

**UNIVERSIDADE FEDERAL DE MINAS GERAIS**  
**ESCOLA DE VETERINÁRIA**  
**PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA**

**REGULAÇÃO DO CONSUMO DE DIETAS COM**  
**DIFERENTES RELAÇÕES DE ENERGIA:PROTEÍNA**  
**PELO BAGRE NEOTROPICAL *Lophiosilurus alexandri***

**DISSERTAÇÃO DE MESTRADO**

**FABIO AREMIL COSTA DOS SANTOS**

**Belo Horizonte, MG**  
**2019**

**Fabio Aremil Costa dos Santos**

**REGULAÇÃO DO CONSUMO DE DIETAS COM  
DIFERENTES RELAÇÕES DE ENERGIA:PROTEÍNA  
PELO BAGRE NEOTROPICAL *Lophiosilurus alexandri***

Dissertação apresentada ao Programa de Pós-Graduação em Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais, como requisito parcial para obtenção do grau de mestre em Zootecnia.

Área de Concentração: Nutrição e Alimentação Animal Prof<sup>ª</sup>. Orientadora: Dra. Paula Adriane Perez Ribeiro

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Regulação do consumo de água com diferentes níveis  
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final para a obtenção do Grau de Mestre em Zootecnia, área de Concentração em Nutrição Animal.

Abrindo a sessão, a Presidente da Comissão, Prof.<sup>a</sup> Paula Adriane Perez Ribeiro, após dar a conhecer aos presentes o teor das Normas Regulamentares da Defesa de Dissertação, passou a palavra ao candidato (a), para apresentação de seu trabalho. Seguiu-se a arguição pelos examinadores, com a respectiva defesa do(a) candidato(a). Logo após, a Comissão se reuniu, sem a presença do(a) candidato(a) e do público, para julgamento da dissertação, tendo sido atribuídas as seguintes indicações:

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## **Dedico esta Dissertação**

Primeiramente a Deus, aos meus pais que são minha base, minha família pelo apoio, aos meus amigos por não me deixar desistir e a todos que de alguma forma contribuíram para essa caminhada até aqui.

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*“Você nunca sabe que resultados virão da sua ação. Mas se você não fizer nada, não existirão resultados.”*

*(Mahatma Gandhi)*



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## RESUMO

Santos, F. A. C. **REGULAÇÃO DO CONSUMO VOLUNTÁRIO DA RELAÇÃO ENERGIA:PROTEÍNA, COM BASE NA COMPOSIÇÃO DA DIETA DO BAGRE NEOTROPICAL *Lophiosilurus alexandri***. 2019. XXp. Dissertação de mestrado (Zootecnia – Nutrição animal) - Escola de Veterinária, Universidade Federal de Minas Gerais, 2019.

Investigou-se o efeito de diferentes relações energia:proteína no consumo voluntário de ração pelo bagre *Lophiosilurus alexandri*, com o objetivo de avaliar seu comportamento alimentar. Dois ensaios foram realizados. No primeiro experimento foram utilizados 16 peixes, distribuídos em 16 tanques, aos quais foi permitido selecionar uma entre duas dietas encapsuladas oferecidas (24% vs 42% de proteína bruta ou relação energia:proteína de 0,068 vs 0,041 MJ g<sup>-1</sup>). Para o segundo experimento, seis grupos experimentais foram delineados, com aumentos sucessivos de proteína, para avaliar a seleção da dieta pelo método de autoalimentação: Grupo 1 - 24% de proteína bruta x 30% de proteína bruta; G2 - 24% x 36%; G3 - 24% x 42%; G4 - 30% x 36%; G5 - 30% x 42%; e G6 - 36% x 42%. Estes grupos tiveram as seguintes relações energia:proteína (E:P, em MJ g<sup>-1</sup> proteína): G1 - 0,068 x 0,057; G2 - 0,068 x 0,048; G3 - 0,068 x 0,041; G4 - 0,057 x 0,048; G5 - 0,057 x 0,041; e G6 - 0,048 x 0,041. Os dados de seleção de dieta foram transformados em arcsen ( $\sqrt{\phantom{x}}$ ) e então submetidos ao teste t de Student. As médias de proteína (g/kg de peso vivo), energia:proteína (energia MJ/g proteína bruta/kg peso vivo) e energia (energia MJ/kg peso vivo) foram submetidas à ANOVA e teste de Tukey (p<0,05). Os resultados do primeiro experimento revelaram uma clara preferência pela dieta contendo 42% de proteína bruta e relação energia:proteína de 0,041 MJ g<sup>-1</sup> de proteína (p<0,05). Mesmo com ausência de percepção de sabor, o peixe retornou ao padrão inicial de seleção depois que o conteúdo das cápsulas foi alterado. O experimento 2 revelou que com dietas contendo menos de 30% de proteína bruta (G1), os peixes selecionaram dietas com maior energia (p<0,05). Nos grupos G4 e G6 os animais exibiram um padrão pouco claro de seleção da dieta, provavelmente, em função da estreita faixa de opção de nutrientes ou pelos requerimentos de proteína e energia terem sido atendidos pelas dietas. Animais dos grupos G2, G3 e G5 escolheram dietas com maior teor de proteína (p<0,05), mesmo após

serem trocadas entre os alimentadores. Os animais mantiveram o padrão de ingestão de proteína no G4 (de 36 a 38% ou 20 g/ peso corporal) e consumo de energia (8,42 MJ kg<sup>-1</sup>), para todos os tratamentos. Com base no comportamento alimentar de *L. alexandri*, no presente estudo, conclui-se que a relação energia:proteína escolhida pelos animais é de aproximadamente 0,028 MJ g<sup>-1</sup> de proteína.

**Palavras-chave:** Auto seleção, comportamento alimentar, nutrição, proteína, produção

## ABSTRACT

The effect of different energy to protein ratios on voluntary feed intake was investigated in the carnivorous catfish *Lophiosilurus alexandri*, a species recently introduced into Brazilian aquaculture. Two experimental trials were performed. The first trial involved 16 fish distributed among 16 tanks, which were allowed to select one of two experimental encapsulated diets (24% vs 42% of crude protein or E:P of 0.068 x 0.041 MJ g<sup>-1</sup> protein). The objective was to isolate sensorial influence on the choice of diet. For the second trial, six experimental groups were designed with successive increases in protein to evaluate diet selection by the self-feeding method: Group 1 - 24% of crude protein x 30% of crude protein; G2 - 24% x 36%; G3 - 24% x 42%; G4 - 30% x 36%; G5 - 30% x 42%; and G6 - 36% x 42%. These groups had the following energy to protein ratios (E:P MJ g<sup>-1</sup> protein): G1 – 0.068 x 0.057; G2 – 0.068 x 0.048; G3 – 0.068 x 0.041; G4 – 0.057 x 0.048; G5 – 0.057 x 0.041; and G6 – 0.048 x 0.041. Data were subjected to Levene's test for homoscedasticity and the Shapiro-Wilk test for normality. Diet selection data were arcsin ( $\sqrt{\quad}$ ) transformed and then subjected to the Student T test. Mean protein (g / kg of BW), energy: protein (MJ energy / g crude protein / kg BW) and energy (MJ energy / kg BW) consumption were submitted to ANOVA and Tukey test. Statistical significance was considered at P<0.05. The results of Trial 1 revealed a clear preference for diet with 42% crude protein and an energy to protein ratio of 0.041 MJ g<sup>-1</sup> protein (P<0.05); even with the absence of taste the fish returned to the initial pattern of selection after the contents of the capsules were changed. Trial 2 revealed that with diets containing less than 30% crude protein (G1), the fish selected diets with higher energy (P<0.05). In G2 and G6 the fish exhibited an unclear pattern of diet selection probably due to the narrow range of nutrient options or because the requirements for protein and energy were met by the diets, respectively. For the G2, G3 and G5, fish choose diets with higher protein content (P<0.05), even after the diets were switched between feeders. The animals kept the protein intake pattern from G4 (~36-38% or 20g/BW) and energy consumption (8.42 MJ kg<sup>-1</sup>) for all treatments. In conclusion, levels of voluntary protein and energy intake depend on the availability or range of dietary nutrients. Based on the feeding behavior of *L. alexandri* in the present study, we infer that the target E:P is around 0.028 MJ g<sup>-1</sup> protein.

**Keywords:** Food behaviour, nutrition, protein, production, self-selection

## 1. INTRODUÇÃO

Segundo a Organização das Nações Unidas para Alimentação e Agricultura (FAO), aquacultura é o cultivo de organismos aquáticos de águas interiores ou costeiras, sendo o processo de criação controlado por meio de intervenções, com o intuito de melhorar a produção. A crescente demanda por fontes de proteína de alta qualidade e a estabilização do volume da pesca extrativista, no fim da década de 1980, contribuíram para que a aquacultura se tornasse o setor de produção de alimento que mais cresce no mundo. O pescado é um dos produtos alimentícios mais comercializados mundialmente, sendo metade do valor das exportações oriunda de países em desenvolvimento (FAO, 2016).

A produção de pescado global em 2016 alcançou 171 milhões de toneladas, sendo 47% representados pela aquacultura, (ou 53%, se desconsiderada a utilização para fins não alimentícios). Desse total, 88% foram destinados à alimentação humana. Estima-se que o pescado proveniente da aquacultura tenha movimentado cerca de 382 bilhões de dólares no mesmo ano (FAO, 2018). De acordo com o relatório da FAO (2018), 54,1 milhões de toneladas do total da produção de pescado (incluindo peixes, crustáceos, moluscos e outros organismos aquáticos), em 2016, são representadas por peixes. No Brasil, a produção de peixes de água doce atingiu 507.122 toneladas em 2016, avaliadas em R\$ 3,7 bilhões, segundo o Instituto Brasileiro de Geografia e Estatística (IBGE).

A alimentação representa cerca de 60% do custo de produção na aquacultura (Emerenciano et al., 2013). Em sistemas de cultivo, os peixes são dependentes de alimento balanceado, sendo imprescindível que este contenha todos os nutrientes necessários para seu desenvolvimento satisfatório. Neste sentido, pesquisas na área de nutrição vêm demonstrando que a dieta influencia o comportamento, integridade estrutural, saúde, funções fisiológicas, reprodução e o crescimento dos peixes.

O comportamento alimentar é complexo e abrange várias respostas associadas à alimentação e hábitos alimentares, dentre elas, o modo de alimentação, mecanismos de detecção de alimentos, frequência de alimentação e preferências alimentares. Estudos mostram que os animais podem escolher diferentes alimentos ou níveis de nutrientes para compor uma dieta equilibrada, que atenda suas necessidades nutricionais, e isso também ocorre em peixes, sendo denominado “sabedoria nutricional”.

Em peixes, os experimentos de auto seleção mostram que estes animais são



capazes de compor uma dieta balanceada e detectar diferenças no teor de nutrientes (Fortes Silva et al., 2011; Montoya et al., 2012; Brännäs e Strand, 2015). A seleção de alimentos é moldada por meio de aprendizagem associativa entre a ingestão do alimento e a recompensa nutricional. A auto seleção de macronutrientes é uma das metodologias empregadas para este tipo de estudo, sendo considerada uma ferramenta valiosa na investigação das preferências nutricionais de peixes e para melhorar a produção em cativeiro (Vivas et al., 2006; Simpson e Raubenheimer, 2001).

A determinação da concentração ideal de nutrientes, para uma melhor eficiência produtiva dos animais, é de extrema importância. A exigência de proteína ideal para cada espécie é influenciada por fatores como: qualidade da proteína, tamanho do animal, taxa de arraçoamento, temperatura da água, sistema de produção e participação de fontes energéticas não proteicas. As concentrações de proteína e energia para peixes devem estar balanceadas para obtenção de crescimento ótimo, com taxas de eficiência alimentar e retenção de proteína na carcaça elevadas (Ellis & Reigh, 1991).

## **2. OBJETIVOS**

### **2.1 Objetivo geral**

O objetivo do presente estudo foi avaliar o padrão comportamental da preferência e consumo de dietas com diferentes proporções energia:proteína por *L. alexandri*.

### **2.2 Objetivos específicos**

- Determinar o comportamento alimentar dos animais frente à dietas contendo diferentes relações energia:proteína;
- Avaliar a atividade alimentar dos animais quando submetidos à auto alimentação;
- Determinar o alvo de ingestão de proteína e energia pelo método de alimentadores de auto-demanda;
- Determinar o alvo de ingestão de proteína e energia por características pós ingestivas, isolando propriedades orosensoriais proporcionadas pela dieta.

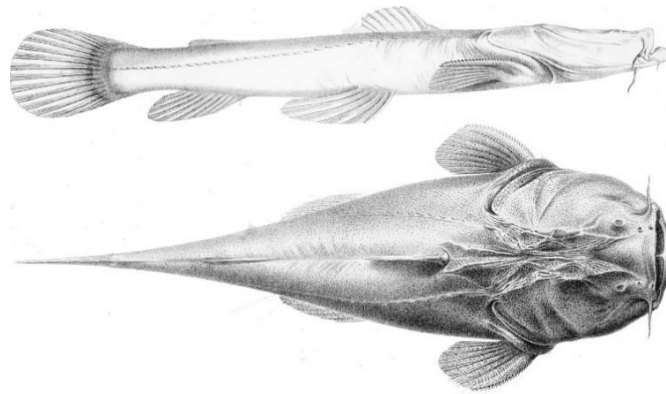
### 3. REVISÃO BIBLIOGRÁFICA

#### 3.1 Pacamã (*Lophiosilurus alexandri*)

O *Lophiosilurus alexandri* (Figura 1), é uma espécie nativa da bacia do rio São Francisco, conhecida popularmente por niquim em outra região do Rio São Francisco, por pacamã no alto São Francisco e também como pacamão (Travassos, 1959). É um siluriforme de hábito bentônico, da família Pseudopimelodidae (Travassos, 1959; Tenório, 2003). No seu habitat natural, apresenta preferências por ambientes lânticos, em locais com fundo de areia ou pedras. O pacamã é considerado uma das espécies mais representativas do São Francisco, e vem sofrendo drástica redução na população devido à pressão no ambiente natural (Tenório et al., 2006), sendo classificada como espécie vulnerável, de acordo com o livro vermelho da fauna brasileira ameaçada de extinção (ICMBIO, 2016).

Tornou-se uma espécie muito estudada em vários aspectos relacionados à produção animal. Sua reprodução se dá por desovas parceladas, onde constroem ninhos para postura (Travassos, 1959). Durante a reprodução, a desova pode ser realizada de forma natural em ambientes com condições controladas (Costa et al., 2015). Na literatura há alguns estudos relacionados à nutrição, como o efeito do processamento da ração no desempenho e sobrevivência em juvenis (Santos et al., 2012), exigência proteica para juvenis (Souza et al., 2013), relação proteína:carboidrato no desempenho e metabolismo (Figueredo et al., 2014), fornecimento de alimento processado de diferentes formas (Souza et al., 2014), digestibilidade de ingredientes em dietas para juvenis (Melo et al., 2016), entre outros.

O "pacamã" tem grande potencial para aquicultura, uma vez que possui características interessantes ao mercado, como filé de cor clara e sem espinhos intramusculares e sabor apreciável (Luz et al., 2013). De acordo com Cardoso et al. (1996), um exemplar adulto dessa espécie pode atingir cerca de 8 Kg.



**FIGURA 1** Exemplar de *Lophiosilurus alexandri* (Fonte: de.wikipedia.org).

### **3.2 Relação energia:proteína em dietas para peixes**

Os peixes apresentam menor exigência energética em relação aos demais animais de produção. Um fator que contribui para isso é serem animais heterotérmicos. Desse modo, a exigência energética dos peixes varia de acordo com a temperatura do meio em que ele está inserido (Smith, 1989).

Na formulação de dietas para peixes são levadas em consideração as necessidades proteicas e energéticas de cada espécie. Com isso, a relação energia:proteína da dieta deve estar balanceada. Dietas com altos níveis energéticos diminuirão a ingestão de outros nutrientes pelos animais (NRC, 1993). Por outro lado, dietas com níveis de energia muito abaixo do recomendado para a espécie resultarão no catabolismo de proteínas, que seriam utilizadas para o crescimento e metabolismo basal do animal. De acordo com o NRC (1993), a relação ideal proteína:energia para diferentes espécies, varia de 81 a 117 mg de proteína/Kcal de energia digestível.

Estudos feitos com espécies de peixes carnívoros mostram que o melhor desempenho é obtido quando metade da energia da dieta é suprida pela proteína, sendo que dietas para carnívoros devem possuir cerca de 40 a 50% de proteína (Jobling et al., 1994). Estudos realizados com tucunaré, avaliando o efetivo da relação energia:proteína sobre o desempenho e composição corporal desses animais, mostram que a medida que aumenta o tamanho dos animais, a exigência proteica diminui (Sampaio et al., 2000). Cho et al. (1985) também destacam que a exigência proteica varia de acordo com o tamanho do animal. Determinar e balancear a exigência proteica das espécies, em diferentes fases de cultivo, é muito importante para evitar o fornecimento excessivo deste nutriente para o

animais (Silva e Anderson, 1995; Boscolo et al., 2004), minimizando custo e impactos ambientais (Furuya et al., 1996).

Estudos feitos com bagre do canal mostraram que animais que consumiram dietas com energia de 275 e 341 Kcal/100g de ração apresentaram excelente relação energia:proteína, aproximadamente 88 mg de proteína/Kcal (24 a 36% de proteína bruta) (Garling & Wilson, 1976). De acordo com Bomfim et al. (2005), a melhor relação energia digestível:proteína bruta para curimatá é de 10,36 Kcal de energia digestível/g de proteína bruta, pois, com essa relação os animais obtiveram melhor desempenho e composição de carcaça.

### **3.3 Comportamento alimentar em peixes**

O comportamento alimentar é complexo e está associado diretamente à ingestão de alimentos, sendo regulado por uma série de fatores, dentre eles, fatores ambientais e mecanismos homeostáticos, que envolvem fatores hormonais, periféricos e produção de metabólitos (Volkoff e Peter, 2006).

Os peixes são considerados bons modelos experimentais para estudar a regulação da ingestão de determinados nutrientes (Volkoff e Peter, 2006). Segundo Simpson e Raubenheimer (2001), os peixes são capazes de regular a ingestão de nutrientes e defender um alvo nutricional. Esta capacidade é baseada na “sabedoria nutricional”, observada em estudos realizados com espécies carnívoras como *Solea senegalensis* (Rubio et al., 2009), *Oncorhynchus mykiss* (Sánchez-vázquez et al., 1999) e *Dicentrarchus labrax* (Rubio et al., 2003), e onívoras como *Oreochromis niloticus* (Fortes-Silva e Sánchez-Vázquez, 2012) e *Carassius auratus* (Sánchez-Vázquez et al., 1998).

### **3.4 Auto seleção em dietas para peixes**

A seleção de alimentos é baseada na premissa de que animais, incluindo peixes, possuem "sabedoria alimentar" e, assim, são capazes de selecionar uma dieta que restaure um desequilíbrio metabólico, resultante de um desafio nutricional (Simpson e

Raubenheimer, 2001). De acordo com essa hipótese, a fonte de nutrientes pode ser detectada por receptores gastrointestinais durante a digestão, liberados dentro do estômago e depois no intestino. Esses receptores desencadeariam sinais (atividade neural e hormônios), que informariam ao cérebro as propriedades nutricionais dos alimentos e, assim, modificariam o comportamento alimentar do animal (Vivas et al., 2003; Almada-Pagan et al., 2006; Rubio et al., 2009). Além da abordagem do "consumo de ração" amplamente utilizado na nutrição animal, outros métodos podem melhorar a compreensão dos mecanismos fisiológicos, como o uso de auto-alimentadores na seleção de dieta. Os peixes podem aprender a se alimentar e selecionar dietas, de acordo com suas necessidades nutricionais (Sánchez-Vázquez et al., 1998; Sánchez-Vázquez et al., 1999; Aranda et al., 2000; Vivas et al., 2006).

Estudos avaliaram a seleção de dietas com diferentes ingredientes, como zinco para truta (Cuenca et al., 1993), metionina para robalo europeu (Hidalgo et al., 1988), e estimulantes de alimentação para truta (Adron e Mackie, 1978). Estes trabalhos confirmam que os peixes podem discriminar entre os níveis das substâncias testadas na dieta, e sugerem que o método de alimentação por auto-seleção é útil para determinar os requisitos nutricionais e ajudar a desenvolver dietas de qualidade para peixes.

O sistema de alimentação por demanda tornou-se uma ferramenta útil para avaliar os ritmos de alimentação em peixes e suas preferências (Azzaydi et al., 1998; Montoya et al., 2012). A auto seleção de nutrientes poderia ser usada para otimizar a composição da dieta fornecida para animais de cativeiro (Fortes-Silva et al., 2016), e possibilitar o estabelecimento de horários de alimentação mais eficientes para as diferentes espécies na aquacultura (Kitagawa et al., 2015).

### **3.5 Dietas encapsuladas na auto seleção pelos peixes**

O odor, sabor e a textura do alimento podem influenciar na preferência e quantidade de alimento a ser ingerido (Fortes-Silva et al., 2011). O método encapsulação de dietas é empregado com o propósito de igualar a textura, forma, além de isolar o efeito do sabor e do cheiro no processo de seleção dos macronutrientes pelos peixes (Rubio et al., 2003).

Rubio et al. (2003) desenvolveram uma metodologia capaz de fornecer diferentes macronutrientes, com mesmo sabor e textura, protegidos dentro de uma cápsula de gelatina. Esta metodologia foi desenvolvida com o intuito de avaliar a capacidade dos

peixes em compor uma dieta balanceada em macronutrientes, apenas por receptores pós ingestivos (endógenos), eliminando as características sensoriais do alimento. Dessa forma, foi possível estudar mais a fundo os mecanismos que envolvem a regulação de ingestão de alimentos pelos peixes. Todas as propriedades organolépticas da dieta, como cheiro sabor e textura são igualadas no momento da encapsulação, para que a seleção de macronutrientes seja feita exclusivamente pela associação dos mecanismos de percepção pós ingestão.

Quando a diferença entre a propriedades nutricionais dos alimentos não geram diferenças sensoriais, os animais só serão capazes de distinguí-los, se for disponibilizado algum sinal sensorial adicional (Forbes et al., 2001). Rubio et al. (2003), registraram a primeira evidência de que uma espécie animal é capaz de discriminar a composição em macronutriente do alimento encapsulado, usando apenas a cor como estímulo condicionado. Em um estudo com robalo europeu foi testado a capacidade em selecionar cápsulas distintas contendo proteína, carboidrato e lipídio. A espécie estudada foi suficientemente hábil em regular sua ingestão de energia e selecionar macronutrientes.

Diversos estudos comprovam a capacidade de algumas espécies de peixes de associar as cápsulas coloridas com o seu conteúdo, distinguindo mudanças espectrais na gama de cores vermelha, laranja e amarela (Rubio et al., 2003).

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## **CAPÍTULO 2**

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**REGULATION OF VOLUNTARY PROTEIN/ENERGY INTAKE BASED  
PRACTICAL DIET COMPOSITION FOR THE CARNIVOROUS  
NEOTROPICAL CATFISH *Lophiosilurus alexandri***

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## ABSTRACT

The effect of different energy to protein ratios on voluntary feed intake was investigated in the carnivorous catfish *Lophiosilurus alexandri*, in order to evaluate their feeding behaviour responses. Two trials were performed. The first trial involved 16 fish distributed among 16 tanks, which were allowed to select one of two experimental encapsulated diets (24% vs 42% of crude protein or Energy (MJ g<sup>-1</sup>): Protein (g) ratio of 0.068 × 0.041). The objective was to isolate sensorial influence on the choice of diet. For the second trial, six experimental groups were designed with successive increases in protein and successive decreases in energy to evaluate diet selection by the self-feeding method: Group 1 – 24% of crude protein x 30% of crude protein; G2 – 24% x 36%; G3 – 24% x 42%; G4 – 30% x 36%; G5 – 30% x 42%; and G6 – 36% x 42%. These groups had the following energy to protein ratios (E:P MJ g<sup>-1</sup> protein): G1 – 0.068 x 0.057; G2 – 0.068 x 0.048; G3 – 0.068 x 0.041; G4 – 0.057 x 0.048; G5 – 0.057 x 0.041; and G6 – 0.048 x 0.041. Diet selection data were arcsin ( $\sqrt{\quad}$ ) transformed and then subjected to the Student t-test. Mean protein (g/kg of BW), energy:protein (MJ energy/g crude protein/kg BW) and energy (MJ energy/kg BW) consumption were submitted to ANOVA and Tukey test (P < .05). The results of Trial 1 revealed a clear preference for diet with 42% crude protein and an energy to protein ratio of 0.041 MJ g<sup>-1</sup> protein (P < .05); even with the absence of taste the fish returned to the initial pattern of selection after the contents of the capsules were changed. Trial 2 revealed that with diets containing < 30% crude protein (G1), the fish selected diets with higher energy (P < .05). In G2 and G6 the fish exhibited an unclear pattern of diet selection probably due to the narrow range of nutrient options or because the requirements for protein and energy were met by the diets, respectively. For the G2, G3 and G5, fish choose diets with higher protein content (P < .05), even after the diets were switched between feeders. The animals kept the protein intake pattern from G4 (~36–38% or 20 g/BW) and energy consumption (8.42 MJ kg<sup>-1</sup>) for all treatments. In conclusion, based on the feeding behavior of *L. alexandri* in the present study, we infer that the target E:P is around 0.028 MJ g<sup>-1</sup> protein.

**Keywords:** self-selection; food behaviour; nutrition; protein; production

## 1. INTRODUCTION

Protein is the most expensive component of fish feed (Yuan et al., 2010), and feed profitability depends on the cost of production and the price of raw dietary ingredients (Jauralde et al., 2016). At high levels of dietary protein (high protein to energy ratios), part of the protein is catabolized to meet energy demands, however, at low levels of dietary protein (low protein to energy ratios) reduced growth and no impact on protein efficiency are expected (Haidar et al., 2018). Dietary protein elicits a wide range of metabolic and physiologic functions that are far beyond its traditional role as a source indispensable amino acid (Greco et al., 2017). In addition, for better use of the protein, an optimal energy to dietary protein ratio (E:P) should be considered when formulating fish diets (Yuan et al., 2010).

Besides, the obvious effects of dietary protein and energy, sensory properties, such as odor, flavor, and texture can affect food intake. Thus, studies on diet acceptability have been undertaken to assess the relationship between fish and their nutritionally-developed diets. European sea bass (*Dicentrarchus labrax*), for example, have exhibited the ability to select fishmeal diet over soybean meal diet, but increased consumption of the soybean diet when containing exogenous phytase enzyme, which is capable of improve the nutritional value of diet (Fortes-Silva et al., 2011a, 2011b). This feeding behavior suggests that the nutritional value of the diet could also influence the acceptability of fish. Several studies observed similar behavior in face of the nutritional composition of the diet (Raubenheimer and Simpson, 1998; AlmadaPagán et al., 2006, 2008; Fortes-Silva et al., 2012; Mattos et al., 2017).

The behavioral feeding studies may be determined by the triggering of self-feeding systems, which leads to a new perspective for investigating food intake regulation in aquaculture (Da Silva et al., 2016). This system can be an important tool for evaluating the acceptance of a new diet formulation by farmed fish before it is used commercially (Carlberg et al., 2015). Self-feeding has been validated for several cultured fish species, including rainbow trout (*Oncorhynchus mykiss*) (Boujard and Gouvello, 1997; Geurden et al., 2006); sea bass (*D. labrax*) (Hidalgo et al., 1988; Aranda et al., 2001); common carp (*Cyprinus carpio*) (Yamamoto et al., 2003); snout seabream (*Diplodus puntazzo*) (Atienza et al., 2004); Senegal sole (*Solea senegalensis*) (Rubio et al., 2009); and Pirarucu (*Arapaima gigas*) (Mattos et al., 2016, 2017). However, few studies have been considered neotropical species.

The catfish called pacamã, *Lophiosilurus alexandri* is a benthic fish and endemic to the São Francisco River (Tenório et al., 2006). This specie has potential of aquaculture (Santos and Luz,

2009; dos Santos et al., 2013; Martins et al., 2014) due to the quality of the filet, market price and also their suitability for ornamental fish culture (Santos and Luz, 2009). Costa et al. (2015) reported that *L. alexandri* reproduces naturally in captivity, and juveniles can be training to accept fed commercial diets (Melilo Filho et al., 2014) presenting good adaptation to these diets (Souza et al., 2019).

The aim of the present study was to evaluate the behavioral pattern of diet preference and protein and energy intake of *L. alexandri* provided a free choice of diets with different protein-energy ratios. Towards this aim, two complementary trials with different approach of feeding behavior studies were performed: Trial 1, employing the encapsulation method to control for sensory properties; and Trial 2, employing self-feeding methods.

## **1. MATERIAL AND METHODS**

### **2.1. Ethical approval**

All procedures herein described were approved by the Committee for Ethics in Animals Use (CEUA / UFMG - n° 208/2018).

### **2.2. Animal housing**

Fish obtained by spawning at the Federal University of Minas Gerais (UFMG, Brazil) were reared in the laboratory. They were acclimatized for 14 days in indoor tanks provided with a recirculation water system, and mechanical and biofilters filters, with water renewal according to daily water evaporation. The following conditions were maintained during acclimatization: temperature below  $28.0 \pm 0.1^\circ\text{C}$ , oxygen  $5.2 \pm 0.12$  mg/L (HI9146, Hanna), ammonia  $0.5 \pm 0.02$  mg/L, (Labcon Test), pH  $7.2 \pm 0.36$  (Labcon Test) and a 12L:12D photoperiod (Key West group DNI, digital timer).

### **2.3. Experimental diets**

We formulated four isocaloric ( $\sim 17$  MJ  $\text{kg}^{-1}$  diet) diets containing 24%, 30%, 36% and 42% crude protein, and 0.068, 0.057, 0.048 and 0.041 energy-protein ratio (E:P, MJ  $\text{g}^{-1}$  protein), respectively (Table 1). The dietary ingredients and its inclusion levels was based on previous studies in neotropical carnivorous fish *Salminus brasiliensis* (Lorenz et al., 2018), *Hoplias lacerdae* (Veras et al., 2010), and *Pseudoplatystoma spp.* (Teixeira et al., 2013). Diet compositions

were designed take into account the minimum and maximum of the range of protein that the fish can freely select from experimental diets. The range of protein and energy in the experimental diets encompassed that required by *L. alexandri* as determined by a previous study (Souza et al., 2013; Figueiredo et al., 2014). The diets were extruded (Imbramx40, Imbramaq Ltda., Ribeirão Preto, São Paulo, Brazil) as 6- and 8-mm pellets and then stored in a freezer ( $-20\text{ }^{\circ}\text{C}$ ).

The amino acid profiles of the diets were assessed using chromatography (HPLC) (White et al., 1986) and the enzymatic method (Lucas and Sotelo, 1980). Crude protein was determined by the Kjeldahl method ( $\text{N} \times 6.25$ ), crude fat by diethyl ether extraction using the Soxhlet Tecator system, ash by burning samples in a muffle furnace at  $550\text{ }^{\circ}\text{C}$  for 12 h and moisture by drying at  $90\text{ }^{\circ}\text{C}$  until constant weight. Samples were determined according to the methodology of AOAC (2005).

#### 2.4. Trial 1: Experimental design with encapsulated diets

We first evaluated whether individuals of *L. alexandri* exhibited a selection pattern in the form of post-ingestive signals when fed with encapsulated diet (food without orosensory properties) (Rubio et al., 2003). The independent variables were E:P and location (within the tank) of the encapsulated diets. Juvenile *L. alexandri* (mean weight:  $124.11 \pm 23.07\text{ g}$ ) were kept in 16 tanks (one fish per tank) each with a submerged tubular feeder (floating container) to control the dispersion of capsules (Fig. 1). The size of the animals in this trial was adopted in order to favour that fish can eat the capsules (Da Silva et al., 2016). During acclimatization (10 days), fish were fed manually in both floating containers with commercial encapsulated feed (SUPRA Acqua line, with 36% crude protein, 12% moisture content and 13% mineral matter). Capsules were made by filling No. 4 (0.2-ml) gelatin capsules (Capsugel Brasil Ltda., Rio de Janeiro, Brazil) using a semi-automatic encapsulator (GA Tech, Belo Horizonte, Brazil) (Fortes-Silva et al., 2011a, 2011b). Capsules of the same color were used to avoid an effect of color preference. After acclimatization, the fish were weighed and kept in their respective tanks where they would receive experimental diets (24% vs. 42% of PB or  $0.068 \times 0.041$  of E:P ratio,  $\text{MJ g}^{-1}$  protein). Five capsules were given per tank at the beginning of the experiment, with capsule consumption being adjusted by an increase of 25% daily according to the consumption of the previous day, in order to avoid ingestive limitation on behalf of the fish and thus a bias to selection. The capsules were offered once daily on a floating vessel, with the remaining capsules being removed after 5 min and counted to determine intake. Fish were also submitted to a behavioral nutritional challenge, whereby the contents of the capsules were changed after 10 days. Photocells were located on each float feeder



to record percent feeding activity. Feed intake was calculated based on pre-filling weight of each capsule type (total capsule weight – empty capsule) (Pereira et al., 2018); the wall of the capsule was also considered since it consisted of gelatine protein (Rubio et al., 2003; Fortes-Silva et al., 2011a, 2011b). Trial 1 lasted 20 days, at the end of which diet preference was calculated as the percent diet intake of 100% offered diet.

## 2.5. Trial 2: Experimental design with self-feeding

Trial 1 was used to guide the experimental design of Trial 2. With the knowledge that the fish have an endogenous diet preference when sensory properties are isolated, we decided to evaluate this preference considering different ranges of protein-energy ratio in practical diets using the self-feeding method. A total of 144 individual *L. alexandri* (mean weight:  $16.78 \pm 1.39$  g) were randomly stocked in 24 tanks (40 L). The size of the animals in this trial was suggested since self-demand method with presence sensors (infrared light) is adapted for small fish (Da Silva et al., 2016). The fish of each tank were allowed access to operating self-feeders (EHEIM 3581, Deizisau, Stuttgart, Germany) connected to an infrared photocell (model Omron, E3SAD62, Japan) located 3 cm below the surface of the water (Fig. 2). Each time a fish crossed the beam of infrared light of the photocell, the automatic feeder was activated to deliver 0.2 g of pellets. Feeding activity was recorded every 10 min using a Measurement Computing usb-1024ls data logger (USA) and DIO98USB software (University of Murcia, Spain), which provide accurate trigger information for each feeder. Sensor triggering and feeding intake by fish throughout the entire experimental period were observed, with a decreasing amount of leftover food, showing that the fish adapted self-demand following the system settings. Fish were fed a commercial diet (SUPRA Acqua line, 36% crude protein) and maintained in this experimental condition for 1 week. During this period, the ability of fish to relate feeders to the food supply, and possible preferences among feeders, were evaluated. When these issues were set, the trial 2 was started. Six experimental groups with four replicates each were designed to evaluate diet selection and nutrient (protein and energy) intake patterns: Group 1 – 24% of crude protein x 30% of crude protein; G2 – 24% x 36%; G3 – 24% x 42%; G4 – 30% x 36%; G5 – 30% x 42%; and G6 – 36% x 42%; corresponding to energyprotein ratios (MJ g<sup>-1</sup> protein) of G1 – 0.068 x 0.057; G2 – 0.068 x 0.048; G3 – 0.068 x 0.041; G4 – 0.057 x 0.048; G5 – 0.057 x 0.041; G6 – 0.048 x 0.041. After 25 days of experimentation, fish were subjected to a behavioral nutritional challenge by changing the content of the self-feeders. Uneaten feed pellets in the self-feeders and tanks were collected and weighed daily after active feeding for consumption analysis. In case of possible remainders in the

tanks, the wet diets were forced-air dried for subsequent weight calculation. Trial 1 lasted 49 days, at the end of which diet preference (expressed as the % of diet intake of 100% offered), protein consumption (g/kg of BW) and energy-protein ratio (MJ of energy/g crude protein/kg BW) were calculated. Triggering of feeders by fish was recorded as feeding activity (%).

## **2.6. Data analysis**

The results are expressed as the mean  $\pm$  standard error of the mean (SEM). Data were subjected to Levene's test for homoscedasticity and the Shapiro-Wilk test for normality. Diet selection data were arcsin ( $\sqrt{\quad}$ ) transformed and then subjected to the Student t-test. Mean protein (g/ kg of BW), energy: protein (MJ energy/g crude protein/kg BW) and energy (MJ energy/kg BW) consumption were submitted to ANOVA and Tukey test. Statistical significance was considered at  $P < .05$ . Data were analyzed by InfoStat® (version 2016, Universidade de Cordoba, Argentina).

## **2. RESULTS**

### **3.1. Trial 1: Experiment with encapsulated diets**

Experiment with encapsulated diets In general, fish exhibited a mean weight of  $164.97 \pm 34.30$ , without difference among groups. The results of the self-selection of encapsulated diets with isolation of diet sensory properties are presented in Fig. 3A,B. In the first 10 days of the experiment, the fish exhibited a clear preference for diets containing 42% crude protein and comparatively low energy content ( $E:P = .041 \text{ MJ g}^{-1}$ ;  $P < .05$ ). This pattern was established quickly, beginning on the second day, and accounted for 83.73% ( $P < .05$ , Student t-test) of the feeding activity recorded by the photocells. When diets were changed, the fish reestablished their diet preference (Phase 2), with a similar presence at the float feeder (feeding activity) of around 82.5% ( $P < .05$ ).

### **3.2. Trial 2: Experiment with self-feeding**

In general, fish exhibited a mean weight of  $50.45 \pm 3.87$ , without difference among groups. The results of self-feeding diet selection are presented in Fig. 4. The fish of Group 1 (Fig. 4) exhibited a clear preference for diets containing 24% of crude protein (E:P = .068 MJ g<sup>-1</sup> protein) beginning on the 9th day ( $P < .05$ ), which corresponded to 72% of feeder activation. This pattern returned after the nutritional challenge of changing the diets between feeders. For G2 (30% vs. 36% CP and 57 vs. 48 E:P), fish selected more protein (36% CP, E:P ratio = 0.048 MJ g<sup>-1</sup> protein) in Phase 1 ( $P < .05$ ), although this pattern was not observed in Phase 2 (after the shift of diets between feeders). For groups G3, G4 and G5, a preference for diets with the highest proportion of protein ( $P < .05$ ) but low energy-protein ratio were observed in both phases 1 and 2. Moreover, G3 fish exhibited high activation (89%) of the feeder containing high crude protein or comparatively low energy-protein ratio. Lastly, the G6 fish did not exhibit a clear pattern of selection for diets with higher percentages of protein. The triggering of feeders by fish revealed a similar behavioral pattern (~50% of feeding activity between feeders).

A lower percentage of crude protein consumption was observed for G1, G2 and G3, than for G4, G5 and G6 ( $P < .05$ ) (Fig. 5A,B), with a similar pattern being observed for average consumption/BW of protein (Fig. 5A,B). Thus, fish stabilized their protein composition at around 36–38% of protein intake, or 20 g/BW, when protein ranges were higher. No differences were observed for energy intake and energyprotein ratio for all groups (Fig. 6A,B) ( $P > .05$ ) with respective intakes of 8.42 MJ/kg BW and 0.028 MJ EB/g PB/kg BW. Considering the contribution of each diet to energy intake, fish allowed to trigger feeders exhibited higher protein intake/BW and energy intake ( $P < .05$ ) from the diet containing 42% CP (E:P = .041 MJ g<sup>-1</sup> protein) only within G3.

#### 4. DISCUSSION

The gustatory system of fish is the most important for food acquisition and ingestion, but is difficult to study and evaluate because the sensory system of fish responds to a multitude of stimuli from food and the aqueous chemical environment (Lamb, 2001). Considering isolated effect of dietary nutrients, the composition of proteins has been shown to affect food intake regulation through pre- or post-absorptive mechanisms in tilapia (*Oreochromis niloticus*) (Fortes-Silva et al., 2012). The effect of amino acids on diet choice can be attributed to the sensitivity of taste receptors, mainly in carnivorous species (Goli et al., 2015). However, these effects were isolated in Trial 1 by employing diet encapsulation. Fish clearly revealed a high consumption of

capsules containing diets with 42% protein than low protein capsules, even after the capsule content was switched. Several studies using diet encapsulation have shown that fish can exhibit diet preferences based on non-dietary organoleptic characteristics and that they defend a protein intake target (Rubio et al., 2003; Almaida- Brännäs and Strand, 2015; Pereira et al., 2018).

The neotropical *C. macropomum* fed with encapsulate diets regulate their energy intake to maintain an energy-protein ratio of approximately 0.0502–0.0586 MJ g<sup>-1</sup> of protein (Filho et al., 2018). According to Almaida-Pagán et al. (2008), the omnivorous sharpnose seabream (*D. puntazzo*), subjected to encapsulated diet were able to regulate protein intake (~67%) and energy intake (~21 kJ/100 g BW), even when challenged with very low fat quantities in their diets. *L. alexandri* exhibited a clear preference for diets containing 42% (E:P = .041 MJ g<sup>-1</sup> ; P < .05) in both phases of the trial 1, confirmed by observed behavioral preference, which revealed alimentary activity of above 80% for the preferred 42% diet in both phases of the trial.

The path of practical diet selection was also traced by examining different ranges of energy-protein ratios using the self-feeding method. In Group 1 (24% x 30%), fish exhibited a clear dietary preference for diet containing low crude protein and high E:P. In this condition, we hypothesized that fish chose to cover their energy requirements since the amount of protein in both diets was low for a carnivorous species. When a fish is constrained from reaching its intake target by suboptimal foods (e.g. low protein), it is forced to make behavioral compromises, resulting in the overconsumption of energy (Simpson and Raubenheimer, 2001). For example, voluntary feed intake was upregulated for the gilthead sea bream (*S. aurata*) when fed a low protein diet and subjected to varying protein-energy ratios (García-Meilán et al., 2013). Likewise, when protein was restricted, pirarucu (*A. gigas*) increased carbohydrate and fat intake (Mattos et al., 2016).

It was suggested that animals subjected to protein deficient feed will increase their daily feed intake, and consequently increase their rate of energy intake (Emmans, 1981). Likewise, when a minimum requirement of protein was recovered, Atlantic salmon (*Salmo salar*) choose to eat less energetic diets (Paspatis and Boujard, 1996). These studies seem to converge on one commonality; diet intake regulation is related to the balance between energy and protein, and non-proteic components of diet can, in turn, be related to a reduction in the oxidation of amino acids (Shiau and Lan, 1996; Castro et al., 2016; Jasour et al., 2018). This, therefore, may explain why *L. alexandri* selected diets with higher energy; that is, to save depleted protein. When the option for protein selection increased to above 30%, the fish exhibited a different behavior pattern and choose diets with high protein content. To another carnivorous fish, rainbow trout (*Oncorhynchus mykiss*) fed by selffeeding showed higher weight gain when fed low dietary energy than for those

fed the high energy diet (Boujard and Medale, 1994). Possibly these fish ingest more protein in the diets with lower energy, reaching the target of protein intake.

When an animal is not constrained on nutritional content of diet, it would be expected to make the best possible compromise to achieve optimal nutrient consumption based on their feeding behavior (Raubenheimer and Simpson, 1998). When *L. alexandri* was allowed to select between high protein diets (above 36%), they revealed another interesting behavior and did not show a clear preference among diets. In this case, protein and energy requirements were probably covered and so the fish did not express a clean selection pattern. When protein was not restricted, sea bass (*D. labrax*) were found to choose a proteinrich diet (51%), which is in accordance with that reported for carnivorous fish (Aranda et al., 2001). Comparatively, omnivorous Nile tilapia (*Oreochromis niloticus*), adjust their protein intake to approximately 24% (Pereira-da-silva et al., 2004). However, in the present study, *L. alexandri* increased their consumption until experimental Group 4 (being exposed to 30 vs. 36% crude protein), after which protein consumption was stabilized for all self-selection possibilities (~36–38% of protein intake or 20 g/BW). These results are in agreement with Souza et al. (2013) that recommended feed containing 36.2% of crude protein for juvenile *L. alexandri*.

In addition, *L. alexandri* exhibited a constant pattern of energy consumption. There has been no research on energy consumption for this species; however, many studies have shown the ability of fish to maintain a constant pattern of energy consumption. For example, sharpsnout seabream (*D. puntazzo*) challenged with fat dilution and deprivation, were capable of sustaining their energy intake pattern at around 21 kJ/100 g BW (Almáida-Pagán et al., 2008). When protein/fat-containing diets were diluted by 50% with cellulose, these fish increased their food intake to compensate for the energy dilution (170 kJ/kg BW/day before dilution vs. 168 kJ/kg BW/day after dilution) (Vivas et al., 2006). *S. senegalensis* were able to sustain their energy intake at 5.0 kJ/kg BW/day, even after dietary energy dilution with an inert substance (Rubio et al., 2009). Increasing the non-protein energy level in a 40% protein diet revealed that juvenile rainbow trout (*O. mykiss*) were able to regulate, with great accuracy, their feed intake using selffeeder (Boujard and Medale, 1994).

## 5. CONCLUSIONS

From the feeding behaviour point of view, the findings presented here emphasize the ability of *L. alexandri* to adjust their dietary protein and energy intake. Based on all of the feeding behavior results, voluntary protein and energy intake targets were maintained around 36–38% or 20 g/BW and 8.42 MJ kg<sup>-1</sup>, respectively, which corresponds to an energy-protein ratio of 0.028 MJ g<sup>-1</sup> protein. For neotropical species newly introduced to aquaculture, aquafeed preference and its nutrient content should be taken into account prior to diet formulation in order to achieve efficient feeding management, which is mandatory in modern aquaculture.

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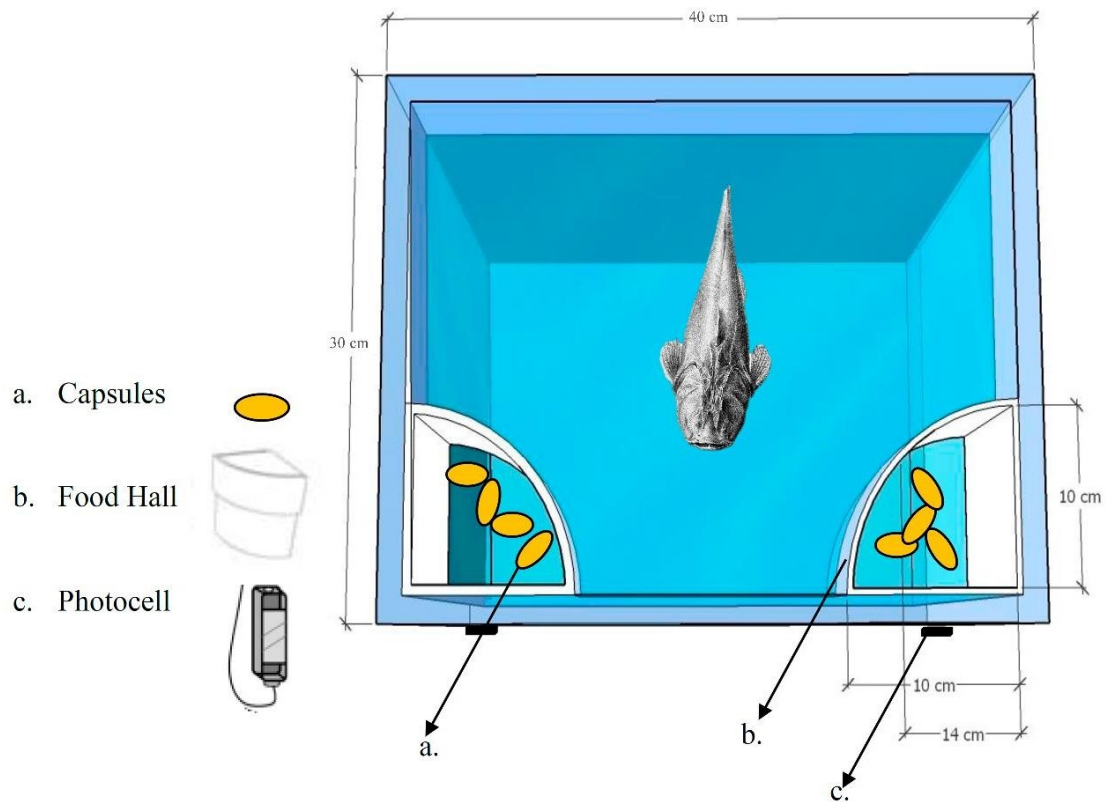
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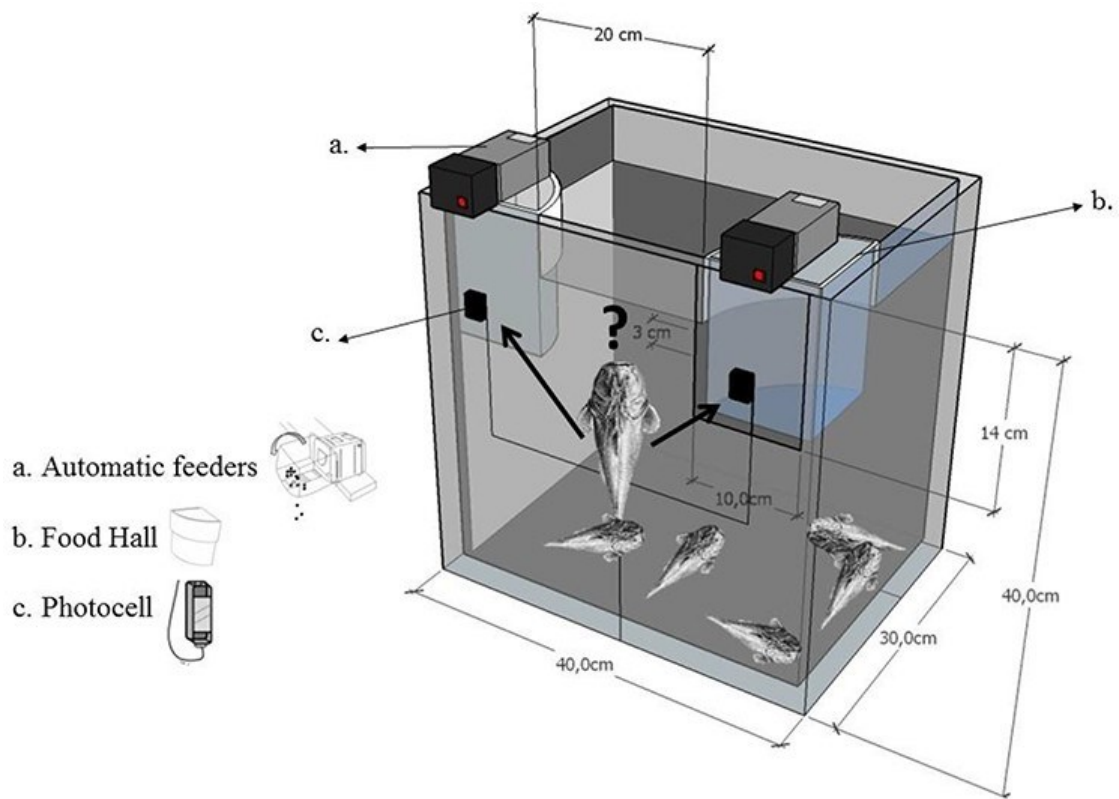
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**Table 1.** Composition of experimental diets

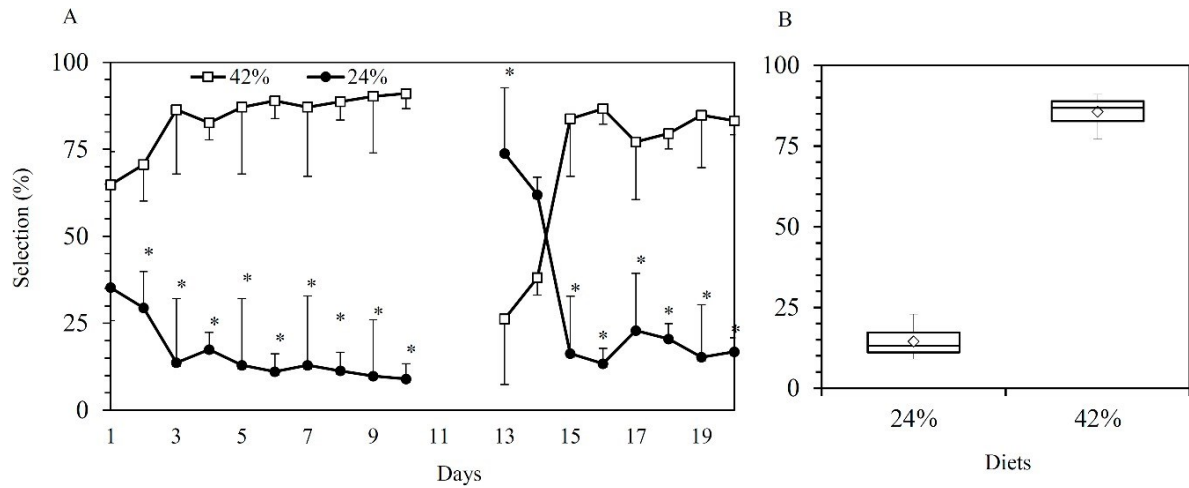
Ingredients	Protein levels in the diet (%)			
	24	30	36	42
Fish meal	15.50	21.00	24.00	29.00
Soybean meal	24.00	31.00	33.00	37.00
Corn	40.00	31.50	22.90	13.80
Sour Rice	5.00	5.00	5.00	5.00
Albumin	1.00	1.60	6.50	9.10
Soy oil	8.80	6.60	5.30	3.70
Cellulose	3.00	1.00	1.00	0.00
Dicalcium phosphate	0.50	0.50	0.50	0.50
<sup>1</sup> Vitamin/Mineral Supplement	0.50	0.50	0.50	0.50
<sup>2</sup> BHT	0.02	0.02	0.02	0.02
Salt	0.50	0.50	0.50	0.50
Inert	1.18	0.78	0.78	0.88
<b><sup>3</sup>Composition analyzed</b>				
Crude protein (%)	25.14	31.12	36.60	42.05
Gross energy (MJ kg <sup>-1</sup> diet)	17.27	17.85	17.91	17.59
Cinzas (%)	6.66	7.02	7.49	10.08
Extrato etéreo (%)	9.98	7.77	7.86	6.35
E: P ratio (kJ g <sup>-1</sup> protein)	68.69	57.36	48.93	41.83
<b><sup>4</sup>Amino acid profile (g/100g)</b>				
Thr	10.20	13.80	15.30	18.00
Val	12.60	16.20	19.30	21.70
Met	4.90	6.50	7.30	9.50
Ile	11.50	14.90	17.60	20.30
Leu	21.30	26.30	29.80	32.90
Phe	11.30	14.50	17.50	19.80
Lys	16.50	22.80	26.10	30.00
His	6.30	8.40	9.90	11.10
Arg	17.00	24.10	27.70	31.30
∑ EAA	111.60	147.50	170.50	194.60
Asp	26.20	36.90	41.20	46.50
Glu	39.50	50.30	56.10	64.50
Gly	15.20	22.30	24.40	29.70
Ala	15.50	19.90	22.40	26.00
Pro	14.50	18.00	19.90	22.60
Tyr	8.80	12.30	13.30	15.60
Cys	2.80	3.90	5.10	6.60
Ser	12.70	17.10	19.80	23.70
∑ NEAA	135.20	180.70	202.20	235.20
∑ EAA/∑ NEAA	0.83	0.82	0.84	0.83



**Figure 1.** Scheme of dietary selection based on encapsulated diets and post-ingestive signals. All three capsule types have the same chemosensory properties. Infrared sensors (one in each floating feeder) were installed to record the feeding activity.

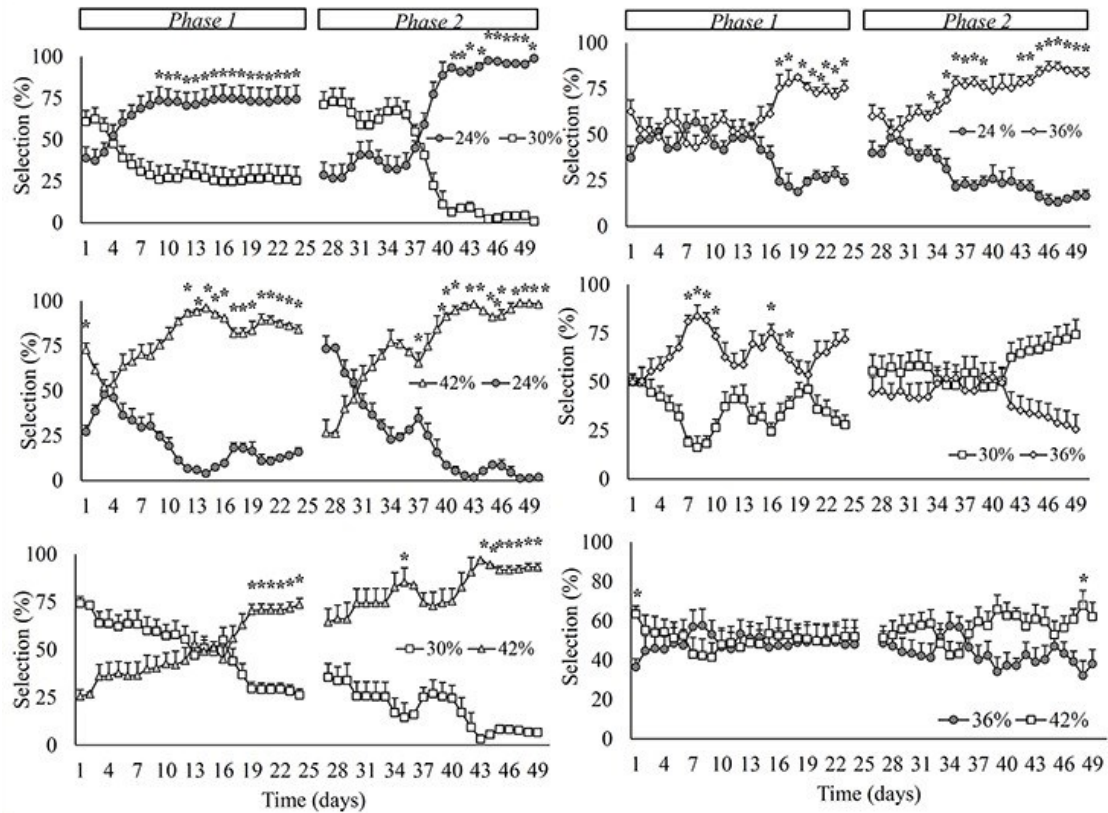


**Figure 2.** Self-feeding with infrared sensor. The feeder is trigger by fish approaching a photocell in the water. The feeder is only triggered by the presence of the fish inside of food hall, thereby avoiding accidental activations.

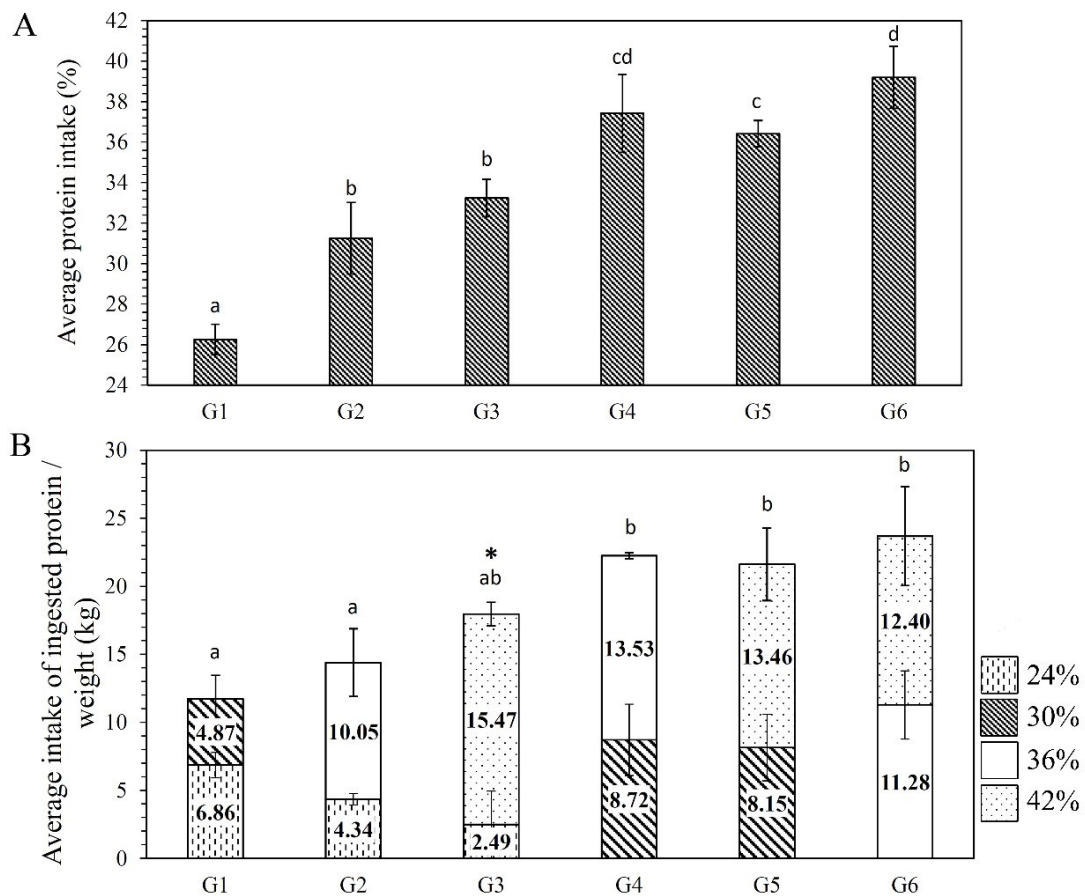


**Figure 3.** A: Daily evolution of encapsulated diet preferences (24% vs. 42% of PB or 0.068 x 0.041 of E:P ratio, MJ g<sup>-1</sup> protein) by post-ingestive signals and expressed as percentage of total diet intake during 20 days of trial. B: boxplot of diet intake g/BW. After 10 days, fish was challenged with shift diets between the capsules. Asterisks represent significant differences by the Student test (P<0.05, n=16).

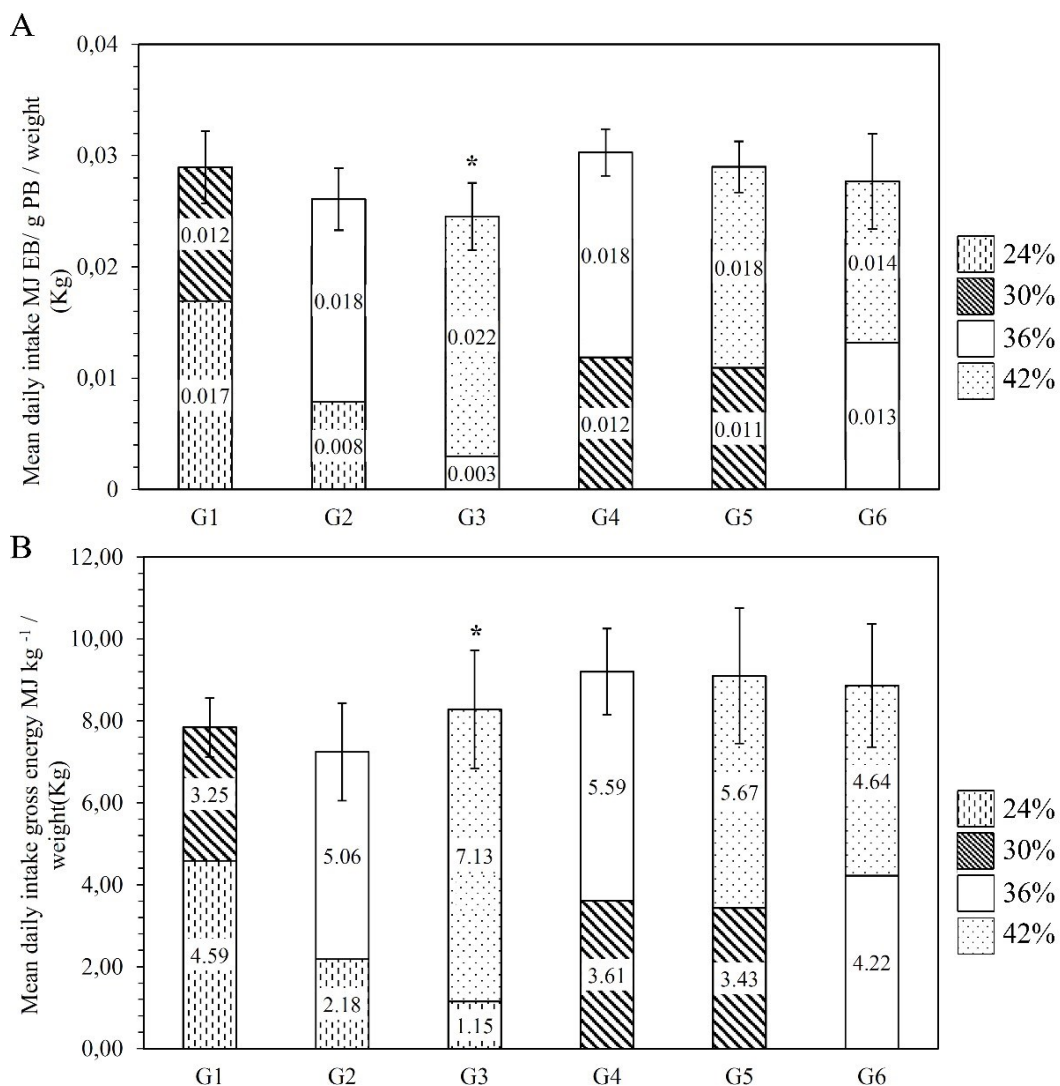




**Figure 4.** Daily evolution of diet preferences expressed as percentage of total diet intake during 49 days of trial. The experimental groups were: G1 - 24% of crude protein x 30% of crude protein; G2 - 24% x 36%; G3 - 24% x 42%; G4 - 30% x 36%; G5 - 30% x 42%; G6 - 36% x 42%. Those experimental groups correspond to following energy:protein ratio (MJ g<sup>-1</sup> protein): G1 – 0.068 x 0.057; G2 – 0.068 x 0.048; G3 – 0.068 x 0.041; G4 – 0.057 x 0.048; G5 – 0.057 x 0.041; G6 – 0.048 x 0.041. After 25 days, fish was challenged with shift diets between the feeders. Asterisks represent significant differences by the Student test (P<0.05, n=24).



**Figure 5.** Effect of the dietary range of protein quantity and protein: energy ratio on protein consumption in catfish (*Lophiosilurus alexandri*). A: Average crude protein intake (%) of each experimental group. B: Average protein intake (g) in relation to the weight of the animals. Values are means  $\pm$  SD (n = 24). The symbol (\*) represents the difference in protein intake between the diets of the same experimental group (Student's T test (P <0.05). Bar without common letter are different, Tukey's test, P <0.05.



**Figure 6.** Effect of the dietary range of the amount protein: energy ratio and gross energy in the consumption of catfish (*Lophiosilurus alexandri*). A: Average energy consumption: protein (MJ EB / g PB) by weight (kg). B: Average energy consumption (MJ kg<sup>-1</sup>) per weight (kg). Values are means ±SD (n=24). The symbol (\*) represents the energy intake difference between the diets of the same experimental group (Student's T test (P<0.05). Bar without common letter are different, Tukey test, P<0.05).