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Thamara Luísa Staudt Schneider

REDUÇÃO DE PROTEÍNA E SUPLEMENTAÇÃO COM LISINA E METIONINA EM DIETAS PARA JUNDIÁS (*Rhamdia quelen*)

Santa Maria, RS
2020

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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 obtenção do título de **Mestre em Zootecnia**

Orientador: Prof. Dr. Rafael Lazzari

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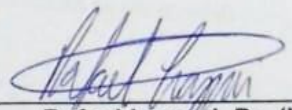

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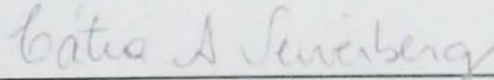

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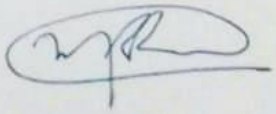

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“Dizem que a vida é para quem sabe viver, mas ninguém nasce pronto. A vida é para quem é corajoso o suficiente para se arriscar e humilde o bastante para aprender.”

(Clarice Lispector)

Dedico a Deus e aos meus familiares esta conquista.

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RESUMO

REDUÇÃO DE PROTEÍNA E SUPLEMENTAÇÃO COM LISINA E METIONINA EM DIETAS PARA JUNDIÁS (*Rhamdia quelen*)

AUTORA: Thamara Luísa Staudt Schneider
ORIENTADOR: Rafael Lazzari

A produção intensiva de peixes requer o uso de estratégias nutricionais sustentáveis. A redução do nível de proteína e a suplementação com lisina e metionina nas dietas podem otimizar o ganho em peso e melhorar a eficiência alimentar dos peixes. O jundiá, *Rhamdia quelen,* apresenta características favoráveis para produção em cativeiro. Neste estudo foram avaliados desempenho, composição corporal, metabolismo e morfometria intestinal de jundiás alimentados com redução do nível de proteína e suplementação com lisina e metionina na dieta. O delineamento experimental utilizado foi inteiramente casualizado, composto por cinco dietas e quatro repetições. Foram formuladas cinco dietas: controle positivo contendo 38% proteína bruta (PB) (38PB), controle negativo contendo 34% PB (34PB); dieta 34PB foi suplementada com: lisina (34L); metionina (34M) e lisina mais metionina (34LM). Durante 63 dias, 320 juvenis de jundiá (peso médio inicial de 26,33 ± 0,40 g) foram mantidos em um sistema de recirculação de água constituído de dois filtros biológicos, caixa de decantação, reservatório de água, aquecimento e 20 tanques (250 L). Foram distribuídos aleatoriamente 16 peixes/tanque e alimentados com as dietas experimentais três vezes ao dia (8, 13 e 18 horas) até a saciedade aparente. Ao final do experimento, dados de peso e comprimento, sangue e tecidos (fígado, músculo, brânquias e intestino) foram coletados para análises de: composição, sanguíneas, bioquímicas e morfometria. Os dados obtidos foram submetidos a ANOVA de uma via e as médias comparadas pelo teste de Tukey ($P < 0,05$). O peso final, taxa de crescimento específico e fator de condição foram maiores nos peixes alimentados com a dieta 34LM do que os peixes alimentados com dieta com 34PB ($P < 0,05$). A deposição de proteína corporal foi maior nos peixes alimentados com a dieta 34LM do que os peixes que receberam a dieta 38PB ($P = 0,03$). No filé, houve menor deposição de lipídios nos peixes alimentados com dieta de 34L do que nos peixes alimentados com dietas de 34M e 34LM ($P < 0,0001$). Os peixes alimentados com a dieta de 34LM apresentaram maior conteúdo de AA no plasma do que os peixes alimentados com as dietas de 34L e 34PB ($P < 0,0001$). A menor altura das vilosidades foi observada nos peixes alimentados com a dieta 34L em comparação à dieta com 38PB ($P = 0,021$). O número de células caliciformes por vilosidades foi maior em peixes alimentados com a dieta 34M em comparação as outras dietas ($P < 0,0001$). A redução de 4% do nível de proteína na dieta para jundiás pode ser realizada, desde que a dieta seja suplementada com lisina e metionina.

Palavras-chave: Aminoácidos essenciais. Nutrição. Piscicultura.

ABSTRACT

PROTEIN REDUCTION AND SUPPLEMENTATION WITH LYSINE AND METHIONNINE IN DIETS FOR SILVER CATFISH (*Rhamdia quelen*)

AUTHOR: Thamara Luísa Staudt Schneider
ADVISOR: Rafael Lazzari

Fish production requires the use of sustainable nutritional strategies. Reduced the level of protein and supplementation with lysine and methionine in diets can optimize weight gain and improve the feeding efficiency of fish. The silver catfish, *Rhamdia quelen*, has favorable characteristics for captive production. In this study, performance, body composition, metabolism and intestinal morphometry of silver catfish fed with reduced protein level and supplementation with lysine and methionine in the diet were evaluated. The experimental design used was completely randomized, composed of five diets and four repetitions. Five diets were formulated: positive control containing 38% crude protein (CP) (38CP), negative control containing 34% CP (34CP); 34CP diet was supplemented with: lysine (34L); methionine (34M) and lysine plus methionine (34LM). For 63 days, 320 juveniles of silver catfish (initial average weight of 26.33 ± 0.40 g) were kept in a water recirculation system consisting of two biological filters, decantation box, water tank, heating and 20 tanks (250 L). 16 fish/tank were randomly distributed and fed with experimental diets three times a day (8, 13 and 18 hours) until apparent satiety. At the end of the experiment, data on weight and length, blood and tissues (liver, muscle, gills and intestine) were collected for analysis of: composition, blood, biochemistry and morphometry. The data obtained were submitted to one-way ANOVA and the means compared by the Tukey test ($P < 0.05$). The final weight, specific growth rate and condition factor were higher in fish fed the 34LM diet than fish fed the 34PB diet ($P < 0.05$). The deposition of body protein was higher in fish fed a 34LM diet than fish that received a 38PB diet ($P = 0.03$). In the fillet, there was lower deposition of lipids in fish fed a 34L diet than in fish fed a 34M and 34LM diet ($P < 0.0001$). Fish fed the 34LM diet had higher AA content in plasma than fish fed the 34L and 34PB diets ($P < 0.0001$). The lower villus height was observed in fish fed the 34L diet compared to the 38PB diet ($P = 0.02$). The number of goblet cells per villus was higher in fish fed the 34M diet compared to 34PB, 34L and 34LM ($P < 0.0001$). The reduction of 4% of the level of protein in the diet for silver catfish can be accomplished, since the diet is supplemented with lysine and methionine.

Key-words: Essential amino acids. Fish farming. Nutrition.

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1 INTRODUÇÃO GERAL

A aquicultura é o setor na produção de alimentos com maior crescimento relativo e grande parte da produção é destinado ao consumo humano (FOOD AND AGRICULTURE ORGANIZATION - FAO, 2018). A demanda constante de alimentos fortalece a cadeia produtiva e estimula a produção. No período de 2011 a 2016, a produção de peixes aumentou 29,44%. Em 2016, contribuiu com quase metade da produção mundial. O aumento da população mundial, a preocupação com a segurança alimentar e interesse pela produção de alimento sustentável são desafios a serem enfrentados pelos países produtores de alimentos. O Brasil está entre os 15 países com maior produção de peixes (FAO, 2018). Em 2019, a produção brasileira somou 722.560 toneladas de peixes, crescimento de 4,5% em comparação ao ano de 2018. A produção nacional foi liderada pela região Sul, sendo 27,5% da produção provenientes das espécies: tilápia, carpa, o jundiá e outros nativos (ASSOCIAÇÃO BRASILEIRA DA PISCICULTURA - PEIXE BR, 2018; 2019).

Atualmente, os investimentos na produção buscam alcançar altos índices de produtividade com eficiência, qualidade, em tecnologias focada na redução dos custos de produção. A alimentação é o insumo mais oneroso, representa mais de 50% dos custos variáveis na produção de peixes (LOVELL, 2002). Em sistemas intensivos, há dependência de alimentos exógenos aos animais e, por consequência, pode haver aumento de emissões de compostos nitrogenados na água (FAO, 2018). A substituição da farinha de peixe, a utilização de alimentos alternativos e a redução de proteína na dieta são estratégias nutricionais sustentáveis que viabilizam a produção, melhoram a eficiência alimentar e reduzem o desperdício (GAN et al., 2012; TEODÓSIO et al., 2019; WANG et al., 2020).

Em 2019, a produção de rações para piscicultura no Brasil somou 940 mil toneladas, crescimento de 5% em relação ao ano anterior (SINDIRAÇÕES, 2020). Os ingredientes utilizados na formulação das rações são constituídos de nutrientes exigidos pelos animais. A proteína é um dos nutrientes mais importantes para crescimento, reprodução e saúde dos peixes (KAUSHIK E SEILIEZ, 2010), a mesma é formada pelo conjunto de aminoácidos (AA). Os peixes exigem dez AAE, entre eles, a lisina e metionina, sendo esses os AA mais limitantes na formulação de dietas (LOVELL, 1989).

O crescimento dos peixes é determinado pelo primeiro AA limitante e pela relação de equilíbrio entre eles (GAYLORD et al., 2017; NATIONAL RESEARCH COUNCIL - NRC, 2011). A lisina e a metionina além de participaram do crescimento, participam no metabolismo energético e no sistema de defesa antioxidante, na síntese proteica e carnitina (WU, 2009). Em alguns trabalhos tem sido documentado que a redução de proteína e a suplementação com AA essenciais (AAE) melhoram a eficiência alimentar e ganho em peso (AHMED et al., 2019; JIANG et al., 2016), diminuem o impacto ambiental (REN et al., 2019; TEODÓSIO et al., 2019) e o custo da dieta (GÜROY et al., 2017). No entanto, o crescimento de peixe-gato do canal, *Ictalurus punctatus,* não foi influenciado com a suplementação de AA, mesmo sendo alimentado com dietas com redução de proteína ou à base de proteínas vegetais (GAYLORD; SEALEY; GATLIN, 2002; LI; ROBINSON, 1998; WEBSTER; TIUL; MORGAN, 2000). Em geral, os estudos tem sido direcionados as respostas de desempenho e deposição de nutrientes em peixes. Embora, o intestino atue na digestão e absorção dos nutrientes e na regulação do crescimento, são poucas as investigações voltadas a alterações morfológicas teciduais em peixes.

Entre as espécies promissoras para cultivo na região Sul, o jundiá, *Rhamdia quelen*, é um bagre de hábito alimentar onívoro que se destaca por apresentar rápido crescimento e boa eficiência alimentar (SANTOS; MEURER, 2018). Em 2017, no Rio Grande do Sul a produção da espécie juntamente com outros peixes representou 8% da produção total (PEIXE BR, 2019). Os estudos de nutrição para jundiás, de modo geral, são restritos a quantidade de proteína na dieta e as exigências nutricionais, ainda existindo dúvidas sobre os benefícios da adição de AA na dieta (RADÜNZ-NETO; BORBA, 2012). Dessa forma, este estudo tem como objetivo avaliar o desempenho zootécnico, composição corporal, metabolismo e morfometria intestinal de jundiás alimentados com redução do nível de proteína e suplementação com lisina e metionina na dieta.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar o desempenho de crescimento, composição corporal, metabolismo e morfometria intestinal de jundiás (*Rhamdia quelen*) alimentados com redução do nível de proteína e suplementação com lisina e metionina na dieta.

2.2 OBJETIVOS ESPECÍFICOS

- Avaliar se há efeito da redução do nível de proteína e suplementação com AA limitantes na dieta em resposta ao crescimento e aos índices digestivos de jundiás.
- Examinar se há efeito das dietas sobre a composição e a deposição dos nutrientes no corpo e no filé de jundiás.
- Identificar se há alterações nos parâmetros plasmáticos e nas respostas hematológicas quando os peixes são alimentados com redução do nível de proteína e suplementação com lisina e metionina na dieta.
- Identificar se há alterações metabólicas e na morfometria intestinal de jundiás alimentados com as dietas experimentais.

3 ESTUDO BIBLIOGRÁFICO

3.1. JUNDIÁ

O jundiá (*Rhamdia quelen*) pertence à ordem Siluriforme e à família Heptateridae. É um bagre nativo da América do Sul, sendo encontrado desde o México até a Argentina (SILFVERGRIP, 1996). No Brasil, sua produção está concentrada nos estados da região Sul, principalmente, no Rio Grande do Sul. Em 2017, o jundiá e outros peixes nativos representaram 8% da produção total do estado, segundo a PEIXE BR (2018). Neste mesmo ano, durante a Semana Santa foram comercializadas 154 toneladas de jundiá no estado (EMATER, 2017). O pescado possui aceitação pelos consumidores por ser desprovido de espinhas intramusculares e pelo seu sabor agradável (CARNEIRO et al., 2004).

O jundiá destaca-se como espécie com potencial de cultivo. Em cativeiro a espécie tem preferência por locais de baixa luminosidade, apresenta rápido crescimento, em tanques escavados podem atingir 1kg de peso médio em doze meses, boa conversão alimentar, em média 1,5 a 1,8, e comportamento dócil em manejos de captura (CARNEIRO, 2004; SANTOS; MEURER, 2018). Hábito alimentar onívoro com tendência a carnívora devido possuir características gastrointestinais específicas como exemplo o intestino curto semelhante ao de peixes carnívoros (RODRIGUES et al., 2012).

Quanto a alimentação com dietas práticas, os jundiás apresentaram boa aceitação de distintas fontes proteicas, que resultaram em crescimento satisfatório e maior deposição de proteína corporal e muscular (LAZZARI et al., 2008; SIGNOR et al., 2004). O nível proteico exigido pela espécie tem sido relatado em muitos estudos, mas mesmo em condições ideais de experimentação e manejo, diferenças quanto à fase de crescimento e à dieta utilizada podem ocasionar variações nos valores de exigência. Nesse sentido, Meyer; Fracalossi (2004), em estudo com alevinos de jundiá (1,52 ± 0,34 g), avaliaram dois níveis de proteína e duas concentrações de energia com dieta purificada e observaram melhor desempenho e composição corporal dos peixes alimentados com 37,3% PB e 3200 kcal EM/ kg e maior deposição de gordura e efeito poupador de proteína na dieta com 32% PB e 3650 kcal EM/ kg. Salhi et al. (2004) verificaram melhor desempenho de jundiás (0,3 g de peso inicial) alimentados com dieta prática contendo 37% PB e 3390 kcal ED/ kg, correspondendo a relação

PD/ED de 23,6 mg/ kj. Quando utilizado farinhas e farelos na dieta, foi observado melhor desempenho e maior deposição de proteína corporal na dieta com 38% PB e 3600 Kcal ED/ kg (SIGNOR et al., 2004). Adorian et al. (2018) alimentaram jundiás (130,05 ± 0,14 g) com 28 e 32% PB e observaram melhor eficiência produtiva nas fêmeas, entretanto, independentemente do sexo, os peixes apresentaram melhor desempenho quando submetidos a dieta comercial com 32% PB e 3489 kcal ED/ kg.

O nível de proteína na dieta deve assegurar quantidade adequada de AA para atender as exigências da espécie, permitindo que o organismo sintetize suas próprias proteínas para manutenção um desenvolvimento e crescimento adequado (PORTZ; FURUYA, 2012). O estudo de Montes-Girao; Fracalossi (2006) foi estimada a exigência dietética de lisina para o jundiá (1,5±0,1 g de peso inicial) de 4,5 ou 5,1% da proteína, dependendo do modelo estatístico: via *broken line* ou regressão polinomial, respectivamente. Considerando o máximo ganho em peso e a taxa de crescimento específico, foi determinada a exigência de metionina, respectivamente, em 3,46 e 3,59% da fração proteica para jundiás (3,26±0,3 g de peso inicial) (ROTILI et al., 2018).

Na formulação de dietas isoproteicas (20% de PB) foram adicionadas diferentes proporções de uma mistura com cinco AAE (0;3;6;12% do peso da ração), os jundiás (*R. quelen*) alimentados com essas dietas apresentaram um mecanismo de resposta adaptativo observado entre a dieta e a atividade proteolítica alcalina (UNGAR et al., 2009). Bittencourt et al. (2018) testaram a suplementação de quatro níveis de lisina (1,30; 1,40; 1,60 e 1,95% da dieta) em dietas com 30% de PB, os autores concluíram que a adição de lisina resultou em acúmulo de gordura visceral e afetou os parâmetros reprodutivos de jundiás (*R. voulezi*). O aumento do ganho em peso, do volume seminal e a diminuição da conversão alimentar foi observado em jundiás (*R. voulezi*) alimentados com o aumento dos níveis de lisina na dieta (1,20; 1,40; 1,60 e 1,80% da dieta) (DIEMER et al., 2014). No entanto, são necessários estudos sobre os efeitos nutricionais e o papel dos AAE no desempenho, composição corporal e metabolismo de jundiás (*R. quelen*).

3.2. PROTEINA NA NUTRIÇÃO DE PEIXES

As proteínas são as macromoléculas mais abundantes que ocorrem em todas as células e são importantes para os tecidos, pois auxiliam na regulação do metabolismo, transporte de nutrientes e sistema imunológico do organismo (PORTZ;

FURUYA, 2012). As proteínas são constituídas de AA que são formados por um átomo central (carbono alfa - α) ligado ao grupo amina, átomo de hidrogênio e grupo carboxílico. Na hidrólise das proteínas, os AA são absorvidos na membrana apical do enterócito, através de simportes AA/Na^+, transportadores não dependentes de Na^+ e por difusão, no trato intestinal e distribuídos pelo sangue aos órgãos e tecidos (LI et al., 2009; NELSON; COX, 2004; PORTZ; FURUYA, 2012).

O aproveitamento das proteínas pelos peixes depende de sua quantidade e estrutura, do equilíbrio entre os AA essenciais (AAE) e não essenciais (AANE) e da energia disponível de carboidratos e lipídios (WILSON, 2002). A quantidade de proteína inadequada resulta em diminuição ou interrupção do crescimento e na mobilização de proteína dos tecidos para manutenção das funções vitais (LOVELL, 1998; MILLWARD, 1989). O excedente da proteína pode ser transformado em energia e/ou eliminado na forma de nitrogênio amoniacal no ambiente (BOMFIM et al., 2008). Estudos recentes relacionaram a diminuição de proteína e a adição de AA observaram a redução na excreção de compostos nitrogenados (TEODÓSIO et al., 2019), maior crescimento e diminuição do desperdício de nutrientes (AHMED et al., 2019; GAYLORD et al., 2017), melhora do sistema de defesa antioxidante (JIANG et al., 2018) e maior custo benefício da ração (GÜROY et al., 2017; REN et al., 2017).

As fontes vegetais proteicas são muito utilizadas na formulação de dietas para espécies de hábito alimentar onívoro. Para o jundiá a combinação de fontes proteicas na dieta resultou em melhor desenvolvimento e crescimento (LAZZARI et al., 2006, 2008). Além disso, na região Sul do Brasil há boa disponibilidade de grãos que proporciona qualidade de produto e preços competitivos (SANTOS; MEURER, 2018). Para a espécie em estudo foi documentado que a farinha de peixe, comumente utilizada nas formulações de rações, pode ser substituída por outros ingredientes de origem animal, como a farinha de carne e ossos (LAZZARI et al., 2007). Contudo, a farinha de carne e ossos apresenta menor quantidade de AAE, como lisina e metionina, se comparada com a farinha de peixe (ROSTAGNO, 2011).

3.3. LISINA E METIONINA

Os peixes exigem uma relação de AAE e AANE na dieta, para síntese proteica e crescimento. Os AAE são aqueles que os animais não podem sintetizar, ou não sintetizam em quantidade suficiente para a manutenção de boas taxas de crescimento. Os AANE podem ser sintetizados a partir de outros compostos. Os AAE

são arginina, histidina, isoleucina, leucina, lisina, metionina, fenilalanina, treonina, triptofano e valina. Os AANE são alanina, aspartato, glutamato, glicina, serina, tirosina, cisteína, glutamina, prolina, hidroxiprolina e taurina (LI et al., 2009).

Os AA são classificados como: polares que são solúveis em água (lisina, arginina, histidina, ácido glutâmico, ácido aspártico) e apolares, que são insolúveis em água (alanina, fenilalanina, leucina, isoleucina, metionina, prolina, triptofano e valina) (NELSON; COX, 2004). Quando advindos da digestão, os AA podem seguir duas rotas: anabólica e catabólica, essa última rota consiste na desaminação das proteínas no músculo e, por consequência, produzirão esqueletos carbônicos usados como fonte de energia, sendo esta, a rota metabólica não desejada (WALTON; COWEY; ADRON, 1984; YANG et al., 2010).

A lisina é o AA mais limitante na formulação de dietas para peixes, sendo utilizada exclusivamente para a síntese de proteína corporal e deposição de tecido muscular (MAI et al., 2006a; WU, 2009). A adição de lisina permite economizar a proteína da dieta sem afetar o desempenho de crescimento dos peixes (MAI et al., 2006a). A deficiência ou o desequilíbrio de lisina na dieta resulta em menor crescimento, maior deposição de gordura (RAMPE et al., 2014) e aumento da excreção de amônia (GAN et al., 2012).

A metionina é um AA limitante, principalmente, quando a dieta é formulada com alta quantidade de proteínas vegetas, como exemplo, o farelo de soja (MAI et al., 2006b; MICHELATO; FURUYA; GATLIN, 2018). A metionina é precursora na síntese de alguns produtos, como: cisteína, taurina e colina e serve como fonte de enxofre para as células (LI et al., 2009; WU, 2009). A deficiência de metionina resulta em menor crescimento (GAO et al., 2019), redução da síntese proteica (JIANG et al., 2017) e catarata (COWEY et al., 1992).

A relação de equilíbrio entre os AA e a exigência pelo primeiro AA limitante se atendido na dieta estabelece um bom desempenho de crescimento aos peixes (GAYLORD et al., 2017; NRC, 2011). A variação de valores da relação de lisina:metionina em algumas espécies onívoras pode ser explicada pela qualidade e proporções das fontes proteicas utilizadas na formulação das dietas, além disso, espécie, categoria, idade também são fatores de influência (Tabela 1). Para o jundiá, os hidrolisados vegetal e animal como fontes principais de proteína na dieta resultaram em peixes de maior peso final com a maior relação lisina:metionina (UCZAY et al., 2019a;b). No entanto, a combinação de farinha de peixe, caseína e gelatina como

fontes de proteína intactas na dieta para a espécie (peso médio inicial de 0,3 ± 3,26 g) resultaram em peixes de maior peso final com a menor relação lisina:metionina (ROTILI et al., 2018). Pianesso et al. (2015) utilizaram as mesmas fontes de proteína que o estudo anterior, no entanto obtiveram peixes (peso médio inicial de 4,65 ± 0,68 g) com menor peso final com a relação lisina:metionina menor.

Tabela 1- Relação Lisina:Metionina em dietas para espécies onívoras

Espécie	Relação L:M		Referência
	Maior*	Menor*	
Catfish (*Pelteobagrus fulvidraco*)	2,98	3,82	JIANG et al. (2018)
Carpa comum (*Cyprinus carpio var. Jian*)	2,10	3,00	REN et al. (2019)
Pacu (*Piaractus mesopotamicus*)	3,92	3,70	ABIMORAD et al. (2009)
Carpa Rohu (*Labeo rohita H.*)	2,00	4,17	SARDAR et al. (2009)
Tilápia híbrida (*Oreochromis niloticus* × *Oreochromis mossambicus*)	1,75	3,96	FIGUEIREDO-SILVA et al. (2014)
Catfish (*Ictalarus punctatus*)	3,63	4,36	JIANG et al. (2019)
	2,25	2,12	LI; ROBINSON, (1998)
Carpa (*Carassius auratus gibeilo*)	2,91	7,13	WANG et al. (2016)
	2,24	2,30	HU et al. (2008)
Jundiá (*Rhamdia quelen*)	1,64	6,12	ROTILI et al. (2018)
	1,34	1,04	UCZAY et al. (2019a)
	1,35	1,26	PIANESSO et al. (2015)
	1,37	1,14	UCZAY et al. (2019b)
Tilápia (*Oreochromis niloticus*)	2,48	2,15	TEODÓSIO et al. (2019)
	2,56	1,88	PRABU et al. (2019)
	2,19	1,97	MICHELATO et al. (2016)

*Valores baseados no peso final maior e menor dos peixes. L:M= Lisina:Metionina

Em conjunto, a lisina e a metionina agem na síntese da carnitina, que transporta ácidos graxos de cadeia longa do citosol para a mitocôndria para a oxidação (LI et al., 2009; WU, 2009). A carnitina pode provocar o efeito poupador de proteínas por meio do catabolismo lipídico (HARPAZ, 2005). A velocidade constante da disponibilidade dos AA pode ocorrer com a combinação de fontes proteicas na dieta (AMBARDEKAR; REIGH; WILLIAMS, 2009). No entanto, o desequilíbrio do perfil de AA resulta em metabólicos que afetam a ingestão de alimentos e síntese de proteína. Por consequência, diminui o crescimento, piora a conversão alimentar e a capacidade digestiva e antioxidante, aumento da deposição de gordura e da excreção de resíduos nitrogenados (GAO et al., 2019; JIANG et al., 2018; KAUSHIK; SEILIEZ, 2010; LI; ROBINSON, 1998; WU, 2009).

3.4. MORFOMETRIA INTESTINAL

O sistema digestório varia com o hábito alimentar das espécies e com o tipo de dieta. O intestino tem como função principal completar a digestão iniciada no estômago e absorver os nutrientes da dieta. O comprimento intestinal implica no tempo de retenção do alimento e na eficiência de absorção dos nutrientes. Normalmente, espécies de intestino curto, como o jundiá, apresentam uma mucosa mais espessa, com maiores dobras, que permitem a adaptação do trato digestivo em função do hábito alimentar. A exigência é por alimento de alta qualidade, uma vez que a quantidade ingerida de alimento é menor e a passagem do alimento mais lenta (PORTZ; FURUYA, 2012). O tempo mais lento permite que os nutrientes sejam difundidos nas vilosidades intestinais antes que sejam absorvidos. No entanto, em consequência do hábito alimentar onívoro a espécie altera estrutura e as propriedades absortivas do sistema digestório em resposta a mudança na dieta, isso pode justificar a aceitação de alto teor de proteínas de origem vegetal na dieta de jundiás (HERNÁNDEZ; PÉREZ GIANESELLI; DOMITROVIC, 2009). A morfometria intestinal é uma ferramenta auxiliar na determinação de alterações nutricionais (SMIRNOV et al., 2006). A inclusão de hidrolisados pécticos na dieta de jundiás resultou maior renovação celular e melhor integridade epitelial do intestino (MOMBACH et al., 2019). Os jundiás alimentados com diferentes concentrados fibrosos apresentaram efeitos positivos nos parâmetros de crescimento e na morfologia intestinal (GOULART et al., 2018).

A parede do intestino é constituída pela camada da mucosa, que fica exposta aos agentes exógenos durante a ingestão, digestão e absorção de nutrientes. A superfície da mucosa é formada por projeções digitiformes, denominadas vilosidades e microvilosidades, que aumentam a área superficial de absorção, permitindo maior contato entre células e nutrientes. A unidade celular intestinal é formada pelos enterócitos, caliciformes e epiteliais. As células caliciformes sintetizam mucina que além de auxiliar no mecanismo de defesa, também apresenta capacidade de digerir e absorver os nutrientes da dieta. Assim, o número de células caliciformes serve como indicador imunológico do sistema digestivo (ADORIAN et al., 2016). Truta arco íris (*Oncorhynchus mykiss*) alimentadas com dietas comerciais apresentaram menor número de células caliciformes, no entanto quando os autores fornecem a dieta experimental com maior proporção de vegetais proteicos o número de células caliciformes aumenta (OSTASZEWSKA et al., 2011). Semelhante resultado foi

observado em jundiás alimentados com fibra de linhaça na dieta, os autores sugeriram que a mucosa intestinal desses peixes estava mais protegida de possíveis infecções com o aumento do número de células caliciformes (ADORIAN et al., 2016).

A melhora da estrutura intestinal e o bom desenvolvimento intestinal com a suplementação de AA em dietas foi relatado para a garoupa (*Epinephelus malabaricus*) (CHENG et al., 2018), carpa comum (*Cyprinus carpio* var. Jian) (YAN; QIU-ZHOU, 2006) e robalo híbrido (*Morone chrysops* × *M. Saxatilis*) (CHENG; GATLIN; BUENTELLO, 2012). A deficiência de metionina na dieta resultou em menor altura de vilosidade, consequentemente, menor capacidade de absorção de nutrientes e menor peso final em pregado (*Scophthalmus maximus* L.) (GAO et al., 2019). Trutas arco íris (*O. mykiss*) alimentadas com dietas deficiente em lisina apresentaram menores dobras intestinais e maior número de células caliciformes no intestino (OSTASZEWSKA et al., 2011). A adição de lisina sintética na dieta de carpa comum (*C. carpio* Var. Jian) resultou em menor altura de vilosidades e profundidade de criptas, possivelmente pode ter ocorrido um estímulo alimentar menor no intestino com a rápida absorção e alta digestibilidade do AA sintético, resultando em menor desenvolvimento das estruturas intestinais (ZHOU et al., 2007). Embora o intestino atua na digestão e absorção dos nutrientes e na regulação do crescimento, investigações direcionados as alterações morfológicas teciduais são limitados.

Em meio a importância dos parâmetros para o desenvolvimento produtivo, os mesmos devem ser avaliados em experimentos, neste caso, nos jundiás que serão alimentados com dietas contendo nível menor de proteína e suplementação de AAE, avaliar não somente o crescimento, mas também relacionar aspectos nutritivos e composição corporal, assim como verificar se há alterações na morfometria intestinal de jundiás alimentados com dietas suplementadas com lisina e metionina.

O seguinte e único Capítulo intitulado “Effects of reduced protein level and dietary amino acid supplementation on growth, body composition and intestinal morphometry of silver catfish (*Rhamdia quelen*)” foi submetido ao periódico **Aquaculture Research**.

4 CAPÍTULO 1

Effects of reduced protein level and dietary amino acid supplementation on growth, body composition and intestinal morphometry of silver catfish (*Rhamdia quelen*)

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Abstract

The reduction of the protein level and the supplementation of lysine (Lys) and methionine (Met) in the diet were evaluated in the performance and intestinal morphometry of silver catfish (*Rhamdia quelen*). The study was used in a completely randomized design for 63 days, where 320 fish (26.33 ± 0.40 g) were distributed in 20 tanks (250 L, 16 fish each) and fed until the apparent satiety was reached. Five diets were formulated, a positive control [38% of crude protein (CP) (38CP)], a negative control [34% of CP (34CP)] and three test diets [34% of CP supplemented with Lys (34L), 34% of CP supplemented with Met (34M) and 34% of CP supplemented with Lys plus Met (34LM)]. Final weight and condition factor were greater in fish fed the 34LM diet than in those fed the 34CP diet ($p < .05$). Body lipid deposition was lower in fish fed the 34CP diet than other diets ($p = .0002$). In the fillet, there was a lower deposition of lipids in fish fed with 34L diet than in fish fed with 34M and 34LM diets ($p < .0001$). Fish fed the 34LM diet had a higher of AA content in the plasma than those 34L and 34CP diets ($p < .0001$). The lower villus height was observed in fish fed the 34L diet compared to the 38CP diet ($p = .021$). It is possible to reduce 4% of the CP level in silver catfish diets with supplementation of Lys and Met.

Keywords: amino acids, aquaculture, goblet cells, intestine, silver catfish

1. Introduction

Feeding fish can make up more than 50% of production costs (Food and Agriculture Organization - FAO, 2018). Protein is one of the most expensive nutrients in the formulation of diets. The use of vegetable by-products and proteins to replace fishmeal allows for a reduction in food costs (Güroy et al., 2017; Lazzari et al., 2008; Santos & Meurer, 2018). However, plant sources have antinutritional factors, deficiency of essential amino acids (AA) and lower digestibility and palatability of nutrients. Soybean meal has a low palatability and concentration of lysine (Lys) and methionine (Met), which can limit the use and growth of animals (National Research Council - NRC, 2011).

An investigate the reduction of crude protein (CP) levels in the diet, adding essentials AA, especially Lys and Met, to ensure that nutritional needs are met (Jiang et al., 2018; Mamauag, Ragaza, & Nacionales, 2019). By increasing the level of Lys and Met, the supplemented diet can improve the use of other excess AA, optimize anabolism and maximize the use of proteins in the diet. Recent studies show that dietary supplementation of Lys and Met in diet improves growth, food intake, nutrient deposition, digestive enzyme activity, antioxidant capacity and ammonia excretion in fish (Gan et al., 2012; Güroy et al., 2017; Jiang et al., 2016; Jiang et al., 2018; Yang, Liu, Tian, Liang, & Lin, 2010).

In general, investigations have focused on performance parameters and nutrient deposition in fish. Studies targeting tissue morphological changes are limited. The intestine and liver act directly on the digestion, absorption and metabolism of nutrients. Both act in the regulation of growth and in the use of dietary nutrients. Some studies report morphological changes in the epithelium and villi of the intestine of fish fed with the addition of AA in the diet (Cai et al., 2018; Gao et al., 2019; Ostaszewska, Dabrowski, Kwasek, Verri, & Kamaszewski, 2011). The cells goblets are secretors of mucin in the intestine, the increase in the number of these cells can occur in response to diets and serves as an immune indicator of

the digestive system (Adorian et al., 2016). The inclusion of Met in the diet increased the number of goblet cells in the intestine, but did not prevent the growth of turbot (*Scophthalmus maximus* L.) (Gao et al., 2019).

The silver catfish, *Rhamdia quelen*, is a specie with potential for aquaculture production due to its acceptance in the market, desirable flavor, rapid growth and quality of the fillet (Santos & Meurer, 2018). This fish, an omnivorous feeding habit, performed well and deposited body protein when fed with 38% of the crude protein (CP) and 3600 kcal energy digestive (ED) kg^{-1} in the diet (Signor, Signor, Feiden, Reidel, & Hayashi, 2004). Lys requirements was estimated based on weight gain and apparent net protein utilization 4.50 and 5.10% of the CP (Montes-Girao & Fracalossi, 2006) and Met was based on weight gain and specific growth rate: 3.43 and 3.57% of the CP (Rotili et al., 2018).

Thus, this study aimed to evaluate the effects of reducing the level of CP and supplementation of Lys and Met in practical diets on parameters of growth, metabolism, body composition and intestinal morphometry of juveniles of silver catfish.

2. Materials and methods

The experimental procedures were conducted at the Fish Farming Laboratory, Federal University of Santa Maria, *Campus* Palmeira das Missões, Rio Grande do Sul, Brazil, under the approval of the institution's Animal Use Ethics Committee (process number 7315131119).

2.1. Experimental diets

The formulation and composition of the diets are listed in Table 1 and 2. Five isoenergetic diets were formulated to contain two levels of CP in the diet, positive control [38% of CP (38CP)] and negative control [34% of CP (34CP)], using plant ingredients and meat and bone meal as sources of protein (Lazzari et al., 2008). The positive control diet was formulated to meet the nutritional requirements of juvenile silver catfish (*R. quelen*) (Signor et al., 2004).

Diets with 34% of CP were supplemented with 0.5% Lys (34L), 1.5% Met (34M) and both AA (34LM), above the AA requirements of species (Montes-Girao & Fracalossi, 2006; Rotili et al., 2018). The diets were prepared by mixing the ingredients with the oil using a horizontal mixer. During this process, the pH was adjusted to neutral using 6 N NaOH (Robinson, Wilson, & Poe, 1981) to avoid possible differences in feed intake due to variable palatability. The mixture was pelleted with a matrix of 5 mm in diameter, and pellets were oven dried (55°C) for 24 h before storage (-20°C).

2.2. Experimental procedure

The fish were adapted to the experimental conditions for three weeks and fed the experimental control diet. Subsequently, the initial biometry was performed with 320 juvenile, with an average initial weight of 26.33 ± 0.40 g, distributed in 20 tanks (250 L, 16 fish each) for 63 days. The study was conducted in a completely randomized design, with five treatments (diets) and four replicated tanks per treatment.

The experiment was carried out in a water recirculation system that contained water inlet and outlet in the tanks, individual aeration, contained a decanter, two mechanical and biological filters and a water reservoir (2000 L), equipped with a heating system. The fish were fed the experimental diets until reaching apparent satiety three times a day (08:00 am, 01:00 pm and 06:00 pm). The of feed ingested was measured daily, and the tanks were cleaned twice a day (10:00 am and 03:00 pm).

The water quality was monitored daily by measuring the temperature and the dissolved oxygen (DO) with YSI ProODO dissolved oxygen meter ODO® technology (YSI Inc. Ohio, USA). The pH (with digital equipment), total alkalinity (by neutralization titration), total hardness (by complexation titration), and nitrogen compounds (ammonia, nitrite and nitrate; by colorimetry, with kits) were measured weekly. Water quality parameters were maintained as needed for the specie (Baldisserotto & Silva, 2004) and are listed as follows: temperature: 23.05

± 1.01°C; oxygen: 7.52 ± 0.31 mg L^{-1}; pH: 7.18 ± 0.13; ammonia: 0.002 ± 0.001 mg NH_3 L^{-1}; nitrite: 0.25 ± 0.00 mg NO_2^- L^{-1}; nitrate: 0.44 ± 0.19 mg NO_3^- L^{-1}; alkalinity: 45.45 ± 6.72 mg $CaCO_3$ L^{-1}; and hardness: 91.85 ± 22.69 mg $CaCO_3$ L^{-1}.

2.3. Measurements and analyses

2.3.1 Sample collection

At the end of the experimental period, the fish were fasted for approximately 18 h. Three fish per tank (12 per treatment) were randomly selected for blood sample collection, which was performed by puncturing the caudal vein with 10% EDTA as an anticoagulant. After the animals were anesthetized with a eugenol (50 mg L^{-1}) to measure the weight (g) and length (cm). Fish were euthanized with anesthetic overdose (250 mg L^{-1}) by spinal sectioning to collect tissues.

2.3.2. Growth, survival, feeding cost and indexes digestives

The fish were weighed and measured to obtain the following data:

Specific growth rate (% day^{-1}): SGR = [ln (FW) − ln (IW)/days]×100;

Relative weight gain (g): RWG = (FW-IW/IW)×100;

Condition factor: CF = (FW/total $length^3$)×100;

Survival (%): S = (final fish number×100)/initial fish number;

Feed efficiency: FE = (FW-IW)/FC, where FC = feed consumption;

Protein efficiency rate: PER = (FW-IW)/PC, where PC = protein consumed;

Daily intake (% live weight): DI = [(FC/2)/(FW+IW)/days]×100.

The cost of feeding (US$ kg of weight $gain^{-1}$) was calculated according to the Abimorad et al. (2009). Carcass yield and digestive indexes were calculated using the following formulas:

Carcass yield (%): CY = (gutted weight with head and gills/whole weight)×100;

Digestive somatic index (%): DSI = (digestive tract weight/whole weight)×100;

Hepatosomatic index (%): HSI = (liver weight/whole weight)×100;

Celomic fat index (%): CFI = (abdominal cavity fat weight/whole weight)×100;

Intestinal quotient (%): IQ = (digestive tract length/total fish length).

2.3.3. Composition and deposition of nutrients

The composition of the diets (Table 2) and the whole body and fillet composition were determined following the methodologies described by the Association of Official Analytical Chemists (AOAC, 1995). Lipids were extracted with methanol/chloroform and quantified by the procedure of Bligh & Dyer (1959). The whole body and fillet samples, collected at the end of the experiment, were homogenized to determine the composition. The same procedure was adopted to determine the initial composition by sampling 10 fish. The dry matter content was determined by drying the samples at 105°C until reaching constant weight (method 934.01). The mineral matter content was determined by incineration at 550 °C for 4 h (method 968.08). The CP content was determined using the adapted Kjeldahl method (954.01). The AA composition of the diets was analyzed according to White, Hart & Fry (1986) (Table 2).

The deposition of digestive nutrients was calculated by equations:

Body protein deposition (g): BPD = [FW×(%CPBf/100)]-[IW×(%CPBi/100)];

Body lipid deposition (g): BLD = [FW×(%LBf/100)]-[IW×(%LBi/100)];

Protein deposition in the fillet (g): PDF = [FW×(%CPFf/100)]-[IW×(%CPFi/100)];

Lipids deposition in the fillet (g): LDF = [FW×(%LFf/100)]-[IW×(%LFi/100)].

Where: initial weight (IW), final weight (FW), initial body protein crude (CPBi), final body protein crude (CPBf), initial body lipid (LBi), final body lipid (LBf), initial fillet protein crude (CPFi), final fillet protein crude (CPFf), initial fillet lipid (LFi) and final fillet lipid (LFf).

2.3.4. Erythrocyte parameters

In the analysis of the erythrocyte series were determined: total number of erythrocytes (Neubauer chamber), hematocrit (microhematocrit method) and hemoglobin

(spectrophotometry). Subsequently, the following erythrocyte indices were calculated using the formulas listed:

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

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

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

2.3.5. Plasma biochemical parameters

Blood samples were centrifuged at 1465 *g* for 10 min to obtain plasma. The glucose and total proteins levels were quantified using an enzymatic method from a commercial colorimetric kits (Doles®, Goiânia, GO, Brazil). The total ammonia and AA contents were determined according to Verdouw et al. (1978) and Spies (1957), respectively.

2.3.6. Biochemical parameters and thiobarbituric acid reactive substances (TBARS) in tissues

In the liver and muscle tissues, total proteins (Bradford, 1976), glycogen (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), TBARS (Buege & Aust, 1975) and AA (Spies, 1957) were determined.

2.3.7. Intestinal morphometry

The anterior portion of the intestines of four fish per treatment was collected for the analysis of intestinal morphometry. The samples were subjected to fixation processes in 10% buffered formaldehyde and 70% alcohol. Subsequently, the samples were dehydrated in gradient alcohol solutions and clarified in xylol. Then, the sections were fixed in paraffin and histologically cut using a semiautomatic microtome (Thermo Scientific®, HM355S). Transverse sections (5 μm) were obtained and subjected to the staining process with hematoxylin and eosin. A morphometric image analysis system was used: images were obtained with a Zeiss Axio Imager® microscope and a Canon Power Shot G9® camera and analyzed by the Axiovision® program.

Six villi per fish were selected [the measured values corresponded to a total of 120 villus], and following was measured height of each villus, under a 10× objective, according to the adapted methodology of Mello et al. (2013). For each villus analyzed, the number of goblet cells was counted under the objective of 40×, and each of them was considered a unit.

2.3.8. Statistical Analysis

The data were submitted to the Shapiro-Wilk normality test and analyzed by unidirectional one-way analysis of variance (ANOVA). The means were compared using the Tukey test ($p < .05$). The results are presented as the mean ± standard deviation. Statistical analyses were performed in the Statistical Analysis System Studio®.

3. Results

3.1 Growth, survival, feeding cost and indexes digestives

The final weight, SGR, RWG and FC of the fish fed with the 34LM diet was equal to the fish fed with the 38CP, 34L and 34M diets, but higher compared to fish that received 34CP diet ($p < .05$) (Table 3). The S was high (> 96.87%) and showed no difference between groups ($p = .67$). The total length, FE, PER, DI, CY, HSI, CFI; DSI and IQ were not influenced by dietary treatments ($p > .05$). The 34L diet resulted in a lower feed cost (US$ kg of weight gain^{-1}) among the experimental diets ($p = .01$).

3.2. Composition and deposition of nutrients

The content of whole body dry matter found in fish fed the 34M diet was higher only than the 34LM group ($p = .04$) (Table 4). The mineral content was higher in fish fed with 34M and 34LM diets than in fish fed with 38CP and 34CP diets ($p = .0006$). The protein content was lower in fish that received the 34M diet compared to fish that received 34CP diet ($p = .006$). Fish fed the 34LM diet showed higher protein deposition than fish fed 38CP diet ($p = .03$). Body lipid content ($p = .0001$) and lipid deposition ($p = .0002$) were lower found in fish fed the 34CP diet, which differed from fish fed the other diets.

The dry matter content (p = .0001), lipid content (p = .0001) and lipid deposition (p <.0001) in the fillets were significantly changed with the experimental diets (Table 4). Fish fed the 34LM diet had higher dry matter content in the fillets than those from 34CP, 34L and 34M diets. Higher lipid content and deposition in the fillet were found in fish fed the 34LM diet, which differed from fish fed the other diets.

3.3. Erythrocyte parameters

Fish fed the 34L diet had a higher number of erythrocytes (p = .003) and lower HCM (p = .0002) and VCM (p <.0001) compared to fish that received the other experimental diets (Table 5). The hemoglobina, hematocrit and MCHC were not influenced by dietary treatments (p > .05).

3.4. Plasma biochemical parameters

Glucose level was lower in fish fed the 34L diet (p = .007), but it was higher in animals treated with 34M and 34LM and did not differ from fish fed the 34CP and 38CP diets (Table 6). For the total proteins content, the highest level was found in fish fed with the 34M diet, however, this parameter differed in fish fed 38CP, 34CP and 34L diets, which showed the lower level (p <.0001). Fish fed the 34LM diet had a higher of AA content in the plasma than those 34L and 34CP diets (p <.0001). Plasma ammonia concentration in fish did not differ between dietary treatments (p = .22).

3.5. Biochemical parameters and TBARS in tissues

Fish fed the 38CP and 34M diets had a higher concentration of TBARS (p = .0006), differing from fish fed the 34LM diet, which had a lower concentration of this parameter in the liver (Table 6). The total proteins, AA and glycogen were not influenced by dietary treatments (p > .05).

In the muscle of fish that received the 34L diet, a higher total proteins concentration was observed, differing from fish fed the 34M diet (p = .02) (Table 6). There was no difference in

247 the concentrations of AA (p = .18) and TBARS (p = .12) in the silver catfish muscle fed with
248 experimental diets.

249 *3.6. Intestinal morphometry*

250 The villus morphometry of the fish was influenced by the experimental diets (Figure 1).
251 The lower villus height was observed in fish fed the 34L diet compared to fish that received the
252 38CP diet (p = .02). The number of goblet cells per villus was higher in fish that received the
253 34M diet compared to fish fed with the other diets (p < .0001) (Figure 2).

254

255 **4. Discussion**

256 *4.1. Growth, survival, feeding cost and indexes digestives*

257 In the present study, compared to fish fed with 38% of CP, final weight, SGR, RWG
258 and FC were not affected in fish that received diets with a 4% reduction in CP level. Although
259 CP levels have been reduced, diets have reached the minimum Lys levels established to meet
260 silver catfish requirements, using supplemental sources. However, Met levels were not met in
261 diets with 38% and 34% of CP. The similar performance of fish that received diets with a
262 reduction of 4% of the CP level, supplemented with Lys and Met indicates that silver catfish
263 was able to use amino acids efficiently for growth. This is confirmed by the lower performance
264 of the fish when fed a 34% CP diet without AA supplementation.

265 Some studies have shown that reduced the level of CP and supplementated with AA
266 brings satisfactory results for the growth of various species of fish (Gan et al., 2012; Jiang et
267 al., 2018; Michelato et al., 2016; Ren et al., 2019). The availability of AA in free form results
268 in greater absorption compared to AA bound to proteins (Nguyen & Davis, 2016; Zhou, Zhao,
269 & Lin, 2007). Michelato et al. (2016) observed that higher levels of Lys in the feeding of Nile
270 tilapia (*Oreochromis niloticus*) increased weight gain. The supplementated of Lys and Met in
271 diets for grass carp (*Ctenopharyngodon idella*) made it possible to reduce the CP level from
272 32% to 30% (Gan et al., 2012). A diet containing high inclusion of soybean meal did not hinder

the growth of yellow catfish (*Pelteobagrus fulvidraco*) when supplemented with Lys and Met, and also made it possible to reduce the CP level by 2% (41% to 39% CP) (Jiang et al., 2018). Ren et al. (2019) observed that food intake was no different between diets supplemented with Lys and Met, suggested that the Jian carp (*C. carpio* Var. Jian) was well adapted to different levels of CP and fish meal in the diets. In the present study, FE, PER, DI and somatic indexes of fish were not influenced by dietary treatments, suggesting that the species was adapted to the levels of CP and the supplementation of AA established.

In the present study, the cost of food was lower in the diet supplemented with Lys only. Although supplementation with synthetic AA increases the cost of the diet, the increase in RWG and the reduction in the excretion of nitrogen compounds justify its use (Abimorad et al., 2009; Güroy et al., 2017). On the other hand, the greater RWG in the short term, reduces the slaughter time and can guarantee greater benefit to the producer (Botaro et al., 2007). Higher levels of Lys favors protein synthesis and can improve meat production, as already observed for Nile tilapia (*O. niloticus*) (Michelato et al., 2016; Nguyen & Davis, 2016). Thus, reducted the level of CP and supplementated AA in the diet are nutritional strategies that can reduce food costs, improve performance and promote protein synthesis in silver catfish.

4.2. Composition and deposition of nutrients

In the present study, fish fed the 34M and 34LM diets had higher levels of dry and mineral matter in the body than in fish fed diets without supplementation. In Meager (*A. regius*) and Yellow catfish (*P. fulvidraco*) the body mineral content was not influenced by diets supplemented with Lys and Met (Güroy et al., 2017; Jiang et al., 2018). However, some studies describe that the mineral content is associated with the growth of fish (Rotili et al., 2018). Our results suggest a relationship between the content of body mineral matter and the growth of fish fed diets supplemented with AA.

297 The silver catfish that received the diet supplemented with Met only had a lower body
298 protein content than those of the negative control diet. The 38CP and 34CP diets did not meet
299 the Met requirements for the specie, Gao et al. (2019) observed that Met deficiency resulted in
300 lower protein and body lipid content in turbot (*S. maximus* L.). Closed snout (*Megalobrama*
301 *amblycephala* Yih) fed with Met had lower body protein content and no increase in protein
302 synthesis was observed with higher levels of this AA (Liang et al., 2016). In silver catfish (*R.*
303 *quelen*) fed diets containing Met levels, there was no difference in body protein content (Rotili
304 et al., 2018). The differences between the results can be attributed to dietary treatments and the
305 characteristics of the species such as age, eating habits (Gao et al., 2019; Liang et al., 2016;
306 Rotili et al., 2018). The inadequate balance of AA in the diet can result in increased body fat
307 instead of protein synthesis (Espe et al., 2010; Li & Robinson, 1998). Our results suggest that
308 Met in fish fed a diet supplemented was catabolized and used for lipid synthesis, since the body
309 lipid content was similar to positive control group and in the negative control diet the lipid
310 content was lower.

311 In the present study, fish that received the diet supplemented with Lys had lower lipid
312 content and deposition in the fillet, compared to fish fed diets supplemented with Met (34M
313 and 34LM). Some studies have shown that the inclusion of Lys in fish diets results in increased
314 protein synthesis and reduced lipid in tissues (Abimorad et al., 2017; Nguyen & Davis, 2016).
315 Lys for be a precursor in the biosynthesis of carnitine, which acts as a lipotrophic factor, can
316 contribute to the reduction of body lipid deposition (Harpaz, 2005). On the other hand, in our
317 study, fish that received a diet supplemented with Lys plus Met resulted in greater deposition
318 and lipid content in the fillet. Contradictory results were found (Gan et al., 2012; Güroy et al.,
319 2017; Sardar, Abid, Randhawa, and Prabhakar, 2009; Yang et al., 2010). Possibly, the
320 supplemented Met was catabolized and used for lipid synthesis as previously described. This
321 result is confirmed when compared to fish fed Met only supplementation in the diet.

322 *4.3. Erythrocyte parameters*

323 The blood data allow responses to the health status of fish subjected to dietary
324 manipulations.The blood values obtained in the present study are within the documented range
325 for the specie *R. quelen* (Dal'Bó et al., 2015). However, the diet supplemented with Lys only
326 resulted in an increase in the number of erythrocytes and decrease in MCH and MCV.
327 Erythrocytes are responsible for providing oxygen for tissue formation (Rathore & Yusufzai,
328 2018).

329 Diets supplemented with essential AA allowed a greater supply of oxygen and improved
330 the hematological status of the main indian carp, rohu (*Labeo rohita* H.) (Sardar et al., 2009).
331 Our results suggest an increase in oxygen demand for protein synthesis, which was documented
332 by Dabrowski et al. (2010).

333 *4.4. Plasma biochemical parameters*

334 In the present study, fish that received a diet supplemented with Lys only had a lower
335 plasma concentration of total proteins, AA and glucose. These results may indicate high
336 mobilization of body and muscle proteins with a decrease in glucose (Adorian et al., 2016).
337 However, the values obtained in the present study were lower when compared to other studies
338 with Silver catfish, possibly this can be justified by the hormonal regulation of glycolytic
339 concentrations (Adorian et al., 2016; Rotili et al., 2018).

340 Fish that received the diets supplemented with Met (34M and 34LM) showed higher or
341 similar concentrations of total proteins, AA and glucose when compared to positive control
342 group. Sardar et al. (2009) observed that supplementation with Lys plus Met in the diet for rohu
343 (*Labeo rohita* H.) resulted in a better hematochemical status with the increase in glucose and
344 total proteins. In our study, the ammonia concentration in fish was not altered by dietary
345 treatments. Ren et al. (2018) had a similar result and was attributed to the rapid release of
346 ammonia from the plasma by the gills.

4.5. Biochemical parameters and TBARS in tissues

The liver is responsible for the synthesis of metabolites that include total circulating proteins and amino acids (Mozanzadeh, Yaghoubi, Marammazi, Safari, & Gisbert, 2018). Lower levels of these metabolites in the liver may indicate that fish are using proteins to supply their energy needs and maintain plasma glucose. However, in our study the concentrations of total proteins and amino acids were not altered by dietary treatments, suggesting a balance of metabolites in the liver.

The results of the present study demonstrate greater oxidative damage to the liver in fish fed with positive control diets and supplemented with Met only. Lower damage was observed in the diet with a 4% reduction in CP level and supplemented with Lys plus Met. Jiang et al. (2018) observed that yellow catfish (*P. fulvidraco*) fed diets that contained a high level of soybean meal showed oxidative damage, however with the supplementation of Lys plus Met in the diets, the fish showed good antioxidant capacity.

4.6. Intestinal morphometry

The identification of intestinal morphological parameters is essential in understanding the different feeding strategies of fish. In the present study, fish fed a positive control diet had greater height of villi, differing only from the group that received a diet supplemented with Lys only. The reduction of the level of 4% of the CP without AA supplementation did not influence intestinal morphological changes. Silver catfish fed by-products not resulted in changes in the height of intestinal villi and showed good growth (Mombach et al., 2019). Some studies have reported intestinal changes in fish fed with AA supplementation in the diet (Cai et al., 2018; Gao et al., 2019; Ostaszewska et al., 2011; Zhou et al., 2007). Lys supplementation resulted in higher villi length and stimulated intestinal development and growth of *Megalobrama amblycephala* (Cai et al., 2018) and Jian carp (*Cyprinus carpio* Var. Jian) (Zhou et al., 2007). Intestinal homeostasis and growth parameters were impaired in rainbow trout (*Oncorhynchus*

372 *mykiss*) fed a Lys deficiency diet (Ostaszewska et al., 2011). Intestinal development can be
373 limited or impaired with a low intake and/or high digestibility of AA, since there may be a low
374 food stimulus in the intestine and, consequently, cause a smaller absorption area (Zhou et al.,
375 2007). Possibly, in our study, Lys was rapidly absorbed and caused changes in intestinal
376 structures without impairing fish growth.

377 The number of goblet cells is an important immunological indicator (Adorian et al.,
378 2016). In the present study, the highest number of goblet cells was observed in fish fed a diet
379 supplemented with Met only. Gao et al. (2019) observed that Met stimulated an increase in the
380 number of goblet cells in the villi of turbot (*S. maximus* L.). The mucin produced by the goblets
381 cells are glycoproteins formed by many AA, mainly serine, proline and threonine (Johansson
382 & Hansson, 2016). The regulation of glycoprotein synthesis is triggered by factors such as
383 fibrous foods, microorganisms, bacteria, CP level and AA in diets (Adorian et al., 2016; Gao et
384 al., 2019; Johansson & Hansson, 2016; Mello et al., 2013). Fermented copra as a source of
385 vegetable protein to replace soybean meal with the inclusion of Lys plus Met in diets for grouper
386 (*Epinephelus fuscoguttatus*) did not impair intestinal morphology and integrity (Mamauag et
387 al., 2019). Thus, our results indicated that supplementation with Met only stimulated greater
388 synthesis of goblet cells and the experimental diets did not cause negative effects on intestinal
389 health and guaranteed the zootechnical performance of the fish.

390

391 **5. Conclusion**

392 In conclusion, the 4% reduction in CP level and AA supplementation influenced
393 performance, body composition, hematochemical status and intestinal morphometry. The diet
394 supplemented with Lys resulted in lower fillet fat and the cost of feeding the silver catfish.

Declaration of Interest

The authors declare that they have no competing interests.

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570 Table 1

571 Experimental diets of silver catfish juveniles

572

Ingredient (g kg^{-1})	US$ kg^{-1}	Diet[a]				
		38CP	34CP	34L	34M	34LM
Meat and bone meal	0.42	390	340	340	340	340
Soybean meal	0.60	360	280	290	290	290
Wheat bran	0.12	90	160	130	130	130
Corn	0.17	109.8	179.8	179.8	179.8	179.8
Soy oil	1.20	30	20	20	20	20
Vitamin and mineral mixture[b]	10.00	10	10	10	10	10
Dicalcium phosphate	0.30	5	5	5	5	5
Salt	0.05	5	5	5	5	5
BHT[c]	2.30	0.2	0.2	0.2	0.2	0.2
L-Lysine HCl 99%	1.20	-	-	5	-	5
DL-Methionine 98%	5.00	-	-	-	15	15
Cellulose	3.00	-	-	15	5	-
Price[d]	US$ Kg^{-1}	0.55	0.49	0.54	0.58	0.57

573

574 [a] Diets: 38CP, 38% crude protein (CP); 34CP, 34% CP; 34L, 34% CP with lysine; 34M, 34% CP with methionine; 34LM, 34% CP with lysine plus methionine

575 [b] Mixture composition (units/kg of mixture): folic acid: 370 mg; pantothenic acid: 3.900 mg; biotin: 40 mg; copper: 740 mg; choline: 75 g; iron: 7.500 mg; inositol: 10 g;
576 iodine, 43 mg; manganese, 7.600 mg; niacin, 8.800 mg, selenium, 38 mg; vitamin A, 780.000 IU; vitamin B1, 1.400 mg; vitamin B12: 1.900 µg; vitamin B2: 1.450 mg; vitamin
577 B6: 1.400 µg; vitamin C: 19.5 g; vitamin D3: 160.000 IU; vitamin E: 14.800 IU; vitamin K3: 480 mg; zinc: 1.400 mg

578 [c] Butylated hydroxytoluene (antioxidant)

579 [d] The cost of feed ingredients as listed by index mundi (https://www.indexmundi.com/commodities)- January 2020

Table 2
Composition of the experimental diets of silver catfish juveniles (% dry matter)

Analyzed composition (%)	Diet[a]				
	38CP	34CP	34L	34M	34LM
Dry matter	94.64	93.78	90.65	95.31	93.12
Mineral matter	18.11	17.04	15.22	15.44	15.58
Crude protein	38.40	34.51	34.12	34.90	34.74
Lipids	11.72	11.52	11.56	10.59	10.79
Neutral detergent fiber	20.90	23.44	21.40	20.88	20.96
DE (MJ kg^{-1})[b]	13.21	13.24	13.01	12.78	12.84
Phosphorus[c]	2.62	2.38	2.35	2.35	2.35
Amino acids (AA) (g kg^{-1})					
Essential AA					
Arginine	24.86	22.05	20.62	21.17	21.55
Histidine	9.15	8.46	8.22	8.20	8.17
Isoleucine	14.10	12.66	11.83	12.26	12.22
Leucine	24.46	22.66	21.51	21.84	21.89
Lysine	21.31	18.73	21.43	18.11	21.71
Methionine	6.01	5.68	5.46	18.13	17.16
Methionine+cysteine	19.78	17.53	17.91	29.48	28.08
Phenylalanine	18.16	16.45	15.65	16.08	16.03
Threonine	12.36	11.49	11.03	10.99	11.15
Valine	15.34	14.21	13.05	13.49	13.45
Nonessential AA					
Alanine	17.76	16.66	15.75	16.46	16.61
Aspartate	31.72	29.37	27.69	25.75	23.73
Glutamate	40.36	38.78	35.11	35.13	34.53
Glycine	29.11	26.18	25.47	26.31	26.06
Hydroxyproline	7.19	6.20	6.32	6.74	6.69
Proline	25.22	23.16	22.40	23.42	23.41
Serine	15.04	13.84	13.09	13.02	13.03
Tyrosine	12.47	11.21	10.67	10.90	10.86

[a] Diets: 38CP, 38% crude protein (CP); 34CP, 34% CP; 34L, 34% CP with lysine; 34M, 34% CP with methionine; 34LM, 34% CP with lysine plus methionine
[b] Digestible energy = [(CP × 23.61 MJ/kg × 0.9) + (Lip × 39.82 MJ/kg × 0.85) + (CHO × 17.21 MJ/kg × 0.50)] (Jobling, 1983)
[c] Calculated according to the Brazilian Poultry and Swine Table (Rostagno, 2011)

Table 3

Growth, survival, feeding cost and indexes digestives of juvenile silver catfish fed with reduced protein level and supplementation of lysine and methionine in the diet for 63 days

Variable	Diet[a]					*p*-value
	38CP	34CP	34L	34M	34LM	
Final weight (g)	54.35±5.30[ab]	48.31±5.24[b]	55.80±0.98[ab]	57.80±3.91[ab]	63.46±5.80[a]	.01
Total length (cm)	18.13±0.92	17.89±0.36	18.32±0.22	18.28±0.11	18.25±0.68	.77
Specific growth rate (% day^{-1})	1.12±0.06[ab]	0.98±0.19[b]	1.22±0.04[ab]	1.23±0.15[ab]	1.36±0.12[a]	.01
Relative weight gain (%)	103.26±8.24[ab]	85.83±21.05[b]	116.34±4.77[ab]	117.59±20.97[ab]	139.30±16.23[a]	.01
Condition factor	0.91±0.05[ab]	0.84±0.76[b]	0.91±0.03[ab]	0.95±0.08[ab]	1.04±0.04[a]	.01
Survival (%)	98.44±3.12	96.87±3.61	100±0.00	98.44±3.12	98.44±3.12	.67
Feed efficiency (g)	0.56±0.05	0.52±0.03	0.58±0.01	0.56±0.05	0.60±0.06	.29
Protein efficiency rate (%)	1.41±0.09	1.44±0.14	1.56±0.04	1.54±0.14	1.61±0.16	.18
Daily intake (% of live weight)	7.08±0.49	7.48±0.50	7.82±0.27	7.51±0.41	7.72±0.38	.23
Carcass yield (%)	85.99±2.04	85.55±3.09	84.81±1.81	85.65±2.81	85.67±2.77	.84
Feeding cost (US$ kg of weight gain^{-1})	1.03±0.10[a]	1.03±0.15[a]	1.01±0.03[b]	1.09±0.09[a]	1.05±0.12[a]	.01
Indexes digestives						
Hepatosomatic index (%)	1.34±0.22	1.50±0.32	1.42±0.30	1.60±0.29	1.50±0.27	.24
Celomic fat index (%)	3.38±1.75	3.08±1.57	2.87±1.22	3.58±1.64	3.19±1.74	.84
Digestive somatic index (%)	3.43±0.65	3.58±0.65	3.45±0.85	3.44±0.55	3.65±0.61	.89
Intestinal quotient (%)	0.98±0.20	0.92±0.18	1.02±0.14	0.87±0.15	0.92±0.12	.20

Values are expressed as the mean ± standard deviation. Means with different letters on the line differ significantly according to Tukey's test ($p < .05$)

[a] Diets: 38CP, 38% crude protein (CP); 34CP, 34% CP; 34L, 34% CP with lysine; 34M, 34% CP with methionine; 34LM, 34% CP with lysine plus methionine

Table 4

Body and fillet composition and nutrient deposition of juvenile silver catfish fed with reduced protein level and supplementation of lysine and methionine in the diet for 63 days (% dry matter)

Variable	Initial	Diet[a]					*p*-value
		38CP	34CP	34L	34M	34LM	
Whole fish							
Dry matter (%)	25.55±0.85	29.27±0.92^{ab}	28.88±1.65^{ab}	28.90±0.71^{ab}	31.11±0.87^{a}	28.58±1.26^{b}	.04
Mineral matter (%)	5.68±0.90	2.47±0.92^{b}	2.43±0.31^{b}	3.20±0.22^{ab}	3.77±0.29^{a}	3.98±0.64^{a}	.0006
Crude protein (%)	15.64±0.85	14.16±1.14^{ab}	15.75±0.62^{a}	14.45±0.65^{ab}	13.28±0.20^{b}	14.49±0.71^{ab}	.006
Lipids (%)	7.09±0.65	11.07±0.44^{a}	7.69±0.32^{b}	10.58±0.12^{a}	11.46±0.45^{a}	11.13±0.80^{a}	.0001
Protein deposition (g)		2.75±0.64^{b}	3.99±0.54^{ab}	3.45±0.28^{ab}	3.10±0.48^{ab}	4.11±0.52^{a}	.03
Lipid deposition (g)		3.82±0.71^{a}	2.17±0.39^{b}	3.70±0.12^{a}	4.48±0.54^{a}	4.30±0.31^{a}	.0002
Fillet							
Dry matter (%)	21.25±0.14	23.84±0.32^{ab}	23.34±0.12^{bc}	23.09±0.25^{c}	23.18±0.18^{c}	24.32±0.40^{a}	.0001
Mineral matter (%)	5.35±0.99	1.56±0.18	1.36±0.20	1.56±0.06	1.63±0.15	1.60±0.15	.25
Crude protein (%)	18.45±0.95	16.58±0.92	16.82±0.54	16.79±0.10	16.92±0.28	16.52±0.26	.81
Lipids (%)	4.42±0.33	2.79±0.16^{bc}	2.71±0.35^{bc}	2.28±0.30^{c}	3.23±0.15^{b}	4.40±0.20^{a}	.0001
Protein deposition (g)		2.97±0.51	3.68±0.74	3.80±0.20	3.92±0.75	3.84±0.83	.33
Lipid deposition (g)		0.22±0.04^{bc}	0.13±0.04^{bc}	-0.07±0.01^{c}	0.51±0.22^{b}	1.24±0.28^{a}	<.0001

Values are expressed as the mean ± standard deviation. Means with different letters on the line differ significantly according to Tukey's test ($p < .05$)

[a] Diets: 38CP, 38% crude protein (CP); 34CP, 34% CP; 34L, 34% CP with lysine; 34M, 34% CP with methionine; 34LM, 34% CP with lysine plus methionine

Table 5

Erythrocyte parameters of silver catfish juveniles fed with reduced protein level and supplementation of lysine and methionine in the diet for 63 days

Variable	Diet[a]					*p*-value
	38CP	34CP	34L	34M	34LM	
Erythrocyte (10^6 µL^{-1})	1.26±0.64[b]	1.32±0.64[b]	3.45±2.38[a]	1.17±0.82[b]	1.42±0.80[b]	.003
Hemoglobin (g dL^{-1})	6.56±1.07	6.79±1.07	5.56±2.54	6.22±2.96	6.39±0.58	.77
Hematocrit (%)	19.88±2.78	18.62±2.77	17.62±4.24	17.12±7.90	19.25±2.71	.75
MCH (pg)[b]	45.05±11.13[a]	45.86±11.13[a]	16.40±7.21[b]	49.47±17.80[a]	42.56±10.33[a]	.0002
MCV (fL)[c]	135.87±30.62[a]	130.19±30.62[a]	48.96±18.74[b]	133.60±42.41[a]	128.68±31.31[a]	<.0001
MCHC (g dL^{-1})[d]	32.74±5.71	36.81±5.71	34.01±4.57	38.24±6.15	33.59±4.22	.20

Values are expressed as the mean ± standard deviation. Means with different letters on the line differ significantly according to Tukey's test ($p < .05$)

[a] Diets: 38CP, 38% crude protein (CP); 34CP, 34% CP; 34L, 34% CP with lysine; 34M, 34% CP with methionine; 34LM, 34% CP with lysine plus methionine

[b] Mean corpuscular hemoglobin

[c] Mean cell volume

[d] Mean corpuscular hemoglobin concentration

Table 6

Biochemical parameters of silver catfish juveniles fed with reduced protein level and supplementation of lysine and methionine in the diet for 63 days

Variable	Diet[a]					*p*-value
	38CP	34CP	34L	34M	34LM	
Plasma						
Glucose (mg dL^{-1})	15.06±3.76^{ab}	14.55±3.33^{ab}	13.08±3.11^{b}	20.66±6.54^{a}	19.82± 4.91^{a}	.007
Total proteins (g dL^{-1})	3.21±0.15^{bc}	2.89±0.43^{c}	2.98±0.39^{c}	3.99±0.38^{a}	3.63±0.32^{ab}	<.0001
AA (µmol mL^{-1})	30.38±2.15^{ab}	24.49±2.90^{bc}	20.98±2.99^{c}	30.04±5.49^{ab}	34.40±2.87^{a}	<.0001
Ammonia (µmol L^{-1})	40.82±10.34	33.10±3.05	33.40±9.55	29.04±12.35	24.74±4.29	.22
Liver						
Total proteins (mg g^{-1})	11.36±0.92	12.21±1.34	11.18±1.40	12.30±1.68	11.15±1.59	.35
AA (µmol g^{-1})	33.57±7.40	35.69±7.21	34.67±7.96	34.64±7.90	34.65±5.59	.99
Glycogen (mg g^{-1})	1.88±0.52	1.99±0.49	2.74±0.93	2.55±0.71	2.62±1.19	.40
TBARS (µmol malondialdehyde g^{-1})[b]	6.39±1.11^{a}	3.81±0.99^{bc}	2.94±0.72^{bc}	4.92±1.72^{ab}	2.22±0.61^{c}	.0006
Muscle						
Total proteins (mg g^{-1})	11.42±1.52^{ab}	11.10±1.13^{ab}	13.16±3.10^{a}	9.52±2.00^{b}	10.87±1.43^{ab}	.02
AA (µmol g^{-1})	28.42±9.44	26.79±6.30	21.41±7.26	22.05±4.95	21.12±4.43	.18
TBARS (µmol malondialdehyde g^{-1})[b]	0.010±0.015	0.002±0.004	0.001±0.003	0.005±0.007	0.014±0.012	.12

Values are expressed as the mean ± standard deviation. Means with different letters on the line differ significantly according to Tukey's test ($p < .05$)

[a] Diets: 38CP, 38% crude protein (CP); 34CP, 34% CP; 34L, 34% CP with lysine; 34M, 34% CP with methionine; 34LM, 34% CP with lysine plus methionine

[b] Thiobarbituric acid reactive substances

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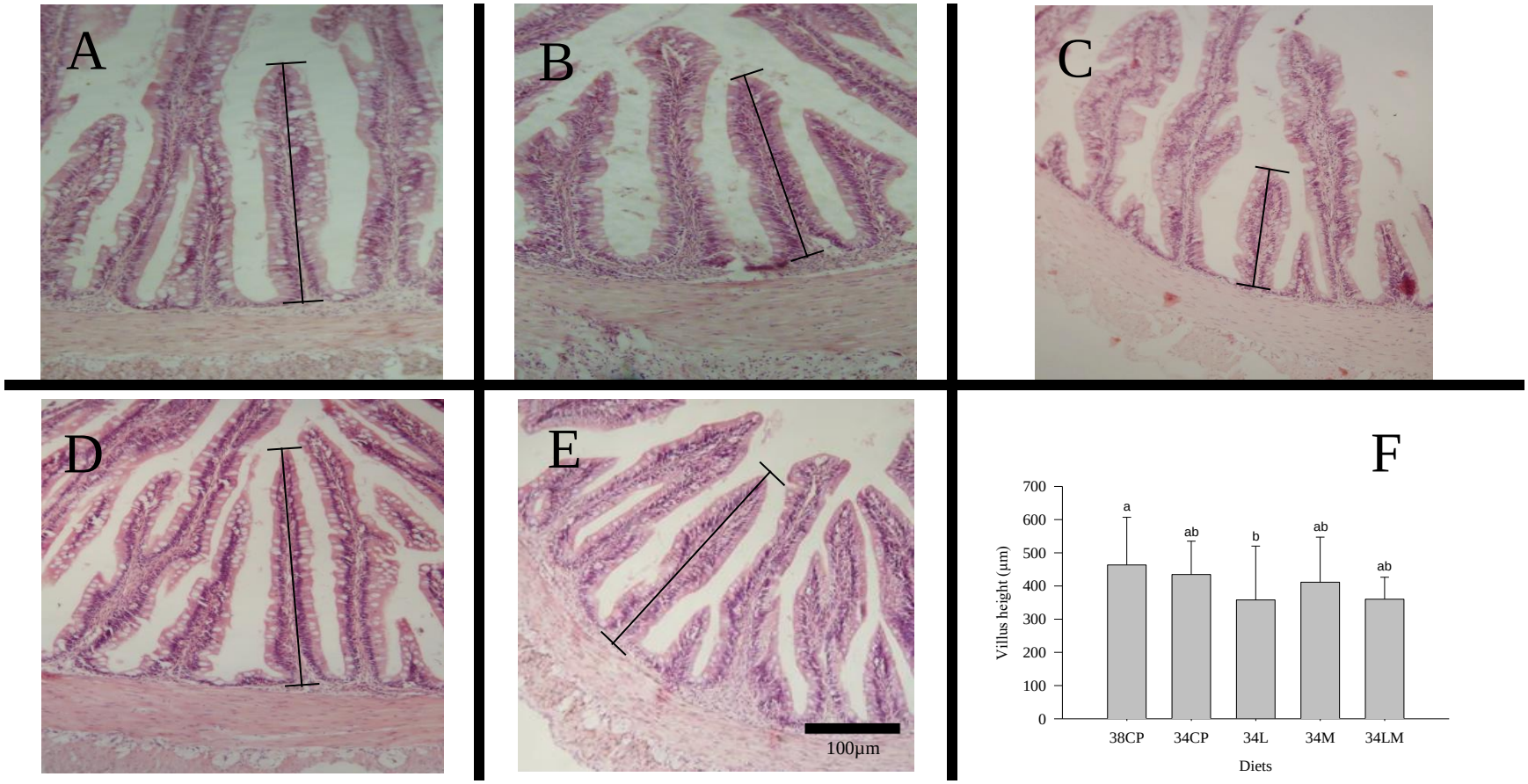

619 Figure 1. Sections of the intestine stained with hematoxylin-eosin (A to E) of juveniles of silver catfish that received diets with reduced protein level and supplementation of
620 lysine and methionine for 63 days. Scale bars = 100 µm. Diets: A: 38CP, 38% crude protein (CP); B: 34CP, 34% CP; C: 34L, 34% CP with lysine; D: 34M, 34% CP with
621 methionine; E: 34LM, 34% CP with lysine plus methionine. Figure F: Mean height of intestinal villi. *Note*: Means with different letters in the column differ significantly according
622 to Tukey's test ($p < .05$)

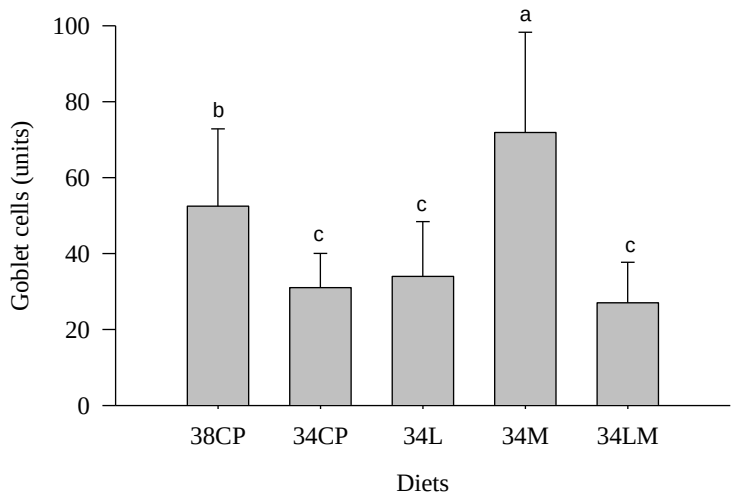

Figure 2. Goblet cell numbers (unit/villus) of the anterior intestinal portion of juvenile silver catfish that received diets with reduced protein level and supplemented with lysine and methionine for 63 days. Diets: 38CP, 38% crude protein (CP); 34CP, 34% CP; 34L, 34% CP with lysine; 34M, 34% CP with methionine; 34LM, 34% CP with lysine plus methionine. *Note*: Means with different letters in the column differ significantly according to Tukey's test ($p < .05$)

5 CONCLUSÕES GERAIS

- A redução do nível de proteína em 4% na dieta diminui o crescimento de juvenis de jundiás (*Rhamdia quelen*).
- A suplementação com lisina e metionina na dieta aumenta em média 18,64% o peso final dos jundiás.
- Menor teor lipídico no filé de peixes alimentados com dieta suplementada com lisina.
- Melhora da capacidade antioxidante no fígado dos peixes alimentados com dieta suplementada com lisina e metionina.
- A adição de metionina na dieta melhorou o estado de saúde do trato gastrointestinal de *R. quelen.*
- É possível a redução do nível de proteína desde que a dieta seja suplementada com lisina e metionina.

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APÊNDICE A – COMPOSIÇÃO CENTESIMAL DOS INGREDIENTES (% MATÉRIA NATURAL)

Ingrediente (%)	Matéria seca*	Matéria mineral*	Proteína bruta*	Lipídeos**
Farinha de carne e ossos[$]	93,26	30,71	47,14	13,68
Farelo de soja	85,43	6,08	43,44	2,71
Farinha de trigo	85,75	3,96	15,30	3,07
Milho	85,55	0,93	7,97	4,75

*AOAC (1995).
**Bligh; Dier (1959).
[$]Bovina

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 "Redução de proteína e suplementação com lisina e metionina em dietas para jundiás (Rhamdia quelen)", protocolada sob o CEUA nº 7315131119 (ID 002895), sob a responsabilidade de **Rafael Lazzari** *e equipe; Mauro Alves da Cunha; Emerson Giuliani Durigon; Nilce Coelho Peixoto; Thamara Luisa Staudt Schneider; Abner Pedroso Fusatto; Karine Francesquetto Costa* - 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 24/03/2020.

We certify that the proposal "Protein reduction and lysine and methionine supplementation in diets for jundiás (Rhamdia quelen)", utilizing 320 Fishes (males and females), protocol number CEUA 7315131119 (ID 002895), under the responsibility of **Rafael Lazzari** *and team; Mauro Alves da Cunha; Emerson Giuliani Durigon; Nilce Coelho Peixoto; Thamara Luisa Staudt Schneider; Abner Pedroso Fusatto; Karine Francesquetto Costa* - 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 03/24/2020.

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ANEXO B- NORMAS DA AQUACULTURE RESEARCH

PREPARING THE SUBMISSION

Main Text File

Line numbering should be included, with numbering to continue from the first line to the end of the text (reference list). Line numbers should be continuous throughout the manuscript and not start again on each page. The text file should be presented in the following order:

i. A short informative title containing the major key words. The title should not contain abbreviations (see Wiley's best practice SEO tips);
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v. Abstract and keywords;
vi. Main text;
vii. Introduction
viii. Materials and Methods (Including Ethics Statement)
ix. Results
x. Discussion
xi. Acknowledgements
xii. Data Availability Statement
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Acknowledgements

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Data Availability Statement

Authors are required to provide a data availability statement to describe the availability or the absence of shared data. When data have been shared, authors are required to include in their data availability statement a link to the repository they have used, and to cite the data they have shared.

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Authors will be asked to provide a conflict of interest statement during the submission process. For details on what to include in this section, see the 'Conflict of Interest' section in the **Editorial Policies and Ethical Considerations** section below. Submitting authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

Abstract

Please provide an abstract of no more than 200 words containing the major keywords.

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References should be prepared according to the Publication Manual of the American Psychological Association (6th edition). This means in text citations should follow the author-date method whereby the author's last name and the year of publication for the source should appear in the text, for example, (Jones, 1998). The use of et al is determined by the number of authors and whether it is the first time a reference has been cited in the paper:

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  Beers, S. R. , & De Bellis, M. D. (2002). Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. The American Journal of Psychiatry, 159, 483–486. doi:10.1176/appi.ajp.159.3.483

- **Book**

  Bradley-Johnson, S. (1994). Psychoeducational assessment of students who are visually impaired or blind: Infancy through high school (2nd ed.). Austin, TX: Pro-ed.

- **Chapter in an Edited Book**

  Borstrøm, I., & Elbro, C. (1997). Prevention of dyslexia in kindergarten: Effects of phoneme awareness training with children of dyslexic parents. In C. Hulme & M. Snowling (Eds.), Dyslexia: Biology, cognition and intervention (pp. 235–253). London: Whurr.

- **Internet Document**

  Norton, R. (2006, November 4). How to train a cat to operate a light switch [Video file]. Retrieved from http://www.youtube.com/watch?v=Vja83KLQXZs

Tables

Tables should be self-contained and complement, not duplicate, information contained in the text. They should be supplied as editable files, not pasted as images. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text. All abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings.

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