equivalents' are acceptable. Normality should be avoided, as it may be ambiguous for certain acids. If analytical standards have been used, they should be specified by name (e.g. yeast RNA) and form (e.g. lactose monohydrate). Percents should only be used when describing a relative increase or decrease in a response. Proportions should be maximum 1.0 or ≤1.0. For more details see the editorial in Vol. 118/3-4.

Percent is *only* used to indicate relative changes. For composition, both w/w (often solids composition g/kg) and w/v (e.g. g/L), v/v (e.g. m/L), mol/mol or M can be accepted depending on the circumstances. Specify units (e.g. g/L) and never as percent.

Digestibility/metabolisability and degradability should always be expressed as a coefficient (not %), and the content of, for example, the digestible component should be expressed as g/kg: thus, the coefficient of digestibility of dry matter is 0.8, while the content of digestible dry matter is 800g/ kg. A distinction between true and apparent digestibility should be made, as well as between faecal and ileal (e.g. coefficient of total tract apparent digestibility - CTTAD). The terms 'availability' and 'bioavailability' should be avoided without definition in context.

In chemical formulae, valence of ions should be given as, e.g. Ca^{2+}, not as Ca^{++}. Isotope numbers should precede the symbols e.g. ^{18}O. The repeated use of chemical formulae in the text is to be avoided where reasonably possible; instead, the name of the compound should be given in full.

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Results should be clear and concise.

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The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

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The abstract should be clear, descriptive and not longer than 400 words. It should contain the following specific information: purpose of study; experimental treatments used; results obtained, preferably with quantitative data; significance of findings; conclusions; implications of results if appropriate.

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References cited together in the text should be arranged chronologically. The list of references should be arranged alphabetically on authors' names, and chronologically per author. If an author's name in the list is also mentioned with co-authors the following order should be used: publications of the single author, arranged according to publication dates - publications of the same author with one co-author - publications of the author with more than one co-author. Publications by the same author(s) in the same year should be listed as 2001a, 2001b, etc.

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Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

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Exceptions may be made in the case of a very long name occurring very frequently or in the case of a compound being described as the end product of a gravimetric determination (e.g. phosphate as P_2O_5).

Math formulae
Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

If differences between treatments are statistically significant, this should be indicated by adding the actual 'P' value obtained. If 0.10 > P > 0.05, then differences can be considered to suggest a trend, or tendency, to a difference, but the actual 'P' value should be stated. Further information on this issue can be found in *Animal Feed Science and Technology* Vol. 129/1-2.

Spaces should be used between all values and units, except for the following: Between the value and degrees or percent. In equations around * and /. In probability expressions (P<0.05). When probability values are given, the 'P' should be a capital letter.

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Electronic artwork
General points
• Make sure you use uniform lettering and sizing of your original artwork.
• Embed the used fonts if the application provides that option.
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A detailed guide on electronic artwork is available.
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All data in figures should have a measure of variation either on the plot (e.g., error bars), in the figure legend itself, or by reference to a table with measures of variation in the figure legend.

Explanations should be given in the figure legend(s). Drawn text in the figures should be kept to a minimum.

If a scale is given, use bar scales (instead of numerical scales) that must be changed with reduction.

Data references
This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

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2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.
Citations may be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999)…. Or, as demonstrated (Jones, 1999; Allan, 2000)… Kramer et al. (2010) have recently shown …'
List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.
Reference to a website:
Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/ aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).
Reference to a dataset:
[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/ xwj98nb39r.1.
Reference to software:

Coon, E., Berndt, M., Jan, A., Svyatsky, D., Atchley, A., Kikinzon, E., Harp, D., Manzini, G., Shelef, E., Lipnikov, K., Garimella, R., Xu, C., Moulton, D., Karra, S., Painter, S., Jafarov, E., & Molins, S., 2020. Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88). Zenodo. https://doi.org/10.5281/zenodo.3727209.

References concerning unpublished data and "personal communications" should not be cited in the reference list but may be mentioned in the text.

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AFTER ACCEPTANCE

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ANEXO C – ARTIGO 2- NORMAS DE SUBMISSÃO DA REVISTA AQUACULTURE

AQUACULTURE

An International Journal

AUTHOR INFORMATION PACK

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ISSN: 0044-8486

DESCRIPTION

The aim of *Aquaculture* is to publish and make available the highest quality international scientific contributions to aquaculture. The Journal publishes disciplinary, interdisciplinary and transdisciplinary aquaculture research. The scope of *Aquaculture* includes the traditional priorities of its sections, but also includes papers from non-traditional scientific areas such as sustainability science, social-ecological systems, ornamental, conservation and restoration related to aquaculture.

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AUDIENCE

Aquaculturists, Fisheries Scientists, Marine Biologists.

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GUIDE FOR AUTHORS

INTRODUCTION

Types of paper

Research Papers should report the results of original research. The material should not have been previously published elsewhere. Articles are expected to contribute new information (e.g. novel methods of analysis with added new insights and impacts) to the knowledge base in the field, not just to confirm previously published work.

Review Articles can cover either narrow disciplinary subjects or broad issues requiring interdisciplinary discussion. They should provide objective critical evaluation of a defined subject. Reviews should not consist solely of a summary of published data. Evaluation of the quality of existing data, the status of knowledge, and the research required to advance knowledge of the subject are essential.

Short Communications are used to communicate results which represent a major breakthrough or startling new discovery and which should therefore be published quickly. They should not be used for preliminary results. Papers must contain sufficient data to establish that the research has achieved reliable and significant results.

Technical Papers should present new methods and procedures for either research methodology or culture-related techniques.

The *Letters to the Editor* section is intended to provide a forum for discussion of aquacultural science emanating from material published in the journal.

Contact details for submission

Papers for consideration should be submitted via the electronic submission system mentioned below to the appropriate Section Editor:

Nutrition:
Vertebrate Nutrition: D.M. Gatlin
Invertebrate Nutrition: M.T. Viana
Larval Nutrition: Q. Ai

The Nutrition Section welcomes high quality research papers presenting novel data as well as original reviews on various aspects of aquatic animal nutrition relevant to aquaculture. Manuscripts addressing the following areas of investigation are encouraged:

1) determination of dietary and metabolic requirements for various nutrients by representative aquatic species. Studies may include environmental/stress effects on animal's physiological responses and requirements at different developmental stages;
2) evaluation of novel or established feedstuffs as well as feed processing and manufacturing procedures with digestibility and growth trials. Such studies should provide comprehensive specifications of the process or evaluated ingredients including nutrients, potential anti-nutrients, and contaminants;
3) comparison of nutrient bioavailability from various ingredients or product forms as well as metabolic kinetics of nutrients, food borne anti-nutrients or toxins;
4) identification of key components in natural diets that influence attractability, palatability, metabolism, growth reproduction and/or immunity of cultured organisms;
5) optimization of diet formulations and feeding practices;
6) characterization of the actions of hormones, cytokines and/or components in intracellular signaling pathway(s) that influence nutrient and/or energy utilization.
7) evaluation of diet supplementation strategies to influence animal performance, metabolism, health and/or flesh quality.
8) evaluation of nutritional strategies oriented to environment-friendly aquaculture and high-quality products.

Manuscripts concerning other areas of nutrition using novel or advanced methods are also welcome. Please note that in regard to various diet additives such as probiotics, prebiotics, herbal extracts, etc., a very large number of papers have already been published. Therefore, Aquaculture will not continue to accept manuscripts that present initial and preliminary investigations of such additives. Manuscripts addressing these and other feed additives will be accepted for review only if they are

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Fish Nutrition, Fish Disease, Gut Health, Gut Microbiota, Fish diet
Francesco Buonocore, University of Tuscia Department for Innovation in Biological Agricultural Food and Forestry Systems, Viterbo, Italy

of the highest scientific quality and they represent a significant advance in our knowledge of the mechanisms involved in their metabolism. Manuscripts may also be considered if they present clinical efficacy data generated in large-scale trials and economic cost-benefit analysis of these applications.

Aquaculture Production Science:
Jian Qin

The Aquaculture Production Science (PS) is dedicated to research on improvements and innovations in aquatic food production.

This section supports worldwide dissemination of the results of innovative, globally important, scientific research on production methods for aquatic foods from fish, crustaceans, mollusks, amphibians, and all types of aquatic plants. Contributions are encouraged in the following areas: 1) Improvement of production systems that results in greater efficiencies of resource usage and sustainability of aquaculture; 2) Effective applications of technologies and methods of aquaculture production for improved stocking regimes; 3) The use of new species and species assemblages; and, 4) Investigations to minimize aquaculture wastes and improve water quality, including technologies for nutrient recycling in aquaculture ecosystems, and potential synergy of aquaculture and other food production systems using methods such as polyculture and integrated aquaculture. Aspects of seafood processing and technology will not be considered in this section although aquaculture techniques that may influence the nutritional value of aq

Physiology:
Vertebrate Physiology: A. Takemura
Invertebrate Physiology: M. Wilder

The Physiology Section welcomes high quality papers that present either novel research data or original reviews. The content must be relevant to solving aquaculture problems on all aspects of the physiology of cultured aquatic animals and plants.

Submitted manuscripts must have a valid hypothesis or objective, clearly state the relevance to aquaculture, have proper experimental design with appropriate controls and utilize appropriate statistical analysis. Mention of trade names is limited to the main text.

Relevant physiological topics include, but are not limited to:Reproductive and endocrine physiology, including control of development and sex differentiation, induced ovulation and spermiation, gamete quality, storage and cryopreservation, physiology of gynogenetic, and triploid and transgenic organismsCardiorespiratory, muscle and exercise physiologyOsmoregulatory physiologyDigestive physiology, including endocrine and environmental regulation of growthLarval physiology and ontogeny, including metamorphosis, smolting and moltingPerformance under variable culture conditions, including temperature, water quality, rearing density, and stress and disease physiologyPhysiology of harvest and handling techniques

Genetics:
J.A.H. Benzie

The Genetics Section welcomes high-quality research papers presenting novel data, as well as critical reviews, on various aspects of selective breeding, genetics and genomics. Submitted manuscripts must have a valid hypothesis or objective, clearly state the relevance to aquaculture, have proper experimental design with appropriate sample size and controls and utilize appropriate statistical analysis.

Relevant genetics topics include, but are not limited to:Breeding programs using classic selection procedures, markers or combining marker assisted selection with classic selectionApplicationsof crossbreeding and interspecific hybridizationEvaluation of commercially important phenotypes among cultured strains, populations or stocksApplications of biotechnology and genetic manipulationmethodsDevelopment of linkage maps, identification of QTL or association of commercially importanttraits with specific gene(s). Where appropriate, linkage maps should include co-dominant markers, such as microsatellite DNA and SNP markers, to enable application to other populations and facilitate comparative mapping.

Aquaculture will NOT accept manuscripts dealing with the application of well-described techniques to yet another species, unless the application solves a specific biological problem important to aquaculture production; or manuscripts dealing with gene cloning, characterizing of microsatellites, species identification using molecular markers, EST papers with small collections, or mapping papers with a small number of markers, unless the papers also deal with solving a biological problem that is relevant to aquaculture production.
Aquaculture will not accept manuscripts focusing mainly on population genetics studies that are based on RAPD and AFLP markers, since the dominance and multilocus nature of the fingerprints are not suitable for making inferences about population genetic diversity and structure.

Sustainability and Society:
M. Dey

The Sustainability and Society section of the journal Aquaculture invites articles at the interface of natural and social sciences that address the broader roles of aquaculture in global food security and trade.

Aims and scope of the Sustainability and Society section are the: global dissemination of interdisciplinary knowledge regarding the management of aquatic resources and resulting impacts on people. Interconnections with other sectors of food production; resource management and implications for societal impact. Going beyond a narrow techno-centric focus, towards more holistic analyses of aquaculture within well-defined contexts. Enquiry based on understanding trajectories of change amid the global challenges of climate change and food security. Mixed methods and approaches that incorporate and integrate both social and natural sciences. Relevance for the diverse range of policy makers, practitioners and other stakeholders involved. Articles that take a value chain approach, rather than being wholly production orientated, are encouraged.

Immunology:
J. Galindo-Villegas

The Immunology section aims to attract high-quality manuscripts dealing with the understanding and characterization of the innate and adaptive immune mechanisms and defense systems, from molecules and cells to tissues impacting the variated aquatic organisms generated through controlled culture means. Functional studies are preferred over those merely descriptive and without a clear scope among aquacultured organisms. Developments and new notions in the understanding of host-microbe interactions, immunostimulation, vaccination, trained immunity, immune-tolerance, etc. determined via using state-of-the-art techniques like (meta)genomics, transcriptomics, metabolomics or proteomics on specific target species, or explicitly demonstrated in lower taxa model-organisms with a clear further application in aquaculture are highly encouraged.

Disease:
Microbial interaction: P. Bossier
Parasites and Parasite Control: M. Longshaw
Viral interactions: F. Kibenge

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