UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CENTRO DE AQUICULTURA DA UNESP

NÍVEIS DE LISINA EM DIETAS PARA REPRODUTORES DE *Rhamdia voulezi* EM TANQUES-REDE

Odair Diemer

Jaboticabal – São Paulo
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Orientadora: Profa. Dra. Elizabeth Romagosa

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DEDICATÓRIA

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1. RESUMO

Objetivou-se avaliar os níveis de lisina em dietas para reprodutores de *Rhamdia voulezi* em tanques-rede por meio do desempenho produtivo, reprodutivo, características hormonais, índices corpóreos e incorporação de aminoácidos. Foram utilizados 400 peixes com peso médio inicial de 35,18±0,25g, distribuídos em um delineamento casualizado com quatro tratamentos e quatro repetições, em 16 tanques-rede com 25 peixes. Os tratamentos foram constituídos por quatro rações elaboradas de modo a conter níveis de lisina total de 1,20; 1,40; 1,60 e 1,80% com 30% de proteína bruta e 3.500 kcal kg^{-1} de energia digestível. Depois de alimentados por 185 dias foram selecionados aleatoriamente 18 machos que liberavam sêmen sob leve compressão da região abdominal e 18 fêmeas que apresentavam abdômen arredondado, papila urogenital intumescida e avermelhada e conduzidos para o laboratório onde foram pesados, medidos, submetidos à hipofisação (extrato bruto de hipófise de carpa - EHC, na dosagem de 2,5 $mg.kg^{-1}$ para os machos e 5,5 $mg.kg^{-1}$ para fêmeas). Após 240 unidades térmicas acumuladas o sêmen foi coletado para avaliar os seguintes parâmetros: volume seminal, tempo de duração e taxa de motilidade espermática, índice de sobrevivência espermática, concentração espermática, porcentagem de espermatozóides normais, pH e osmolaridade. Ao mesmo tempo os ovócitos foram coletados e divididos em parcelas: solução de Gilson (diâmetro dos ovócitos), congeladas (análise de aminoácidos) e o restante fertilizado com o semen pré-analisado e dispostos em incubadoras cônicas (20L) para aferição dos parâmetros reprodutivos. Em seguida, o sangue foi coletado (análise de cortisol, estradiol e testosterona), posteriormente, sacrificados por deslocamento da coluna cervical, dissecados, onde os ovários, testículos, fígado, gordura e vísceras foram retirados, pesados e estimados os respectivos índices corpóreos. As carcaças foram secas para análise dos aminoácidos. Nos machos foi verificado efeito ($p<0,05$) para as médias de peso final, ganho em peso, conversão alimentar aparente e fator de condição. Com relação aos parâmetros reprodutivos, apenas o volume seminal foi afetado ($p<0,05$). Os níveis de testosterona mostraram efeito quadrático ($p<0,05$). A anatomia e a morfologia dos testículos foram semelhantes entre os tratamentos. No que diz respeito aos índices corpóreos e a composição de aminoácidos na carcaça não apresentaram diferenças ($p>0,05$) entre os tratamentos. Em relação às fêmeas houve efeito ($p<0,05$) sobre as médias de peso e comprimento final, ganho em peso, conversão alimentar aparente e fator de condição. As distribuições de frequência de diâmetro dos ovócitos apresentaram padrões semelhantes nos quatro tratamentos, com tendência polimodal. No entanto, no momento da desova, as configurações foram semelhantes para as dietas com níveis de 1,20, 1,60 e 1,80% de lisina. Apenas as fêmeas do tratamento com 1,40% de lisina proporcionaram três modas com diâmetros inferiores a 520; 590 e 790 µm, com a presença de brancos (opacas), irregulares e sangrentas. Os ovócitos liberados, a fecundidade absoluta e ovários remanescentes foram influenciados ($p<0,05$) pela dieta, e os maiores valores médios foram observados no tratamento com 1,80% de lisina. O cortisol e o estradiol não foram afetados ($p>0,05$). Entre os índices corpóreos, apenas a gordura visceral foi influenciada ($p<0,05$). A composição de aminoácidos nos ovócitos não foi ($p>0,05$) influenciada pela dieta, mas, na carcaça as composições de ácido aspártico, ácido glutâmico, serina, alanina, arginina, treonina, valina, metionina e fenilalanina foram afetadas ($p<0,05$). Sumarizando, nos machos o incremento de lisina na dieta proporcionou aumento

linear para o ganho em peso e volume seminal e redução linear para conversão alimentar. Nas fêmeas o nível de 1,80% de lisina promoveu um maior crescimento e, consequentemente, maior quantidade de ovócitos produzidos em relação aos demais tratamentos.

Palavras-chave: Nutrição de peixes, peixes nativos, reprodução induzida, tanques-rede.

2. ABSTRACT

The aim was to evaluate the levels of lysine in diets to breeding of *Rhamdia voulezi* in cages evaluating the productive, reproductive performance, hormonal characteristics, organosomatic indices and amino acid incorporation in oocytes and in the carcass in. Four hundred fish were used with an average initial weight of 35.18 ± 0.25g, distributed in a randomized design with four treatments and four replications in 16 cages with 25 fish each. The treatments consisted of four diets designed to contain levels of total lysine of 1.20; 1.40; 1.60 and 1.80% with 30% crude protein and 3,500 kcal kg^{-1} digestible energy. After fed for 185 days it was randomly selected 18 males that liberated semen under mild compression of the abdomen and 18 females who had rounded abdomen, swollen and reddish urogenital papilla, and taken to the laboratory where they were weighed, measured, submitted to hypophysation (with crude carp pituitary extract (EHC)) at a dose of 2.5 $mg.kg^{-1}$ in males and 5.5 $mg.kg^{-1}$ for females. After 240 thermal units accumulated, the semen was collected for fertilization and evaluation of the following parameters: semen volume, duration and rate of sperm motility, sperm survival index, sperm concentration, percentage of normal sperm, pH and osmolarity. At the same time, the oocyties was colellected and divided into portions: Gilson solution (oocyte diameter), frozen (amino acid analysis) and the remainder in fertilized with pre-analyzed semen and willing conical incubators (20L) for measurement of reproductive parameters. Then, the blood was collected (analysis of cortisol, estradiol and testosterone), after it they were sacrificed by dislocation of the cervical spine, dissected, where the ovaries, testicles, liver, fat and viscera were removed, weighed and estimated the respective organosomatic indices. Carcasses were dried for analysis of amino acids. In males was verified effect ($p<0.05$) for the final weight, weight gain, feed conversion ratio and condition factor. With regard to reproductive parameters, only the seminal volume was affected ($p<0.05$). Testosterone levels showed quadratic effect ($P<0.05$). The anatomy and histomorphology of testis were similar between treatments. In regard to organosomatic index and the amino acid composition in the carcass, showed no differences ($p>0.05$) between treatments. For females there was effect ($p<0.05$) on the average final weight and length, weight gain, feed conversion ratio and condition factor. The frequency distributions of oocyte diameter showed similar patterns in all four treatments, with polymodal trend. However, at the time of spawning, settings were similar for diets with levels of 1.20, 1.60 and 1.80% of lysine. Only females treatment with 1.40% lysine provided three modes, with diameters less than 520; 590 and 790 μm, with the presence of white (opaque), irregular and bloody. Oocytes released, absolute fecundity and remaining ovaries were influenced ($p<0.05$) by the diet, and the higher values were observed in the treatment with 1.80% lysine. The cortisol and estradiol were not affected ($p>0.05$).

Among the organosomatic indices, only visceral fat was influenced ($p<0.05$). The amino acid composition in oocytes was not ($p>0.05$) affected by the diet, but, in the carcass compositions aspartic acid, glutamic acid, serine, alanine, arginine, threonine, valine, phenylalanine and methionine were affected ($p<0.05$). Summarizing, in males the increase of lysine in the diet caused a linear gain to the weight and semen volume and a linear decrease of feed convertion. In females the level of 1.80% lysine promoted greater growth and consequently higher amount of oocytes produced in relation to the other treatments.

Keywords: Fish nutrition, native fish, induced breeding, net cages.

3. INTRODUÇÃO GERAL

Rhamdia voulezi é uma espécie vulgarmente conhecida como jundiá, endêmica do rio Iguaçu, Paraná, Brasil, pertence à classe Osteichtyes, ordem Siluriformes, e família Heptapteridae (Gomes et al., 2000). Particularidades morfológicas e genéticas indicam que seja considerada sinônima do *Rhamdia quelen* (Mise et al., 2013). Estudos mencionam qualidades positivas para a espécie ser utilizada na piscicultura como, rusticidade, docilidade, crescimento acelerado (Fracalossi et al., 2004), fácil reprodução (Sividanes et al., 2012), boa eficiência alimentar (Freitas et al., 2011), características organolépticas desejáveis, boa apreciação pelo mercado consumidor e propriedades propícias para o processamento (Manske et al., 2011). Além disso, tem sido cultivada em tanques-rede (Reis et al., 2012).

A potencialidade do Brasil para a piscicultura é imensa, pelas condições naturais, diversidade de espécies, clima favorável, e por ter aproximadamente, 13% da água doce renovável do planeta, destacando-se a possibilidade de utilização das águas da União, principalmente as de reservatórios de hidrelétricas para a criação de peixes em tanques-rede (Rocha et al., 2013). Esse sistema de produção permite o aumento do número de peixes estocados, aprimora o uso de alimentos artificiais, melhora a eficiência alimentar, facilita o acompanhamento do crescimento e da sobrevivência, além de facilitar, a captura para a utilização na reprodução induzida (Signor et al., 2010). Contudo, uma das principais dificuldades que retarda o avanço da piscicultura em tanques-rede é a falta de larvas em grande escala e de boa qualidade (Santinón et al., 2010) .

Todavia, os problemas existentes na reprodução (desova) e fertilização de peixes de piracema têm mostrado escassas informações na literatura, principalmente quando as matrizes são estocadas em tanques-rede (Godinho, 2007). Contudo, sabe-se que peixes reofílicos não se reproduzem naturalmente, em condições de cativeiro, pois, as fêmeas não atingem a maturação ovocitária final em virtude da influência de fatores externos ou condições inadequadas de manejo (Romagosa, 2010). Nesse contexto, Signor et al. (2013) relataram que exemplares de *Rhamdia voulezi* cultivado em tanques-rede devem ser induzidos artificialmente, assim como aqueles confinados em viveiros escavados. Bittencourt et al. (2012) tiveram êxito na reprodução de *Piaractus mesopotamicus* mantidas em tanques-rede. No entanto, Hainfellner et al. (2012) descreveram que

o processo de maturação ovariana é comprometido nas fêmeas de *Prochilodus lineatus* mantidas em tanques-rede com redução significativa no volume dos ovócitos vitelogênicos.

Apesar da reprodução induzida do *Rhamdia voulezi* ser relativamente fácil, há ocorrência de grande mortalidade após a fertilização, sendo um fator que limita sua criação (Signor et al., 2013). Investimentos em pesquisa, desenvolvimento e inovação são fundamentais para o desenvolvimento produtivo e reprodutivo de espécies nativas como o jundiá, especialmente para disponibilizar e comercializar sistematicamente o número de alevinos (Rocha et al., 2013). Segundo Murgas et al. (2009) a utilização das técnicas de propagação artificial, aliado ao estudo das exigências nutricionais dos reprodutores, podem otimizar a produção e, também, garantir a sobrevivência da prole. Uma adequada nutrição de reprodutores possibilita uma série de benefícios, destacando-se, o desenvolvimento gonadal e a desova, o aumento das taxas de fertilização e eclosão, bem como, a qualidade seminal (Navarro et al., 2010).

Sabe-se que para o desenvolvimento de embriões de peixes, é necessário dietas com nutrientes de qualidade. Para tal, a proteína é um dos nutrientes de grande relevância, uma vez que, são os constituintes do organismo animal em todas as fases de desenvolvimento e, estão presentes nos ovos de peixes na forma de lipoproteínas, hormônios e enzimas, definindo sua qualidade e, consequentemente, a produção de alevinos em grande escala (Parra et al., 2010). Contudo, dietas elaboradas com base na proteína bruta podem ocasionar grandes prejuízos no desempenho dos peixes, principalmente, quando há um desbalanceamento de aminoácidos (Furuya, 2010). O desequilíbrio aminoácidico ou o excesso de proteína é catabolizado e excretado ocasionando o aumento na excreção de nitrogênio envolvendo maior custo metabólico incorporando o aminoácido na cadeia proteica, diminuindo o desenvolvimento dos animais (Atencio et al., 2004). Assim, deve-se formular dietas para peixes com aminoácidos na proporção ideal, de modo que, não existam deficiências ou excessos.

Nos estudos de exigências nutricionais utilizando dietas práticas, a lisina tem sido descrita como um aminoácido limitante para o crescimento dos peixes (Furuya et al., 2013). Do mesmo modo, Takishita et al. (2009) relataram que a lisina é considerado o aminoácido de referência em formulações utilizando o

conceito de proteína ideal, pelo fato de que a relação entre a lisina e os outros aminoácidos essenciais permanecem, em grande parte, inalterada. Além disso, sabe-se que a lisina é um aminoácido essencial cujo principal papel fisiológico consiste na síntese de proteínas musculares, estando envolvida em menores proporções em outros processos metabólicos (Finn e Fyhn, 2010). Adicionalmente, Khan e Abidi (2011) relataram que as análises para determinação dos níveis de lisina nos alimentos são mais simples em comparação aos aminoácidos sulfurosos e ao triptofano.

De acordo com Montes-Girao e Fracalossi (2006) a exigência em lisina para alevinos de *Rhamdia quelen* é de 4,5% da proteína. Todavia, ainda são desconhecidos os efeitos da lisina para reprodutores de jundiás. Contudo, há relatos de Barbosa et al. (1999) mostraram que o efeito da suplementação de lisina na dieta de peixes melhora a eficiência de sua utilização na produção de ovos. Prado (2002) afirma que a lisina é importante para se obter melhor qualidade seminal.

Neste aspecto, estudos envolvendo a avaliação do efeito da lisina no desempenho produtivo e reprodutivo do *Rhamdia voulezi* em tanques-rede, tornam-se necessários para gerar informações que visam melhorar a produção de gametas e, consequentemente, de larvas dessa espécie.

4. REVISÃO DE LITERATURA

4.1. Espécie estudada: *Rhamdia voulezi*

A maior e mais diversificada ictiofauna do planeta, encontra-se no Brasil, principalmente, devido ao grande número de rios existentes, Hoje possuímos 2.122 espécies catalogadas, próximo de 21% das espécies do mundo, porém, há espécies ainda não identificadas (Agostinho et al., 2005). Nesse cenário, o rio Iguaçu no estado do Paraná, Brasil, apresenta uma ictiofauna com várias espécies de peixes endêmicos, característica causada principalmente, pelo isolamento proporcionado pelas cataratas do Iguaçu, pelo grande número de barramentos construídos, associado à compartimentalização geológica da bacia (Oliveira et al., 2008).

No que se refere à ictiofauna do rio Iguaçu, são conhecidas 79 espécies de peixes, distribuídas em 16 famílias e seis ordens. No entanto, este valor deve ser subestimado, pois ainda é insuficiente o número de levantamentos em algumas áreas e falta concordância acerca do status taxonômico de algumas espécies catalogadas (Abilhoa, 2004). Recentemente, Baumgartner et al. (2012) descreveram no baixo rio Iguaçu, 106 espécies de peixes. No local onde foi conduzido o estudo (área aquícola do reservatório de Salto Caxias, rio Iguaçu/PR), Campagnolo (2012) registraram 31 espécies, distribuídas em quatro ordens e 11 famílias.

Em meio às espécies endêmicas do rio Iguaçu, destaca-se o *Rhamdia voulezi*, popularmente chamado de jundiá, congênere do *Rhamdia quelen*, pertence à classe Osteichtyes, ordem Siluriformes, e família Heptapteridae que é considerada a família de bagres neotropicais mais diversificada (Gomes et al., 2000). É uma espécie de hábito alimentar onívoro que em ambiente natural se alimenta preferencialmente de peixes, crustáceos e insetos (Cassemiro et al., 2005); vive em ambientes lênticos, são provavelmente pelágicos (Mise et al., 2013); sua reprodução é anual com desova assincrônica (Signor, et al., 2013); a eclosão das larvas ocorre após 492,75 °C horas-grau (Sividanes et al., 2012).

De acordo com a revisão de Silvefvergrip (1996) muitas espécies do gênero *Rhamdia*, incluindo o *Rhamdia voulezi*, podem ser catalogadas como sendo sinônimas do *Rhamdia quelen*, que tem ampla distribuição geográfica desde o sudeste do México ao norte até o centro da Argentina ao sul, e as poucas diferenças morfológicas existentes estão relacionadas a diferente ocupação geográfica, algumas espécies são restritas à pequenos locais, proporcionando dessa forma, particularidades do individuo. Todavia, estes relatos são contraditórios de acordo com Abucarma e Martins-Santos (2001); Anza, (2006); Mise et al. (2013).

Em uma revisão sobre a biologia do *Rhamdia quelen* Gomes et al. (2000), enfatizaram aspectos morfológicos, como, peixe de couro, corpo alongado e crânio achatado, boca grande sem a presença de dentes com três pares de barbilhões sensitivos, comprimento vai desde a inserção das nadadeiras peitorais até a nadadeira caudal e a cor varia de marrom avermelhado claro para cinza escuro. Segundo os autores, pode atingir 50 cm de comprimento e 3 kg de peso, possui hábito noturno e habita locais calmos e profundos dos rios, é uma espécie

euritérmica, maturidade sexual inicia no primeiro ano de vida com dois picos reprodutivos por ano e desova múltipla sem cuidado parental.

Os jundiás de forma geral, tem atraído atenção de produtores, devido a características favoráveis para a piscicultura comercial (Diemer et al., 2012). Nesse contexto, Fracalossi et al. (2004), relataram que o *Rhamdia quelen* é rustico para o manejo, apresenta rápido crescimento mesmo em meses mais frios do ano e tolera baixos níveis de oxigênio na água. Em relação à reprodução, fêmeas apresentaram altas taxas de fecundação, quando mantidas com nutrição adequada e uma relação espermatozóide.ovócito^{-1} de 89.497 proporcionando taxas de fertilização de 86,68% (Bombardelli et al., 2006).

4.2. Criação de Peixes em Tanques-rede

Em razão da crescente demanda mundial de pescado e estagnação da produção extrativa, a piscicultura é uma das principais alternativas para a produção de alimento com alto valor proteico para consumo humano (Santos e Mattos, 2009). Nesse cenário, o Brasil pode se tornar um dos maiores produtores mundiais de peixes, por possuir seis milhões de hectares de águas represadas em açudes de grandes reservatórios, construídos com a finalidade de produção de energia elétrica e passíveis de criação comercial de peixes em tanques-rede, fomentado essa atividade (Marengoni, 2006).

Tanques-rede são estruturas flutuantes delimitadas por redes ou telas, construídos em uma variedade de formas e materiais, tais como ferro, pvc, nylon e outros materiais sintéticos, que permitem a renovação constante da água em seu interior, promovendo o fornecimento da demanda de oxigênio e a remoção de dejetos e metabólitos produzidos, possibilitando a produção de grandes quantidades de peixes por unidade de volume, sendo a qualidade de água um dos fatores decisivos ao crescimento, conversão alimentar e saúde dos peixes (Teixeira et al., 2006).

A criação de peixes em tanques-rede possibilita a otimização do uso de reservatórios aumentando assim a produção pesqueira, mas por outro lado, há o risco do ambiente ficar eutrofizado, quando não houver manejos apropriados (Neu et al., 2014). No entanto, Diemer et al. (2010) relataram que a criação de peixes

em tanques-rede pode ser praticada com sustentabilidade, desde que se adotem cuidados, como o monitoramento continuo da qualidade da água.

Entretanto, Ramos et al. (2009) relataram algumas alterações na qualidade da água, nas comunidades bentônicas, planctônicas e peixes gerados por pisciculturas em tanques-rede. Nesse sentido, Beveridge (2004) descreveu que até 30% da ração utilizada na produção de pescado em tanques-rede não são aproveitados, sendo lançados no meio na forma de matéria orgânica ocasionando danos ao ecossistema aquático local. Loureiro et al. (2011) constataram no reservatório de Itá, rio Uruguai, uma maior biomassa zooplanctônica nas proximidades da criação de peixes em tanques-rede. Menezes e Beiruth (2003) na represa de Guarapiranga, São Paulo, observaram maior abundância da comunidade bentônica em áreas próximas ao cultivo de peixes demonstrando modificações ocorridas no sedimento.

A criação de peixes em tanques-rede apresenta uma série de vantagens sobre o sistema tradicional, especialmente por proporcionar o aproveitamento de ambientes aquáticos já existentes como mar, estuários, lagos, lagoas, rios, bem como, em represas formadas por nascentes, canais de irrigação, grandes reservatórios, desta forma, dispensando o alagamento de novas áreas e reduzindo os custos com a construção de represas e viveiros escavados (Cavero et al., 2003). Além disso, permite a produção em unidades pequenas, colheita rápida e simples, adaptação flexível às demandas do mercado; densidades elevadas; observação direta dos peixes e à intervenção imediata, mecanização de algumas etapas, etc. (Ono e Kubtiza, 1999).

O potencial do Brasil para a criação de peixes em tanques-rede é enorme, principalmente, em áreas onde o pescado apresenta um elevado valor de mercado ou não está disponível, associado às condições climáticas adequadas e à disponibilidade de rações completas e balanceadas para piscicultura intensiva (Zimmermann e Fitzsimmons, 2004). De acordo com os autores, praticamente todo o território nacional oferece condições necessárias para o sucesso dessa atividade, sendo responsável pelo impulso da aquicultura mundial, em meados de 80.

Para ordenar a criação de peixes em tanques-rede, o Governo Federal editou o Decreto n° 4.895 de 25 de novembro de 2003, que, juntamente com a Instrução Normativa Interministerial n° 6 de 31 de maio de 2004, norteou a

demarcação dos primeiros parques e áreas aquícolas. Assim, tiveram início às primeiras implantações de parques aquícolas no Brasil, no reservatório da Usina Hidrelétrica Itaipu Binacional, no Paraná em 2007 e no Açude Castanhão, no Estado do Ceará em 2008 (Brabo et al., 2014). Recentemente, tem despertado o interesse para a produção de peixes em tanques-rede no rio Iguaçu, estado do Paraná, Brasil, por possuir reservatórios de Hidroelétricas instalados em seu leito, como, Salto Caxias, Segredo, Salto Santiago, Salto Ozório (IAP, 2012).

4.3. Reprodução de peixes nativos

A aquicultura pode ser um ótimo investimento agropecuário, no entanto, deve manter um fornecimento contínuo de produtos (larvas, alevinos e juvenis) com boa qualidade e preços competitivos (Teixeira et al., 2006). Segundo Andrade e Yasui (2003) a piscicultura no Brasil, somente se desenvolverá com o domínio das técnicas de reprodução artificial.

Em cativeiro, peixes migradores não se reproduzem naturalmente, sendo necessário realizar a indução hormonal, ou seja, reprodução por meio da aplicação de hormônios, desencadeando a maturação final dos gametas, na qual possibilita a coleta de ovócitos e sêmen e, subsequente, fertilização artificial (Streit Jr et al., 2002). Essa técnica permanece sendo uma das melhores alternativas para reprodução de peixes reofílicos, sendo conhecida como a técnica de hipofisação e, permite conseguir larvas e alevinos em grande escala (Zaniboni-Filho e Weingartner, 2007).

A escolha da matriz para reprodução induzida deve ser realizada com cuidado, na época reprodutiva da espécie, nas fêmeas, observa-se o abdômen abaulado, macio, papila urogenital proeminente e de coloração rosada ou avermelhada e orifício genital ligeiramente aberto, nos machos, ocorre à liberação de sêmen sob leve massagem na cavidade abdominal (Murgas et al., 2012). Em seguida, deve-se conduzi-los para o laboratório de reprodução, onde os peixes são pesados, identificados e acondicionados em caixas de água, separados por sexo para receberem o respectivo tratamento hormonal. Para isso existem alternativas, sendo que a maioria das terapias hormonais são aplicados intramuscular ou intrabdominal, desencadeando estímulos na hipófise ou nas gônadas (Felizardo et al., 2012).

Ainda hoje, a maior parte das pisciculturas brasileiras utiliza a hipófise desidratada de carpa para realizar a indução hormonal, que é aplicada na forma de extrato bruto, diluída em solução fisiológica, que é usada, sobretudo pela facilidade de obtenção do produto, bem como, pela simplicidade desta metodologia (Andrade e Yasui, 2003).

Para garantia do sucesso da reprodução é de extrema importância avaliar a qualidade dos gametas (sêmen e ovócitos) e que os mesmos estejam em perfeitas condições de utilização. Nesse sentido, torna-se necessário fazer análises qualitativas e quantitativas para determinar a qualidade do sêmen e ovócitos, sendo que as principais avaliações seminais são volume, taxa e duração da motilidade, concentração, sobrevivência e normalidade (Felizardo et al., 2010). Em relação aos ovócitos as principais características observadas, são coloração, diâmetro e a posição periférica da vesícula germinativa (Felizardo et al., 2012).

4.4. Nutrição de reprodutores

Watanabe e Vassalo-Agius (2003) relataram que o adequado manejo nutricional de reprodutores tem influencia direta no desempenho reprodutivo afetando principalmente o desenvolvimento inicial das formas jovens, uma vez que a qualidade das proles é estabelecida nas fêmeas (matrizes) devido à produção de vitelogenina hepática e mobilização destas reservas para os ovócitos, que servirão como fontes de nutrientes no período inicial de desenvolvimento. Além disso, a nutrição contribui para o crescimento e maturação gonadal (Navarro et al., 2010), na fecundidade (Tyler e Sumpter, 1996), e na qualidade dos gametas, dos embriões e das larvas (Izquierdo et al., 2001).

Estudos nutricionais de matrizes estão sendo aprimorados (Navarro et al., 2010; Reidel et al., 2010; Bittencourt et al., 2012; Romagosa et al., 2013, Freccia et al., 2014) e novos avanços científicos são fundamentais para colaborar com o desenvolvimento produtivo (Adewumi, 2006). Pesquisas demonstraram os efeitos benéficos da dieta sobre os parâmetros reprodutivos, com destaque para a proteína (Siddiqui et al., 1998; El Sayed et al., 2003; Parra et al., 2010 e Bittencourt et al., 2012) vitaminas E, C e A (Izquierdo et al., 2001; Lee e Dabrowski, 2004 e Navarro et al., 2009) ácidos graxos (Furuita et al., 2000; El

Sayed et al., 2005; Bombardelli et al., 2009 e Tessaro et al., 2012) e minerais (Srivastav, 1998, Pereira et al., 2009 e Pasa, 2010).

Para reprodutores de jundiá, Diemer et al. (2013) avaliaram a suplementação de vitamina B12 na ração do *Rhamdia voulezi* criados em tanques-rede e constataram que a adição de 1,0 $mg.Kg^{-1}$ proporcionou uma melhora no tempo da motilidade espermática. Nesse sentido, Reidel et al. (2010) sugeriram que dietas com 3250 $kcal.kg^{-1}$ de energia digestível e 35% proteína bruta para fêmeas de *Rhamdia quelen* mantidos em tanques-rede é a mais adequada para o desenvolvimento gonadal e ampliação do período de desova. Parra et al. (2010) registraram alteração na morfometria dos ovos e sobrevivência larval, em decorrência de dietas com diferentes fontes proteicas para a mesma espécie.

Um manejo nutricional inadequado pode trazer consequências negativas para o processo reprodutivo devido principalmente ao desbalanceamento de nutrientes, que compromete o sistema endócrino e, ainda, limita componentes bioquímicos responsáveis pela ovulogênese e espermatogênese (Reidel et al., 2010). A deficiência nutricional pode provocar à absorção de ovócitos vitelogênicos, resultando em menor número de ovócitos maduros ou também impedir o início da vitelogênese (Zaniboni-Filho e Nuñer, 2004). Portanto, uma nutrição adequada deve ser empregada, de maneira a possibilitar um enriquecimento na condição dos tecidos ovarianos e testiculares e, consequentemente, trazer benefícios para reprodução (Coldebella et al., 2011).

4.5. Aminoácidos na nutrição de peixes

As proteínas são nutrientes de elevada importância para os peixes, principalmente por que estão relacionadas ao estado de saúde, podendo ser o principal fator da manutenção da qualidade de vida e resistência as diversas situações de desafio a que os animais possam ser submetidos, e o perfil aminoacídico é decisivo para qualidade da proteína (Portz e Furuya, 2013). São encontrados aproximadamente 20 aminoácidos nas proteínas, contudo apenas dez são essenciais aos peixes, dentre eles: arginina, histidina, isoleucina, leucina, metionina, valina, fenilalanina, treonina, lisina e triptofano (Wu, 2009). Para adequada utilização da proteína nas dietas, os aminoácidos devem estar

presentes em quantidades e proporções ideais para, deste modo, maximizar o desenvolvimento dos peixes (Wilson, 2002).

Nos ingredientes utilizados para elaboração das dietas para peixes há uma grande variação na composição dos aminoácidos, alguns alimentos como a gelatina e glúten de milho, por exemplo, são deficientes em um ou mais aminoácidos. Outros, como a farinha de peixes, possuem um balanço aminoacídico mais adequado para atender as exigências dos peixes, sendo que os aminoácidos são requeridos continuamente pelo organismo, tanto para formar novas proteínas (crescimento e reprodução), quanto para repor proteínas que são degradadas no corpo (manutenção de tecidos e órgãos) (Portz, 2001). Mas, com o surgimento da produção de aminoácidos sintéticos, é possível formular rações com maior variedade de ingredientes, menor custo e níveis mais adequados de aminoácidos (Silva et al., 2010).

Exigências em aminoácidos para o crescimento de diferentes espécies de peixes têm sido reportadas, todavia, ainda pouco se conhece sobre exigências deste nutriente para reprodutores de peixes (Navarro et al., 2010). No entanto, Wright e Fyhn (2001) relataram que os aminoácidos tem um importante papel na formação de ovócitos, embriões e larvas. Do mesmo modo, Rodehutscord et al. (1995) observaram que um aumento da oferta dietética de aminoácidos promoveu um maior crescimento em larvas de *Oncorhynchus mykiss.*

Estudos sobre as exigências de aminoácidos são indispensáveis para a formulação de rações ambientalmente e economicamente sustentáveis que promovam adequado desenvolvimento dos peixes (Furuya e Furuya, 2010). Desse modo, pesquisas nesse sentido devem ser aprimoradas para o desenvolvimento de um pacote tecnológico adequado à cadeia produtiva das espécies nativas (Boscolo et al., 2011).

4.6. Lisina na alimentação de peixes

A lisina é geralmente o primeiro aminoácido limitante em dietas para peixes (Abboudi et al., 2006). Na maior parte das espécies de peixes já estudadas os requerimentos de lisina foram semelhantes, variando de 4 a 5% da proteína da dieta (Wilson, 2002). Presente em elevada proporção no tecido muscular, têm como principal função estar envolvida na síntese proteica, além de atuar como

precursora da carnitina, que está relacionada com o transporte de ácidos graxos de cadeia longa, sua suplementação em dietas proporciona melhora no ganho em peso, na conversão alimentar e reduz a gordura abdominal (Buteri et al., 2009).

Segundo Pezzato et al. (2004) fontes de proteína utilizadas em dietas para peixes devem conter níveis adequados de lisina, caso contrário esta deverá ser suplementada, uma vez que, deficiências em lisina prejudicam consideravelmente o crescimento e a sobrevivência dos peixes. A carência de lisina na fase inicial impede a máxima deposição proteica, enquanto o excesso na fase final representa desperdício, gera gasto calórico adicional devido à excreção (Buteri et al., 2009).

Estudos sobre exigências de lisina para um maior ganho em peso de algumas espécies de peixes nativas de água doce brasileiras foram conduzidos, destacando-se: para o *Piaractus mesopotamicus* requerimento de 16,4 $g.kg^{-1}$ (Abimorad et al., 2010); *Salminus brasiliensis* exigência de 5,8% da proteína (Dairiki et al., 2013); *Pseudoplatystoma spp* estimado em 2,75% da dieta (Prado, 2011) e *Astyanax altiparanae* exigência de 5,74% da proteína (Abimorad e Castellani, 2011).

Para o jundiá são escassas as pesquisas relacionadas às estimativas de aminoácidos essenciais, com exceção do estudo realizado por Montes-Girão e Fracalossi, (2006) com o *Rhamdia quelen,* ao qual estimaram uma exigência em lisina de 4,5 a 5,1% da proteína. Além disso, são praticamente inexistentes estudos relacionados aos requerimentos de lisina para alcançar melhor eficiência reprodutiva (Obi et al., 2013). A carência de conhecimento sobre o efeito da lisina na reprodução de peixes nativos tem ocasionado a formulação de dietas desbalanceadas refletindo em um baixo aproveitamento destas e, consequentemente, comprometendo o desempenho produtivo e reprodutivo (Boscolo et al., 2011). Romagosa et al. (2013) relataram que estudos relacionados a avaliação de aminoácidos para reprodutores mantidos em confinamento são de suma importância para a piscicultura.

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6. ARTIGO 1 – Publicado na **Aquaculture**, v. 434, p. 93-99, 2014.

Lysine in the diet of *Rhamdia voulezi* male broodstocks confined in net cages

6.1 - Abstract

The focus of this study was on evaluating the effect of lysine on the productive (weight and length gain, apparent feed conversion and condition factor) and reproductive performance of *Rhamdia voulezi* males confined in net cages during the first reproductive cycle. The following parameters were assessed: seminal parameters (motility rate and duration, survival, sperm concentration, morphology, pH and osmolarity), hormonal parameters (cortisol and testosterone), testicular tissue (histomorphology), organosomatic indices (gonadosomatic, hepatosomatic and viscerosomatic indices) and composition of essential amino acids, crude protein and moisture of whole carcass. Four hundred fish were used, distributed in a random experimental design with four treatments and four replications in 16 net cages with 25 fish each. The treatments consisted of four different diets prepared so as to contain the following levels of lysine: 1.20; 1.40; 1.60 and 1.80%, with 30% crude protein and 3,500 kcal kg-1 digestible energy for 185 days (Jul./12-Jan./13). Eighteen males were selected per treatment, and they all released semen after slight abdominal pressure. The males were weighed, measured, submitted to hypophysation (2.5 mg kg-1 Carp Pituitary Extract), and then had their semen and blood collected. The fish were sacrificed by cervical dislocation, dissected, and the testes, liver, fat and guts were removed and weighed. The effects ($p<0.05$) for the means of final weight, weight gain, apparent feed conversion and condition factor were observed for the analysis of productive performance. With regard to the reproductive parameters, only the seminal volume was affected ($p<0.05$). Thus, the levels of testosterone showed quadratic effect ($p<0.05$). The anatomy and the histomorphology of the testes were similar between the treatments during the spermiation period. With regard to the organosomatic indices, there was no influence ($p>0.05$) between the treatments. The amino acids in the carcass were not affected ($p>0.05$). The increment of lysine in the diet provided linear increase for weight gain and seminal volume and linear decrease for feed conversion in Rhamdia voulezi
broodstocks confined in net cages.

Keywords: *Rhamdia voulezi***,** male broodstock, net cages, lysine, reproductive performances.

6.2 - Introduction

Rhamdia voulezi (Siluriformes) is regarded as economically interesting, endemic to Brazil, found in the region of the lower Iguaçu river, Paraná/BR (Baumgartner et al., 2006). This catfish adapts easily to different environments (Barcellos et al., 2002, 2004) and artificial diets (Reidel et al., 2010; Coldebella et al., 2011; Tessaro et al., 2012), is easy to manage and well accepted commercially (Diemer et al., 2012).

The process of testicular maturation of catfish begins at one year of age (August to March), when males reach 13.4 cm and present early spermiation, and, when in good nutritional status, can achieve high rates of hatching in response to hormonal induction (Barcellos et al., 2002; Reidel et al., 2010; Tessaro et al., 2012). The increasing demand for fingerlings of this species requires the maximization of their production through efficient reproductive management and development of suitable diets that meet the nutritional requirements of broodstock (Coldebella et al., 2011). However, the available information on *R. voulezi* broodstock reared in intensive culture cages remains unsatisfactory (Romagosa et al., 2013).

In general, the nutritional status of the males can influence the testicular development and limit the amount and the quality of the sperm (Navarro et al., 2010; Reidel et al., 2010). Tessaro et al. (2012), studying *R. quelen* males, verified that the use of 30% digestible protein and 2850 kcal DE kg^{-1} in the diet promoted satisfactory reproduction and productivity indices at low costs.

Furthermore, Romagosa et al. (2013) reported that the diets for broodfish must contain all the amino acids that are essential for the protein synthesis; therefore, they are considered one of the most important components through all the stages of development. However, little is known about the effect of these nutrients on the reproductive success in fish (Bittencourt et al., 2012; Navarro et al., 2010; Reidel et al., 2010; Romagosa et al., 2013; Tessaro et al., 2012). The knowledge of each essential amino acid requirement is inevitable, especially the lysine, becauseit acts in high proportions in the muscular tissue of the fish and is the first limiting amino acid (Furuya et al., 2004). Coldebella et al. (2011) showed that for *R. quelen* the relationship between the level of lysine in the diet and the productive and reproductive aspects is very strong, because nutrition has a

significant effect on the growth, quality of ovarian and testicular tissues, ova, semen and fecundity.

Thus, the objective of this study was to analyze the effect of lysine on the productive (weight and length gain, apparent feed conversion, specific growth rate and condition factor) and reproductive performance of *R. voulezi* males confined in net cages during the first reproductive cycle by evaluating the seminal (motility rate and duration, survival, concentration of spermatozoa, morphology, pH and osmolarity) and hormonal (cortisol and testosterone) parameters, testicular tissue (histomorphology), organosomatic indices (gonadosomatic, hepatosomatic and viscerosomatic indices) and composition of essential amino acids, crude protein and moisture of whole carcass.

6.3 - Materials and methods

This study was conducted at the CDT-Iguaçu (Center for the Development of Technologies for Net Cages of the Iguaçu river), located in the town of Boa Vista da Aparecida-Paraná/Brazil (UTM coordinates: 254,036.84 and 7,183,301.91), and received the support from the Study Group on the Management of Aquaculture (GEMAq) of the State University of West Paraná - UNIOESTE, campus in Toledo, Paraná, Brazil. Four hundred R. voulezi (Haseman, 1911) juveniles (initial mean values of total length and weight 15.1 ± 2.2 cm and 35.18 ± 0.25 g, respectively) were kept in 16 net cages with 0.40m^3 of usable volume and 0.5 cm mesh size for 185 days (Jul./12–Jan./13). Four treatments and four replications were established and distributed randomly. The experimental unit consisted of 25 fishes per net cage (25 × 4 replicates=100 fish per treatment). The treatments consisted of four diets prepared with the following levels of total lysine: 1.20; 1.40; 1.60 and 1.80%, with 30% crude protein and 3500 kcal kg−1 digestible energy, according to the requirements set by Reidel et al. (2010) (Table 1). The ingredients were ground in a hammer mill, with 0.5mm mesh size and submitted to extrusion process, and the requirement for each nutrient was calculated by the software SuperCrac® 5.7Master. The values of the analysis of the amino acid and protein composition in the four diets of *R. voulezi* after the experimental feed had been prepared and can be found in Tables 1 and 2. After the experimental feed had been prepared, it was

sent to a commercial laboratory so that the amino acid and protein composition could be determined, measured by means of high performance liquid chromatography.

Table 1. Ingredients and proximate composition of the experimental diets.

Ingredients (%)	Lysine level (%)			
	1.20	**1.40**	**1.60**	**1.80**
Rice meal	35.00	34.90	34.80	34.70
Corn gluten	22.00	21.67	21.33	21.00
Corn	16.06	16.27	16.49	16.70
Fish meal	14.97	14.91	14.86	14.80
Soybean meal	9.15	9.07	8.98	8.90
Soybean oil	1.65	1.73	1.82	1.90
Mineral and vitamin mix[a]	0.50	0.50	0.50	0.50
Salt (NaCl)	0.30	0.30	0.30	0.30
Antifungal	0.20	0.20	0.20	0.20
L-Threonine	0.15	0.16	0.17	0.18
Antioxidant	0.02	0.02	0.02	0.02
L-Lysine	0.00	0.27	0.53	0.80
Total	100.00	100.00	100.00	100.00
Proximate composition				
Digestible energy (kcal kg^{-1})[b]	3500	3500	3500	3500
Crude protein (%)[c]	30.00	30.00	30.00	30.00
Total lysine (%)[c]	1.20	1.40	1.60	1.80
Total phosphorus (%)[d]	0.80	0.80	0.80	0.80
Fat (%)[d]	4.73	4.73	4.73	4.73
Crude fibre (%)[d]	1.38	1.38	1.38	1.38
Starch (%)[d]	38.62	38.62	38.62	38.62
Calcium (%)[d]	0.97	0.97	0.97	0.97

[a] Basic composition: folic acid: 500 mg, pantotenic acid: 4000 mg; biotin: 40 mg; Cu: 2000 mg; Fe: 12,500 mg; I: 200 mg; Mn: 7500 mg; niacin: 5000 mg; Se: 70 mg; vitamin A: 1,000,000 UI; vitamin B1: 1900 mg; vitamin B12: 3500 mg; vitamin B2: 2000 mg; vitamin B6: 2400 mg; vitamin C: 50,000 mg; vitamin D3: 500,000 UI; vitamin E: 20,000 UI; vitamin K3: 500 mg; Zn: 25,000 mg.
[b] Digestible values to *Rhamdia quelen* according to Oliveira Filho and Fracalossi (2006).
[c] HPLC laboratory analysis.
[d] Calculated values

The extruded feed (ration) assigned was weighed daily, and offered twice a day (09 a.m. and 05 p.m.), until the fishes refused it. Food adjustment and the sampling of fish (3 males from each cage per treatment were randomly captured) were carried out monthly. Thus 18 males, that released semen after abdominal massage were selected per treatment, taken to the laboratory located on the riverbank, were individually anesthetized (1 g benzocaine: 150 mL alcohol 96.0 °C: 20 L water) for 4 min, and had their weight (Wt, g) and length (Lt, cm) measured.

At the end of the experiment, the means of final weight, final length, weight gain (difference between final weight and initial weight), apparent feed conversion (relationship between the mean total feed consumption and weight gain) and condition factor (K = [weight × (total length^{-3}) × 100]) of the fishwere determined according to Tessaro et al. (2012).

Table 2. Analysis of protein and amino acid composition of the experimental diets.

Total amino acids (%)	Lysine level (%)			
	1.20	**1.40**	**1.60**	**1.80**
Aspartic Acid	2.44	2.35	2.28	2.46
Glutamic Acid	5.28	5.13	4.95	5.26
Serine	1.52	1.49	1.42	1.49
Glycine	1.84	1.83	1.79	1.93
Histidine	0.65	0.59	0.62	0.63
Arginine	1.82	1.78	1.75	1.84
Threonine	1.14	1.10	1.04	1.15
Alanine	2.18	2.10	2.01	2.14
Proline	2.40	2.40	2.32	2.48
Tyrosine	1.12	1.13	1.13	1.17
Valine	1.40	1.32	1.34	1.42
Methionine	0.60	0.60	0.56	0.58
Cystine	0.35	0.46	0.59	0.48
Isoleucine	1.25	1.16	1.20	1.27
Leucine	3.44	3.34	3.27	3.46
Phenylalanine	1.47	1.42	1.39	1.46
Lysine	1.30	1.40	1.60	1.95
Taurine	0.08	0.07	0.07	0.08
Crude protein (%)	30.47	29.79	29.61	31.40

The selected males (18) were intraperitoneally injected at one single dose of 2.5 mg of CPE kg−1 (CPE = Carp Pituitary Extract) and after a period of 240 ATU (accumulated thermal units), the semen was collected, the first drop was discarded to avoid possible contamination and the remainder was stored (graded Falcon tube 0.1 mL) in order to measure the volume of released semen according to the reproductive protocol described by Bombardelli et al. (2006). The following seminal parameters were analyzed during the collections: i) sperm motility duration and rate according to Sanches et al. (2013); ii) sperm survival rate measured by the method of eosin–nigrosin staining adapted from Blom (1950); iii) sperm concentration by means of sperm cell count in Neubauer

hematimetric chamber (Wirtz and Steinmann, 2006); iv) percentage of normal spermatozoa by the method of rose bengal staining (Sanches et al., 2010); v) pH measured by indicator paper, and vi) osmolarity using the centrifuged plasma analyzed with an Osmometer (Semimicro osmometer K 7400 Knauer) according to Sanches et al. (2013).

After the semen had been collected, these same 18 males from each treatment were anesthetized with Eugenol® solution (60 mg·L−1), according to recommendations described by Diemer et al. (2012) for the with drawal of blood aliquots by caudal puncture (with syringes). After centrifugation at 3000 rpm for 10 min, the plasma was kept in an Ultra Freezer (-45 °C) for later analysis of the levels of cortisol and testosterone, using Interkit immunoassay commercial kits ELISA, according to Barcellos et al. (2002; 2003).

Then these 18 males were sacrificed by cervical dislocation (CFMV, 2008), dissected, had their organs removed (testes, liver, guts and visceral fat) and weighed (g). Fragments of the testes were fixed in buffered formol and processed according to the routine techniques for light microscopy (Romagosa, 2010). The tissues were dehydrated in a series of ethanol, infiltrated and embedded in glycol methacry, and then 2.5 to 3.0 mm sections were cut on a microtome (Sorvall Type JB-4), mounted onto glass slides, stained with Harris hematoxylin and documented with a NIKON Eclipse-50 microscope. Then the organosomatic indices were calculated using the following formulas: gonadosomatic [GSI = (weight of the testes / total weight of the fish) × 100], hepatosomatic [HSI = (weight of the liver / total weight of the fish) × 100], viscerosomatic [VSI = (weight of the guts / total weight of the fish) × 100] and fat [FI = (weight of the visceral fat / total weight of the fish) × 100], according to Tessaro et al. (2012).

The organs (testes, liver, guts and fat) and the whole carcass were removed from six fish per treatment (three samples each), chopped (±1 cm) with a scalpel, homogenized, dried in a drying oven (105 °C), ground, identified and sent to a commercial laboratory for analysis of the composition of amino acids, protein and moisture of whole carcass by means of high performance liquid chromatography.

The quality of the water of the area covered by the net cages was monitored by analyzing the following variables: temperature (22.1 ± 1.9 °C), pH (7.44 ± 0.42), electrical conductivity (26.8 ± 5.1 µS·cm−1) and dissolved oxygen

(7.43 ± 1.49 mg·L−1) measured weekly “in situ” by means of portable potentiometers by Hanna Instruments® and water transparency (3.1 ± 0.5 m) measured by visual disappearance of the Secchi disk.

Data were subjected to analysis of variance (ANOVA) followed by regression, and then the Duncan test was applied at 5% significance (Zar, 2009). Tests for normality and homoscedasticity of the variances were conducted, and the statistical analysis was carried out by the free Software R-2.15.

6.4 - Results and discussion

Significant differences ($p < 0.05$) were observed in *R. voulezi* for the parameters of productive performance (final weight, weight gain, apparent feed conversion and condition factor) (Table 3), seminal volume (Table 4) and testosterone concentration (Fig. 1). The highest level tested in the *R. voulezi's* diet, 1.80% total lysine, was the one that provided the highest values of weight gain, in accordance with Furuya et al. (2006) and Bomfim et al. (2010) for *Oreochromis niloticus*, but at a different rearing stage. In addition, those authors had reported that the increase in the content of lysine in the feed had gradually raised the values of the zootechnical parameters, exactly what happened in the present study. Similarly, Wilson (2002) stated that in fish, there is usually an increase in weight gain associated to a raise in the amino acid intake up to the point of interruption, which corresponds to the amino acid requirement.

The seminal volume of *R. voulezi* was affected ($p < 0.05$) by the diet, exhibiting linear effect, and therefore larger seminal volume was observed in the treatment with 1.80% total lysine (Table 4). However, effects ($p > 0.05$) were not observed in the means of motility duration, motility rate, survival, sperm concentration, normal spermatozoa, pH and osmolarity.

It is known that the nutritional needs of broodstocks are bigger when compared to the juveniles, but the information about the nutritional demands of fish broodstocks, as stated before, is limited to only a few fish species (Izquierdo et al., 2001). The experiment with *R. voulezi* started with juvenile specimens (initial mean weight 35.18 ± 0.25 g), and continued until they reached their first reproductive cycle, when it was noticed that in net cages the testes continued to mature early. The same was described by Ghiraldelli et al. (2007). Thus, the lysine requirements of *R. voulezi* were similar to the ones observed for the juvenile stage

of other fish species (Bomfim et al., 2010 and Furuya et al., 2006), and may vary in later reproductive cycles. Murgas et al. (2012) recommended the use of broodstocks between the 2nd and the 5th year of gonadal maturation for presenting better results and being easier to be managed.

Table 3. Productive performance of male breeding *Rhamdia voulezi*, fed with diets containing different lysine levels.

Variables	Lysine level (%)				p
	1.20	1.40	1.60	1.80	
Final mean weight (g)	65.15±13.86^{b}	67.42±6.91^{ab}	74.5±8.84^{ab}	88.02±14.83^{a}	0.038*^{1}
Total length mean (cm)	19.62±1.28	19.38±0.59	19.82±0.65	20.98±5.57	0.132
Weight gain (g)	30.01±13.89^{b}	32.46±6.90^{ab}	39.04±8.75^{ab}	52.62±19.33^{a}	0.036*^{2}
Feed conversion	2.06±0.80^{b}	1.81±0.34^{ab}	1.21±0.24^{ab}	0.95±0.19^{a}	0.038*^{3}
Condition factor	0.82±0.05^{b}	0.93±0.05^{ab}	0.95±0.06^{a}	0.95±0.07^{a}	0.015*^{4}

*Different small letters in the line indicates significative difference, ANOVA followed by Duncan test ($p<0.05$) and with linear effect: 1. $y = 37.845x + 17.005$, $R^2 = 0.9002$; 2. $y = 37.205x – 17.275$, $R^2 = 0.8982$; 3. $y = -1.965x + 4.455$, $R^2 = 0.9701$ and 4. $y = 0.205x + 0.605$, $R^2 = 0.7199$.

The present study warns that lysine is basic for the development of *R. voulezi* because it is the only amino acid exclusively involved in the body protein deposition, in addition to being effective in the immune and gastrointestinal responses, a characteristic that is also observed in cobias, *Rachycentron canadum* (Zhou et al., 2007). Furthermore, according to Berge et al. (1998), the supplementation of this amino acid is closely related to the increase in the values of weight gain in *Salmo salar* (35 ± 11 g treatment with 5.9 g·kg^{-1} lysine to 105 ± 4 g with 18 g·kg^{-1} lysine in the diet) and improvement in the values of apparent feed conversion (2.0 ± 0.7 treatment with 5.9 g·kg^{-1} lysine for 0.9 ± 0.1 with 18 g·kg^{-1} lysine in the diet).

Table 4. Reproductive performance of male breeding *Rhamdia voulezi*, fed with diets containing different lysine levels.

Variables	Lysine level (%)				p
	1.20	1.40	1.60	1.80	
Volume (mL)	2.79±0.44[b]	2.79±0.92[b]	2.78±0.56[b]	3.81±0.98[a]	0.04*
Sperm motility (s)	25.61±2.20	26.63±3.17	26.70±1.66	27.84±1.38	0.23
Motility rate (%)	66.85±5.82	60.92±6.11	65.35±10.90	66.57±9.10	0.49
Sperm survival (%)	53.25±17.19	55.28±6.11	63.09±10.90	68.40±9.10	0.15
Concentration (spz 10^9 mL^{-1})	19.90±9.68	23.58±12.08	20.82±10.40	26.1±8.30	0.41
Sperm normality (%)	53.20±11.43	44.73±17.59	49.71±14.76	54.72±8.30	0.25
pH	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	0.42
Osmolarity (mOSM kg^{-1})	249.50±13.74	253.10±19.46	248.70±17.32	245.40±18.77	0.80

*Different small letters in the line indicates significative difference, ANOVA followed by Duncan test ($p<0.05$) and with linear effect: $y = 1.525x + 0.755$, $R^2 = 0.5922$.

It is important to point out that due to the lack of information in literature, many times *R. voulezi* was compared with other fish species that received similar treatments with lysine, but at another rearing stage. Zhou et al. (2010) showed that conflicting results may be associated with the rearing stage (fingerlings, juveniles, growers, adults), the species, feed management and feed formulation, which influence significantly the development of the fish. Montes-Girao and Fracalossi (2006), analyzing the effect of lysine requirement on the values of weight gain (70% higher) when compared with the basal diet for *Rhamdia quelen* fingerlings showed that the level of 1.73% lysine presented similar results to the ones found in this study. However, for *R. voulezi* male broodstock the values were lower than the ones described by Zhou et al. (2007), who estimated a requirement of 2.33% lysine in the diet of *R. canadum* juveniles, and higher than the ones found by Furuya et al. (2004), who determined the requirement of 1.42% lysine for *O. niloticus* fingerlings.

Cabrita et al. (2011) state that, in general, good quality semen is related to the capacity to produce viable embryos in suitable environments, including events such as ability of the spermatozoa to reach the oocytes; ability to penetrate the oocyte envelope through the micropyle; recognize the oolemma and fusion of both cell membranes; correct activation of the metabolic pathways of the egg; and

contribution of the future embryo. It is known that the use of a pool of semen from several males apparently may solve some problems at the moment of fertilization, but it does not ensure good development of the progeny. However, good quality semen (a single male) may fertilize most oocytes, resulting in an unpredictable endogamy. The assessment of the potential of the broodstocks in the process of artificial fertilization is fundamental (Viveiros and Godinho, 2009; Sanches et al., 2010; 2013). In addition, the semen (plasma or seminal fluid and spermatozoa) produced in the testes presents great morphophysiological variability (Cabrita et al., 2011).

In *R. voulezi*, the seminal characteristics were similar to the ones reported for *R. quelen* by Bombardelli et al. (2006), Hilbig et al. (2008) and Tessaro et al. (2012), indicating that the semen was adequate for spawning at the four levels of lysine studied. Watanabe and Vassallo-Agius (2003) stated that nutritional deficiency in broodstocks compromises reproduction (quality of sperm and oocytes), causes decline in growth, and decreases weight gain and resistance.

The seminal volume of *R. voulezi* was higher in the treatment with 1.80% total lysine, which can be attributed to higher values of weight gain of the broodstocks in this treatment. According to Luz et al. (2001), the volume of semen produced by the fish is variable and depends on the weight of the animal. Furthermore, the level of 1.80% lysine used for *R. voulezi* may have allowed the balance of amino acids, and consequently maximized the use of protein, resulting in the values of weight gain and semen volume. According to Gonçalves et al. (2009), the amino acids are precursors of biological compounds and the deficiency or excess of one or more amino acids may cause an imbalance between the amino acids of the diet. Therefore, the amino acids must be adequately balanced in order to promote growth and ideal development.

Lahnsteiner (2009), when studying the role of the free amino acids in the semen of *Oncorhynchus mykiss* and *Cyprinus carpio* reported that these elements, which are present in the seminal plasma, differ in a quali-quantitative way between the fish species, and noticed that the lysine is one of the amino acids present in the constitution of the spermatozoa and the seminal plasma. Besides, the author observed that the amino acids are actively secreted into the seminal fluid, and that the amino acid composition, however, depends on the degree of gonadal maturation and the physiological condition of each fish. Nevertheless,

Tantikitti and March (1995) mentioned that the composition of the amino acids present in the *O. mykiss* semen is directly related to the diet composition.

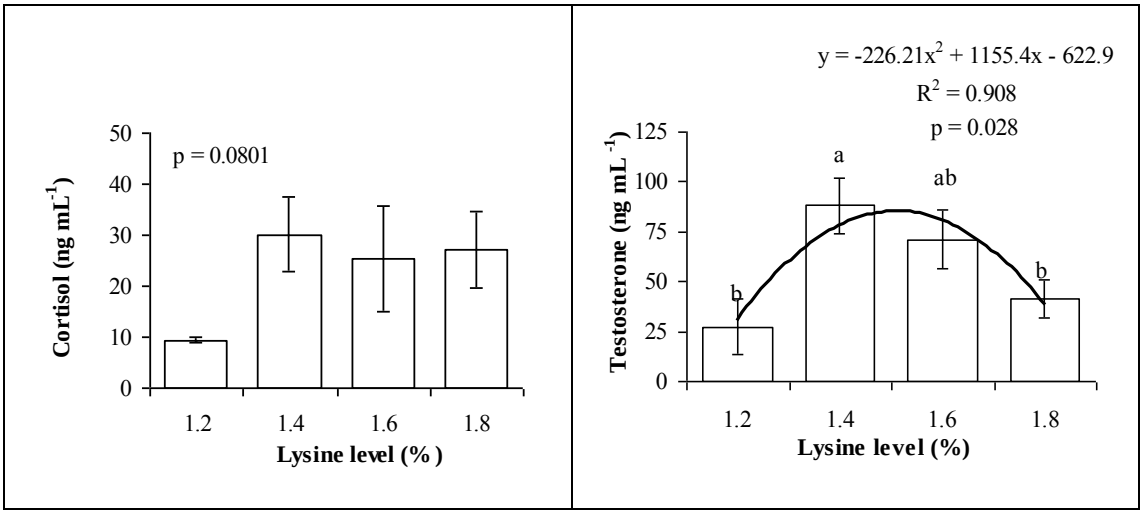

Fig. 1. Effect of lysine in the concentration of cortisol and testosterone of male breeding *Rhamdia voulezi*, fed with diets containing different lysine levels.

Studies demonstrate that the amino acids (glycine and alanine) have effect on the semen of *Morone saxatilis*, raising the values of motility rate of the cryopreserved spermatozoa (He and Woods, 2003). In *Perca fluviatilis*, asparagine, lysine, methionine and valine increased the percentage of motile spermatozoa and their swimming speed (Lahnsteiner, 2010). The same was observed with *R. voulezi*, in which lysine increased seminal volume.

According to Soso et al. (2008), the rise in the levels of cortisol may affect the reproduction of *R. quelen*, but *R. voulezi* males did not show alterations in cortisol levels when the fish received the four diets containing lysine ($p > 0.05$) (Fig. 1). Most times, the increase in blood cortisol concentration in fish is associated with a response to stress (Barcellos et al., 2003), but *R. voulezi* males showed that the daily management in net cages was adequate.

However, the values of testosterone showed influence ($p < 0.05$) with quadratic effect, and the lowest mean concentrations were found in the treatments with 1.20 and 1.80%, and the highest ones were found in the levels of 1.40 and 1.60% lysine (Fig. 1). That profile was similar to the one described by Barcellos et al. (2002) when studying the plasma concentrations of steroids during the reproductive cycle of *R. quelen* males. The variations in the levels of testosterone

observed may be related to different intrinsic responses of each animal to hormonal treatment (carp pituitary extract), since the inducing agent is responsible for the production and secretion of a series of hormones (Streit et al., 2002).

The most distinct characteristic of the testes of this species is the presence of digitiform projections or fringes, which vary in shape, volume, color and vascularization during the development of testicular maturation (Fig. 2A, C, E, G). The fringes are connected to the right and left sperm ducts, gradually tapering into a common duct that extends to the conical urogenital papilla, without fringes, located posterior to the anal opening (Fig. 2A, C, E, G). This species does not have seminal vesicle and secretion in the caudal region was not detected, corroborating what had been observed by Melo et al. (2011) for *Rhamdia aspera*, *Pimelodus maculatus* and *Lophiosilurus alexandri*. Testicular morphology similar to the studied species had been described by Ghiraldelli et al. (2007) for fish kept in earthen ponds and Reidel et al. (2010) with *R. quelen* kept in net cages.

The different stages of testicular maturation of *R. voulezi* males reared in net cages are described in Table 5 and shown in Fig. 2 (A to H) according to the reproductive activity, whose terminology was adapted according to Reidel et al. (2010) and Brown-Peterson et al. (2012). Histomorphologically, it was not possible to observe the variations that occurred in the testes of the fish that had received the four experimental diets. Afterwards, it was possible to characterize the reproductive cycle of this species as a continuous process, consisting of two components: seminiferous lobules and interstitial tissue (Melo et al., 2011; Schulz et al., 2010). The anastomosed seminiferous lobules include the Sertoli cells and the germ cells (spermatogonia, spermatocytes, spermatids and spermatozoa) distributed over the testes, following the pattern described for other native fish (Freitas et al., 2013; Melo et al., 2011). *R. voulezi* presents intense reproductive phase from November to January, coinciding with the testicular cycle described by Reidel et al. (2010) and Sanches et al. (2010; 2013) for *R. quelen* kept in net cages and earthen ponds, respectively. After completing total spermiation, the testes become remarkably smaller, and go through a stage of partial regression, and then total regression, returning to the stage of maturation (Reidel et al., 2010). The *R. voulezi* males exhibited reproductive activity when kept in net cages, with significant increase in the levels of testosterone (Fig. 1). In the treatment with 1.80% lysine, the weight of the testes was slightly higher (Fig. 2).

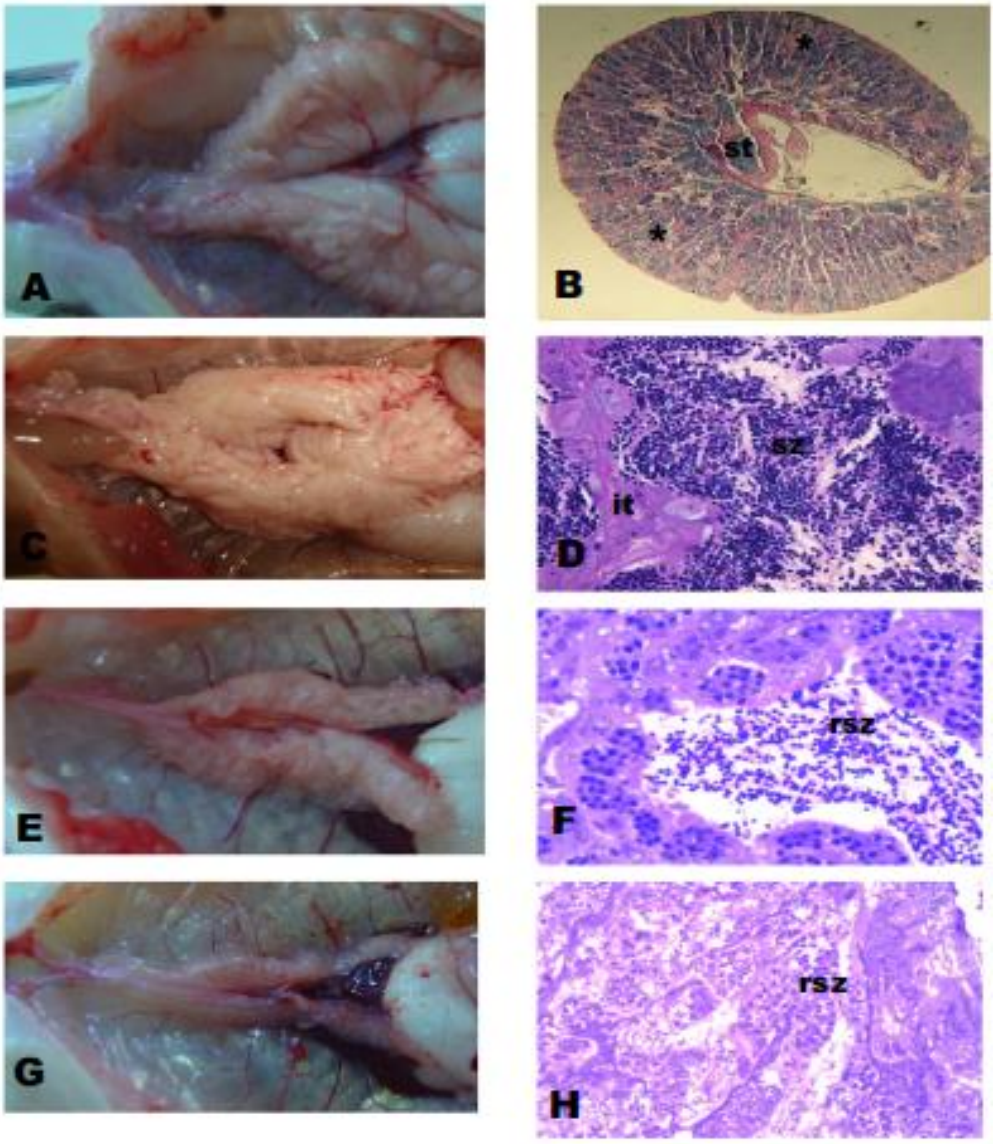

Fig. 2. Anatomical organization (left) and light micrographs (right) of *Rhamdia voulezi* male reproductive system: (A; C; E; G) Fringed testis in developing stage final maturation (A, C, 10×), partial regression (E, 10x×) and (G, 10×) total regression; (B, 10×); (B) anastomosis in the seminiferous tubules with cysts of the spermatogenic lineage cells in different stages of development (asterisk), and spermatic duct (st) filled with spermatozoa (sz); (D, 20×) Spermatozoa (sz) and interstitial tissue (it); (F, 20×) Remnants of spermatozoa (rsz), (H, 20×) disorganization and remnants of spermatozoa (rsz) (hematoxylin–eosin).

Table 5. Description of the testicular phases of *Rhamdia voulezi* in cages.

PHASE	TERMINOLOGY	MACROSCOPIC AND HISTOLOGICAL FEATURES
Spawning Capable (fish will spwan in this cycle)	Late Maturation, Ripe, Ripening	Macroscopically, Testis large but milt freely flowing with gentle press **(Fig. 2A e C)** Microscopically, Spermatozoa in lumen of lobules and/or sperm duc All stages of spermatogenesis can be present. Spermatocysts throughout testis. **(Fig. 2 B e C)**
Parcial Regression	Parcial-spent	Macroscopically, Testes reduced and flacid. **(Fig. 2 E)** Microscopically, Residual spermatozoa present in lumen of lobules and sperm ducts. Widely scattered spermatocyts containing spermati **(Fig. 2 F)**
Total Regression	Total-spent	Macroscopically, Testes well reduced, small and more flacid. The milt not released with pressure. **(Fig. 2G)** Microscopically, Similar the anterior phase, Spermatogonial proliferation in the periphery of test **(Fig. 2H)**

The values of gonadosomatic index ranged from 2.63 to 3.34%, hepatosomatic index from 1.13 to 1.37%, viscerosomatic index from 13.24 to 18.29% and visceral fat from 1.06 to 1.37%. The different levels of lysine assessed did not influence ($p > 0.05$) the organosomatic index. The absence of difference between the parameters might be related to a high coefficient of variation, mainly for the gonadosomatic index (25.4%) and hepatosomatic index (32.6%). The same was reported by Bomfim et al. (2010) when studying the decrease in crude protein with amino acid supplementation in the feed for *O. niloticus*.

The concentrations of essential amino acids, crude protein and moisture f whole carcass of the fish were not affected ($p > 0.05$) by the different levels of lysine in the diet (Table 6).

Table 6. Composition of essential amino acids, crude protein and moisture of whole body of male breeding *Rhamdia voulezi*, fed with diets containing different lysine levels.

Variables (%)	Lysine level (%)				p
	1.20	1.40	1.60	1.80	
Histidine	0.90±0.20	0.77±0.05	0.77±0.06	0.86±0.08	0.287
Arginine	3.70±0.39	3.42±0.22	3.29±0.40	3.58±0.31	0.367
Threonine	2.05±0.40	1.80±0.13	1.73±0.16	2.15±0.22	0.100
Methionine	1.40±0.37	1.18±0.05	1.06±0.13	1.42±0.15	0.088
Isoleucine	2.28±0.58	1.94±0.13	1.95±0.13	2.37±0.25	0.197
Leucine	3.88±0.98	3.29±0.25	3.42±0.22	3.69±0.35	0.457
Phenylalanine	1.93±0.45	1.65±0.10	1.67±0.12	1.93±0.18	0.265
Lysine	4.13±1.17	3.49±0.27	3.65±0.24	4.12±0.39	0.411
Valine	2.27±0.53	1.95±0.10	1.90±0.14	2.51±0.31	0.059
Crude protein	52.42±7.77	46.00±0.84	44.83±3.22	51.22±4.57	0.113
Moisture	68.18±4.01	69.02±2.66	67.31±1.80	71.1±2.26	0.304

The increase in the levels of lysine in the diet was not able to change the composition of essential amino acids, crude protein and moisture of whole carcass because according to Bicudo et al. (2009), and there are no response patterns for the characteristics of carcass composition related to the amino acid requirement. Similar observations were reported by Campos et al. (2006) with *Pseudoplatystoma corruscans*. In addition, those authors concluded that the profile of amino acids in the carcass may be a reference to the inclusion of amino acids in the diet.

The assessment of the effect of lysine on the diet of *R. voulezi* male broodstocks allows adequate supplementation of the diets with this amino acid. Fish farming in net cages is one of the alternatives for the development and strengthening of fish culture. The stocking of broodstocks in this system may be another option, because other studies have proven its viability (Bittencourt et al., 2012). However, research on the nutrition of fish broodstocks is still limited and increasingly necessary to clarify the role of the nutrients in the reproductive physiology and their impact on the production of viable progeny with satisfactory development to maximize the zootechnical indices of the species in question.

The conclusion is that the increase in the levels of lysine in the diet provided linear increase in weight gain and seminal volume and linear decrease in feed conversion in *R. voulezi* broodstocks confined in net cages during the first reproductive cycle.

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7. ARTIGO 2: Enviado em outubro de 2014 para a **Aquaculture**

Lysine in the diet of *Rhamdia voulezi* female broodstocks confined in net cages

7.1 - Abstract

The objective of this study was to evaluate the levels of lysine on the productive performance and reproductive parameters of *Rhamdia voulezi* female broodstocks in net cages, during the first reproductive cycle. Four hundred fish were used, distributed in a random experimental design with four treatments and four replications in 16 net cages with 25 fish each. The treatments consisted of four different diets prepared so as to contain the following levels of lysine: 1.20; 1.40; 1.60 and 1.80%, with 30% crude protein and 3,500 kcal kg^{-1} digestible energy for 185 days. The females broodstocks were previously selected according to their external characteristics. Thus, 72 females (18 per treatment) were weighed, measured, and submitted to hypophysation (0.5 and 5.0 mg kg^{-1} carp pituitary extract). The oocytes were collected before the 1st hormonal dose, and at the moment of release to verify their size. The females were then sacrificed, dissected, had their ovaries, liver, fat and guts removed and the respective organosomatic indices were estimated. There was effect ($p<0.05$) on the means of final weight and length, weight gain, apparent feed conversion and condition factor. The percent frequency distributions of oocyte diameter exhibited similar patterns in the four treatments, with polimodal tendency. However, at the moment of spawning, the configurations were similar for the diets with levels of 1.20, 1.60 and 1.80% lysine. Only the females from treatment 2 (1.40 % lysine) showed three modes with diameters below 520; 590 and 790 μm, with the presence of white (opaque), irregular and bloody residual eggs. With regard to the reproductive parameters, the released oocytes, absolute fecundity and remaining ovaries were influenced ($p<0.05$) by the diet, and the highest mean values were observed in the treatment with 1.80% lysine. The hormonal parameters were not affected ($p<0.05$). Among the organosomatic indices, only the visceral fat was influenced ($p<0.05$). The composition of aspartic acid, glutamic acid, serine, alanine, arginine, threonine, valine, methionine and phenylalanine in the carcass was affected ($p<0.05$). We can conclude that the level of 1.80% lysine promoted greater growth and consequently higher amount of oocytes produced in relation to the other treatments.

Keywords: *Rhamdia voulezi*; female broodfish; amino acid; spawn hormone; oocytes; lysine.

7.2 - Introduction

Rhamdia voulezi (Siluriformes) has attracted the interest of fish farmers especially in Southern Brazil due to its rapid growth, even during the coldest months of the year, good feed conversion, practical management and tasty flesh without intramuscular bones that is well appreciated by the consumers (Gomes et al. 2000; Diemer et al. 2012; Signor et al. 2013). However, it is a species endemic to the Iguaçu river basin in Paraná, Brazil, and its reproductive behavior in commercial rearing has not been deeply studied yet (Freitas et al. 2011).

The scarcity of fingerlings has been one of the bottlenecks to meet the regional demand and allow the systematic distribution to the productive sector. Nevertheless, we have observed significant progress in the development of priority investigations in order to understand the relationship between nutrition and reproduction mainly because it is a species of native fish (Mylonas et al., 2010; Reidel et al. 2010; Coldebella et al. 2011; Freitas et al. 2011; Tessaro et al. 2012; Signor et al. 2013).

Parra et al. (2010) observed changes in the morphometry of the eggs and larvae survival as a result of diets with different protein sources for *R. quelen* kept in captivity. According to Reidel et al. (2010), diets with 3,250 $kcal.kg^{-1}$ digestible energy and 35% crude protein for catfish kept in net cages caused faster gonad development and an extension of the spawning period. Therefore, it is evident that an appropriate nutrition enables the enrichment of the ovarian and testicular tissues, and consequently benefits reproduction (Coldebella et al. 2011; Tessaro et al. 2012). Bittencourt et al. (2012) found that a diet with 18% crude protein was satisfactory, promoting maturation of oocytes and spawning of *Piaractus mesopotamicus* when kept in net cages.

Given the complexity and difficulty in the conduction of experiments, there are few studies involving the nutrition of native broodfish, because they require higher costs, large premises and longer experimental time, in addition to low production scale of the diets by the factories (Freccia et al. 2014).

In this sense, it is believed that amino acids are essential for the diet of broodfish, because they promote increase in vitellogenin production. Vitellogin is the main precursor of yolk sac formation (Fernandéz-Palacios et al. 1997; Izquierdo et al. 2001), mobilizing the endogenous reserves as sources of nutrients

for the initial ontogenetic period until the functional development of the digestive tract is complete (Andrade et al. 2010).

Among the amino acids required by fish, lysine is regarded as reference to diet formulation because it is strictly essential, does not present any endogenous synthesis pathway, has basic metabolism and is the only one intended for body protein deposition. Concomitantly, the laboratory analysis to determine its level in the ingredients, diets and tissues is quite precise (Khan e Abidi, 2011).

Therefore, the objective of this study was to analyze the effect of lysine on the productive performance (weight and length gain, apparent feed conversion, specific growth rate and condition factor), reproductive parameters (position of nucleus, diameter of oocytes and eggs, fertilization and hatching rates), hormonal parameters (cortisol and estradiol), ovarian tissue (histomorphology), organosomatic indices (gonadosomatic, hepatosomatic and viscerosomatic) and composition of total amino acids in the oocytes and in the carcass of *R. voulezi* broodstocks confined in net cages during the first reproductive cycle.

7.3 - Materials and methods

This study was conducted at the CDT-Iguaçu (Center for the Development of Technologies for Net Cages of the Iguaçu river), located in the town of Boa Vista da Aparecida-Paraná/Brazil (UTM coordinates: 254036.84 and 7183301.91), and received the support from the Study Group on the Management of Aquaculture (GEMAq) of the State University of West Paraná-Unioeste, campus in Toledo, Paraná, Brazil. Four hundred *Rhamdia voulezi* (Haseman, 1911) juveniles (initial mean values of total length and weight 15.1±2.2 cm and 35.18±0.25g, respectively) were kept in 16 net cages with 0.40 m³ usable volume and 0.5 cm mesh size for 185 days (Jul./12-Jan./13). Four treatments and four replications were established and distributed randomly. The experimental unit consisted of 25 fish per net cage (25 X 4 replicates =100 fish per treatment). The treatments consisted of four diets prepared with the following levels of total lysine: 1.20; 1.40; 1.60 and 1.80%, with 30% crude protein and 3,500 kcal kg^{-1} digestible energy, according to the requirements set by Reidel et al. (2010) (Table 1). The ingredients were ground in a hammer mill, with 0.5 mm mesh size and submitted to an extrusion process, and the requirement for each nutrient was calculated by the software SuperCrac® 5.7 Master. The values of the analysis of amino acid and

protein composition in the four diets of *Rhamdia voulezi* after the experimental feed had been prepared can be found in Tables 1 and 2. After the experimental feed had been prepared, it was sent to a commercial laboratory so that the amino acid and protein composition could be determined, measured by means of High Performance Liquid Chromatography.

Table 1. Ingredients and proximate composition of the experimental diets.

Ingredients (%)	Lysine level (%)			
	1.20	**1.40**	**1.60**	**1.80**
Rice meal	35.00	34.90	34.80	34.70
Corn gluten	22.00	21.67	21.33	21.00
Corn	16.06	16.27	16.49	16.70
Fish meal	14.97	14.91	14.86	14.80
Soybean meal	9.15	9.07	8.98	8.90
Soybean oil	1.65	1.73	1.82	1.90
Mineral and vitamin mix[a]	0.50	0.50	0.50	0.50
Salt (NaCl)	0.30	0.30	0.30	0.30
Antifungal	0.20	0.20	0.20	0.20
L-Threonine	0.15	0.16	0.17	0.18
Antioxidant	0.02	0.02	0.02	0.02
L-Lysine	0.00	0.27	0.53	0.80
Total	100.00	100.00	100.00	100.00
Proximate composition				
Digestible energy (kcal kg^{-1})[b]	3500	3500	3500	3500
Crude protein (%)[c]	30.00	30.00	30.00	30.00
Total lysine (%)[c]	1.20	1.40	1.60	1.80
Total phosphorus (%)[d]	0.80	0.80	0.80	0.80
Fat (%)[d]	4.73	4.73	4.73	4.73
Crude fibre (%)[d]	1.38	1.38	1.38	1.38
Starch (%)[d]	38.62	38.62	38.62	38.62
Calcium (%)[d]	0.97	0.97	0.97	0.97

[a] Basic composition: folic acid: 500 mg, pantotenic acid: 4000 mg; biotin: 40 mg; Cu: 2000 mg; Fe: 12,500 mg; I: 200 mg; Mn: 7500 mg; niacin: 5000 mg; Se: 70 mg; vitamin A: 1,000,000 UI; vitamin B1: 1900 mg; vitamin B12: 3500 mg; vitamin B2: 2000 mg; vitamin B6: 2400 mg; vitamin C: 50,000 mg; vitamin D3: 500,000 UI; vitamin E: 20,000 UI; vitamin K3: 500 mg; Zn: 25,000 mg.
[b] Digestible values to *Rhamdia quelen* according to Oliveira Filho and Fracalossi (2006).
[c] HPLC laboratory analysis.
[d] Calculated values

The extruded feed assigned was weighed daily, and offered twice a day (9 a.m. and 5 p.m.), until the fish refused it. Food adjustment and fish sampling were carried out monthly.

Table 2. Analysis of protein and amino acid composition of the experimental diets.

Total amino acids (%)	Lysine level (%)			
	1.20	**1.40**	**1.60**	**1.80**
Aspartic Acid	2.44	2.35	2.28	2.46
Glutamic Acid	5.28	5.13	4.95	5.26
Serine	1.52	1.49	1.42	1.49
Glycine	1.84	1.83	1.79	1.93
Histidine	0.65	0.59	0.62	0.63
Arginine	1.82	1.78	1.75	1.84
Threonine	1.14	1.10	1.04	1.15
Alanine	2.18	2.10	2.01	2.14
Proline	2.40	2.40	2.32	2.48
Tyrosine	1.12	1.13	1.13	1.17
Valine	1.40	1.32	1.34	1.42
Methionine	0.60	0.60	0.56	0.58
Cystine	0.35	0.46	0.59	0.48
Isoleucine	1.25	1.16	1.20	1.27
Leucine	3.44	3.34	3.27	3.46
Phenylalanine	1.47	1.42	1.39	1.46
Lysine	1.30	1.40	1.60	1.95
Taurine	0.08	0.07	0.07	0.08
Crude protein (%)	30.47	29.79	29.61	31.40

During the reproductive period, 72 females (18 per treatment) were selected by external characteristics (bulging abdomen that is soft to touch, swollen and reddish genital papilla) (Reidel et al. 2010). The males (18 per treatment) were chosen according to the color and fluidity of the milt extruded after gentle pressure on the abdomen, and the seminal parameters were described by Diemer et al. (2014). The selected fish were transferred to the laboratory located on the riverbank, individually weighed and placed in 250 L tanks. The water temperature ranged from 23.0 to 25.0ºC.

In addition to the females' external characteristics, an ovarian sample was taken by biopsy from each fish per treatment: first sample (before the first hormonal dose) and second sample (at the moment of stripping). Each sample was removed using the intra-ovarian cannulation technique (inserting number 8 commercial urethral plastic catheter, external diameter = 2.0 mm), and separated by experimental diet (treatment). The biopsy sample was subdivided into three equal samples (subsamples): (1) placed in Serra solution (Bruzka, 1979) for 5 minutes to find the position of the germinal vesicle (GV) using a stereomicroscope (oc. 10X; obj. 17) (Bittencourt et al. 2012). Only the females which presented ≥ 60% GVs in a peripheral position were selected and separated by experimental

diet (treatment); (2) preserved in Gilson's solution (Simpson, 1951) for 30 minutes, and then the diameters were measured under a Zeiss Stemi 2000 stereomicroscope, at 170 X magnification (Romagosa et al. 1990; Bittencourt et al. 2012); the data were used to create graphs of frequency distribution; (3) preserved in a buffered formaldehyde solution (for 24h), and embedded in glycol methacrylate to monitor the morpho-structural modifications under light microscopy (Leonardo et al. 2004; Romagosa, 2010; Bittencourt et al. 2012). The material was processed at the Histology Laboratory, Fishery Institute, São Paulo/Brazil, using routine histological techniques. The analysis and photographic documentation were performed with a NIKON Eclipse-50 photomicroscope.

For induction of ovulation, these 18 females selected per treatment were intraperitoneally injected two doses of 0.5 and 5.0 mg CPE kg^{-1} (CPE= Carp Pituitary Extract), with a 12-hour interval between the doses according to the reproductive protocol described by Bombardelli et al. (2006). The males (18 per treatment) received one single dose of 2.5 mg CPE kg^{-1} according to Diemer et al. (2014).

After a period of 240 degree-hours (ATU=Accumulated Thermal Units) the females were massaged so that the oocytes could be released (non-hydrated). The oocytes from each female were collected in beakers (250mL). Then, three 0.1 mL sub-samples were collected, and the total number of released oocytes was estimated by extrusion: (1) 0.1g quantify the number of released oocytes; (2)100 oocytes fixed in Gilson´s solution (Simpson, 1951) to measure the oocyte diameter at the moment of release (=spawning), and (3) the remainder were frozem Ultra Freezer (-45ºC), identified and sent for analysis in a commercial laboratory to determine amino acid composition by means of High Performance Liquid Chromatography. The amount of spawning females (*percentage of females that released oocyte*); absolute fecundity (*total number of oocytes released by each female*) and relative fecundity (*number of oocytes released in relation to body weight*) were calculated from oocytes samples (items 1 and 2).

The oocytes (10 mL) and semen (insemination dose 450,000 spermatozoa) were then homogenized separately for each treatment (Bombardelli et al. 2006). The uniformity of diameter and color of the oocytes was observed, and white (non-viable) oocytes were noticed (Romagosa et al., 1998). Soon after the fertilization and hydration (5-7 min), the eggs from each female were transferred to 20 L conic

fiberglass incubators, with upwelling flow of 1 1/8 s for the first 4 hours and increased to 1 1/10 s afterward. Eight hours after insemination, the progress of cleavage was observed under stereoscopic microscope. The fertilization rate (FR, % = *100 X the number of eggs which were observed to show cleavage - blastoporus closure/ N=total number of oocytes*) for each treatment (Romagosa et al., 1990) was estimated in three 250 mL beakers, using 100 eggs from each unit. The eggs showing irregular or opaque cleavage were discarded. The hatching rate (HR, % = *100 X the number of hatched larvae/N=total number of larvae*) was also estimated according to Romagosa et al. (1990).

These 18 females from each treatment were then anesthetized with Eugenol ® solution (60 mgL^{-1}), as recommended by Diemer et al. (2012) for the withdrawal of blood aliquots by caudal puncture (with syringes). After centrifugation at 3000 rpm for 10 minutes, the plasma was kept in an Ultra Freezer (-45ºC) for later analysis of the levels of cortisol and estradiol, using Interkit immunoassay commercial kits ELISA, according to Barcellos et al. (1999; 2002).

Afterward, all females were sacrificed by cervical dislocation (CFMV, 2008), dissected, had their organs removed (ovaries remnants, liver, guts and visceral fat) and weighed (*g*). Fragments of the ovaries were fixed in buffered formol and processed according to the routine techniques for light microscopy (Romagosa, 2010; Bittencourt et al. 2012). The tissues were dehydrated in a series of ethanol, infiltrated and embedded in glycol methacrylate, and then 2.5 to 3.0 mm sections were cut on a microtome (Sorvall Type JB-4), mounted onto glass slides and stained with hematoxylin-eosin. The organosomatic indices were then calculated: gonadosomatic [GSI=(*weight of the ovaries/total weight of the fish*)x100], hepatosomatic [HSI=(*weight of the liver/total weight of the fish*)x100], viscerosomatic [VSI=(*weight of the guts/total weight of the fish*)x100] and fat [FI=(*weight of the visceral fat/total weight of the fish*)x100], according to Tessaro et al. (2012).

The organs (ovaries, liver, guts and fat) and the whole carcass were removed from six fish per treatment (three samples each), chopped (±1 cm) with a scalpel, homogenized, dried in a drying oven (105°C), ground, identified and sent to a commercial laboratory for analysis of the composition of amino acids, protein and moisture of whole carcass by means of High Performance Liquid Chromatography.

The quality of the water of the area covered by the net cages was monitored by analyzing the variables: temperature (22.1±1.9°C), pH (7.44±0.42), electrical conductivity (26.8±5.1µS.cm^{-1}) and dissolved oxygen (7.43±1.49mg.L^{-1}) measured weekly "*in situ*" by means of portable potentiometers by Hanna Instruments® and water transparency (3,1±0,5m) measured by visual disappearance of the Secchi disk.

Data were submitted to analysis of variance (ANOVA) followed by Tukey test was applied at 5% significance (Zar, 2009). Tests for normality and homoscedasticity of the variances were conducted, and the statistical analysis was carried out by the free Software R-3.02.

7.4 - Results and Discussion

Table 3 shows that the mean values of final weight and length, weight gain, apparent feed conversion and condition factor were significantly influenced ($p<0.05$) by the diets, and the treatment with 1.80% lysine, the highest level evaluated, presented the best results of productive performance.

Table 3. Productive performance of female breeding *Rhamdia voulezi*, fed with diets containing different lysine levels.

Variables	Lysine level (%)				p
	1.20	1.40	1.60	1.80	
Initial mean weight (g)	35.26±0.32	35.06±0.37	35,2±0,77	35,42±0,23	0.754
Initial mean length (cm)	16.25±2.00	13.38±2.40	14.63±1.30	16.00±1.50	0.250
Final mean weight (g)	80.89±28.55^{b}	86.44±27.05^{b}	84.33±20.57^{b}	120.22±30.01^{a}	0.00007*
Total length mean (cm)	20.19±1.92^{b}	20.36±1.98^{b}	20.28±1.40^{b}	22.16±1.97^{a}	0.002*
Weight gain (g)	45.78±20.33^{b}	51.27±23.05^{b}	43.07±16.15^{b}	84.80±18.88^{a}	0.008*
Feed conversion	1.36±0.49^{ab}	1.52±0.54^{b}	1.42±0.55^{ab}	0.74±0,13^{b}	0.041*
Condition factor	0.95±0.10^{b}	1.00±0.11^{ab}	1.00±0.09^{ab}	1.06±0.09^{a}	0.011*

*Different small letters in the line indicates significative difference, ANOVA followed by Tukey test.

During the previous selection of *R. voulezi* females, it is recommended to observe certain external characteristics, such as color and shape of the genital papilla, as well as abdominal bulge. Therefore, fish with no blemishes, distended abdomen, and prominent genital papilla were classified (Signor et al., 2013).

However, Phelps et al. (2011) explained that the selection of mature broodfish can often be more arbitrary and subjective, and so it is a difficult method to quantify and may vary according to the experience of the biologist performing the selection. Other visual indicators of the development of oocytes and eggs (appearance, position of germinal vesicle or nucleus and diameter of oocytes and eggs) were used according to Romagosa et al., (1998); Leonardo et al., (2004) and Bittencourt et al., (2012).

The females selected for hormonal induction exhibited over 60% ($p \geq 0.05$) of relocated germinal vesicle (nucleus). We observed that the oocytes from Treatment 2 (1.40%) showed lower percentage of relocated nucleus (64.72%) when compared with the other diets (72.64; 71.18; 74.17% diets 1, 3 and 4, respectively), and were not different from one another ($p \geq 0.05$). Ovaries at final maturation, showing relocated nucleus may be seen in Figure 1 A (Fig. 1 C), ratifying that although those females were kept in limited environments and confined, the sequence of reproductive events occurred normally, including the process of ovarian maturation (Reidel et al., 2010; Coldebella et al., 2011). Signor et al. (2013) reported that *R. voulezi* presented great potential to be reared in net cages and the broodstocks were viable for 180 days. Released *R. voulezi* oocytes could be seen (Fig. 1 E).

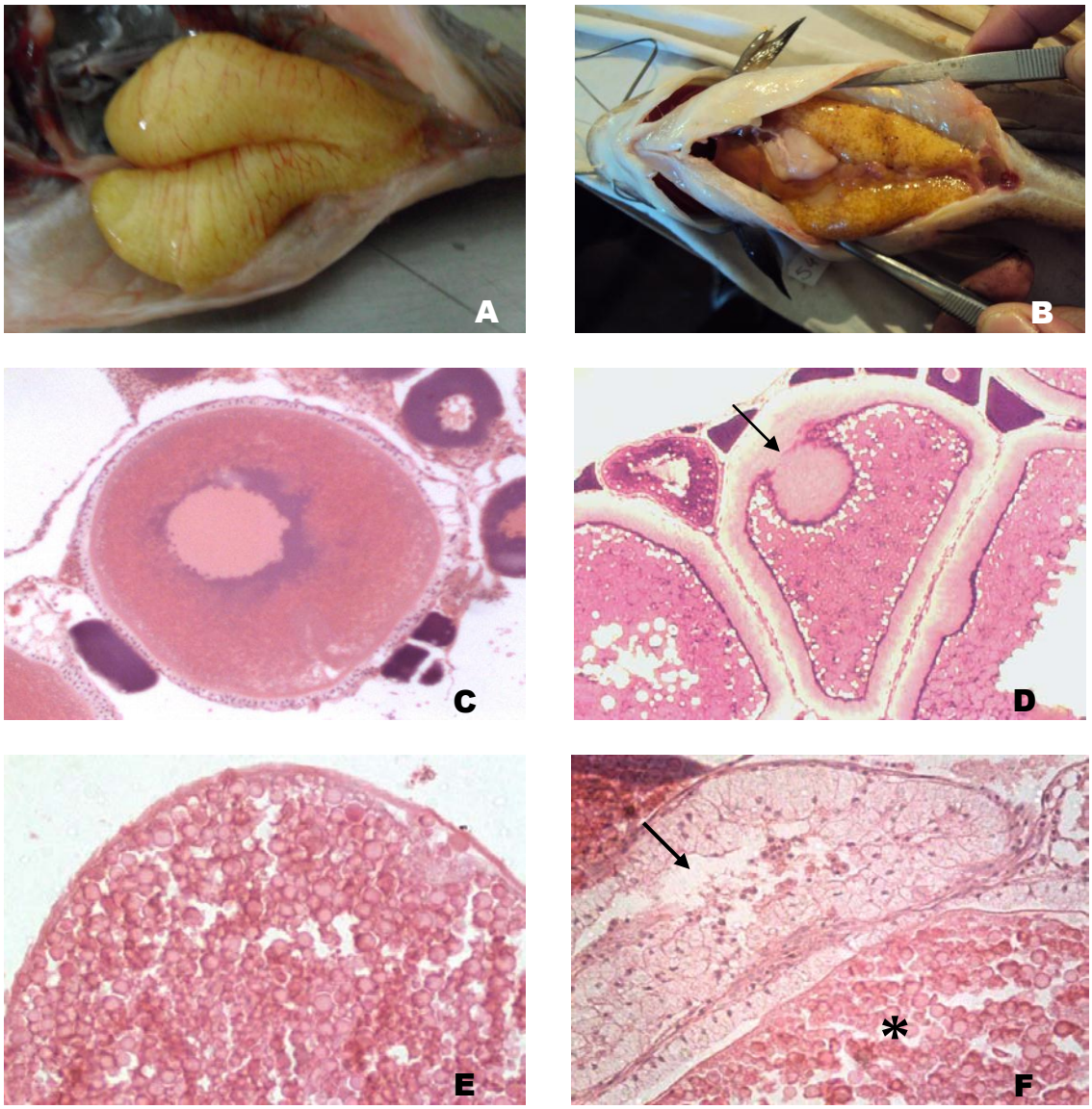

Fig. 1. Anatomical organization (**A** and **B**) and light micrographs (**C**;**D**; **E; F**) of *Rhamdia voulezi* female reproductive system: (**A, 10x**) Ovaries in stage final Maturation; (**C, 20x**) Oocyte before the first hormone - germination vesicle slightly shifted toward the periphery; (**E, 20x**) Time of release of the oocyte; (**B**, **10x**) Ovaries oocyte after spawning; (**D, 10x**) Micropyle (arrow); (**F, 20x**) Mature oocyte (*) and post-ovulatory follicle (arrow).

Another parameter assessed was the size or diameter of the oocytes, which is recommended as an indicator (monitor) of ovarian development (Romagosa et al., 1998; Leonardo et al., 2004; Bittencourt et al., 2012), and also supplies an estimate of parental investment of the progeny (Mylonas et al., 2010; Romagosa et al., 2013). For *R. voulezi,* the percentage distribution of the oocyte diameters before the 1st hormonal injection (1st sample) exhibited similar patterns between the four treatments, with polimodal tendency, with modes of 570; 670; 750 and

860 µm (Fig. 2A). Nevertheless, at the moment of spawning (2nd sample), the configurations were similar for the diets with levels of 1.20, 1.60 and 1.80% lysine keeping the modes at 590, 670 750 and 860 µm, respectively (Figs 2B1; 2B3 and 2B4). That type of distribution for *R. voulezi* had been expected, since the species is characterized by asynchronous development, presenting four modes, released in parcels in the wild, during the reproductive period (Reidel et al., 2010). Those findings corroborate the ones described by Signor et al. (2013), who showed that *R. voulezi* may reproduce several times during the same reproductive season. Still in Figure 2B2 we can verify that the females which had received diets containing 1.40% lysine displayed three modes with smaller diameters (520; 590 and 790 µm) when compared with the other three diets offered. However, it is noteworthy that at the moment of release, the quality of the oocytes (color, aspect and uniformity) was inadequate, with the presence of numerous white (opaque), irregular and bloody cells, indicating residual oocytes.

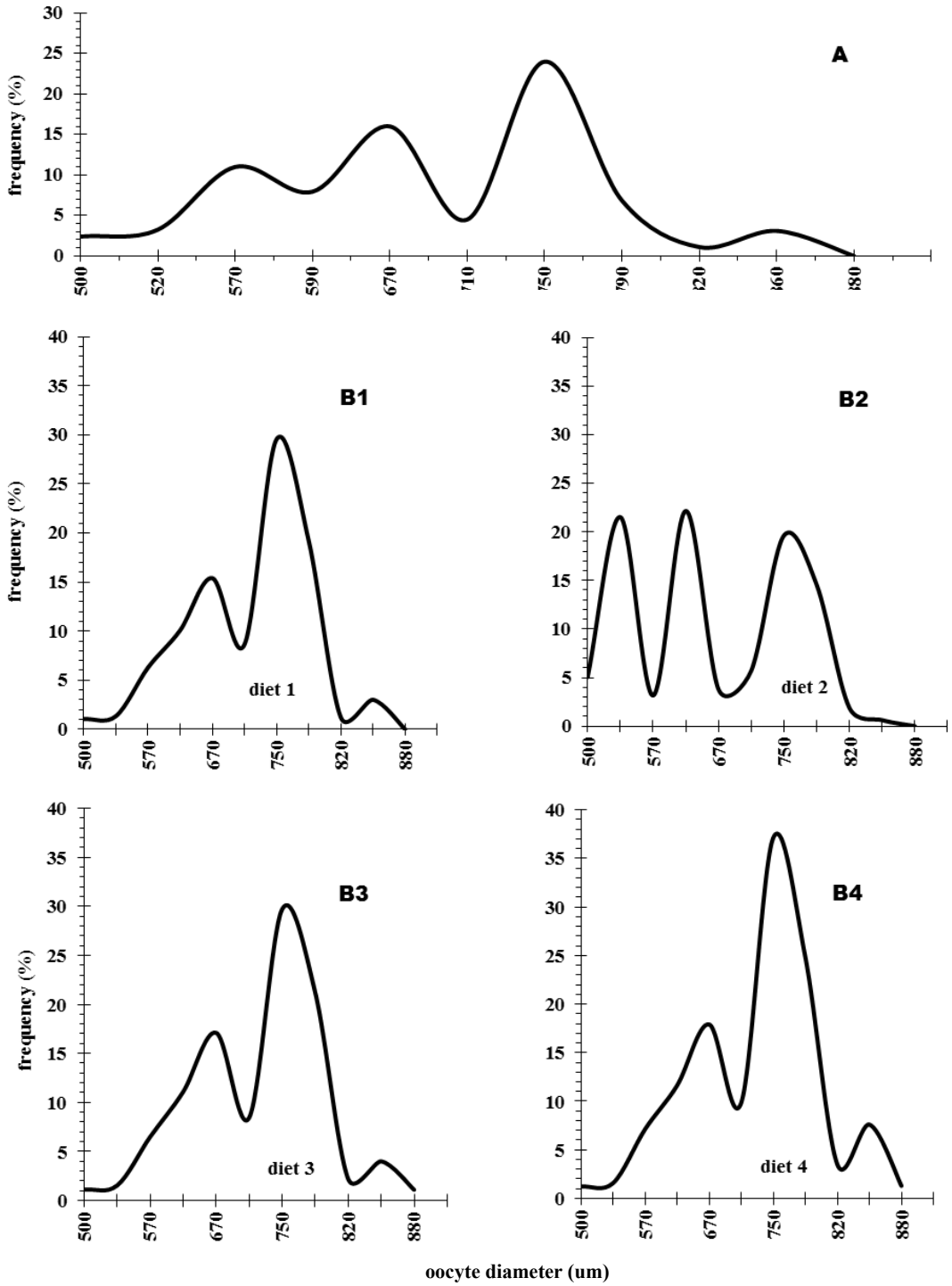

Fig. 2. The diameters of oocyte frequency distributions of *Rhamdia voulezi*: (A) before the 1st application of hormones – the 1st sample of the four treatments grouped, (B) during oocyte release- the 2nd sample, (B1=1.2; B2=1.4; B3=1,6; B4=1.8% lisine). Frequencies are presented as mean values. (n=6 by treatment)

It is known that not all the females reach ovarian maturation at the same time (Phelps et al., 2011). It is important to point out that the *R. voulezi* females used in this study were at 1st maturation (first reproductive cycle, 100% of the fish

were 11 months old), which justified the wide variation in the reproductive parameters (Tab. 4).

The reproductive parameters: released oocytes, fertilization and hatching rates, absolute fecundity and remaining ovaries were influenced ($p<0.05$) by the diet (Tab. 4). According to Signor et al. (2013), the capacity of egg production of *R. voulezi* is directly related to the class of body weight; in other words, larger broodfish produce larger number of oocytes.

Table 4. Reproductive performance of female breeding *Rhamdia voulezi*, fed with diets containing different lysine levels.

Variables	Lysine level (%)				p
	1.20	1.40	1.60	1.80	
Spawning females (%)	66.83±42.05	78.00±26.94	50.00±27.60	66.86±36.51	0.58
Oocytes released (g)	6.41±3.70^{b}	5.77±3.11^{b}	5.35±2.50^{b}	11.00±5.43^{a}	0.002*
Rate of Fertilization (%)	49.92±19.65^{ab}	26.32±16.55^{b}	52.77±20.84^{a}	43.20±34.98^{ab}	0.022*
Rate of Hatching (%)	69.72±29,91^{ab}	3.52±2.59^{c}	80.45±16.97^{a}	49.88±43.64^{b}	0.034*
Absolute Fecundity (oocytes.female $^{-1}$)	7,643±4,638^{b}	7,300±3,568^{b}	7,266±2,621^{b}	13,210±6,520^{a}	0.008*
Relative Fecundity (oocytes.g of female)	97.65±44.47	82.79±31.04	85.60±28.57	111.02±39.17	0.229
Remnants ovaries (g)	4.90±3.36^{b}	4.90±2.22^{b}	3.60±2.80^{b}	7.60±5.22^{a}	0.004*

*Different small letters in the line indicates significative difference, ANOVA followed by Tukey test.

The average percentage of spawning females ranged from 50.00 to 78.00% and was not influenced ($p>0.05$) by the addition of different levels of lysine in the feed. The absence of effect on this parameter was also described by Tessaro et al. (2012) for *R. quelen* females, but the values were slightly higher, ranging between 76.72 and 89.63% with diets supplemented by different energy levels.

According to Bombardelli et al. (2006), the relative fecundity for *Rhamdia quelen* when reared in captivity varied from 116 to 156 oocytes per gram of female. In this study, lysine did not affect ($p>0.05$) relative fecundity, and the results ranged between 82.79 and 111.02, lower than the ones reported for *R. quelen*, probably due to the system of confinement adopted. According to Hainfellner et al. (2012), when assessing comparatively the process of oocyte development of *Prochilodus lineatus* kept in two rearing systems: excavated ponds and net cages, the process of ovarian maturation was compromised in the females kept in net cages with significant reduction in the volume of vitellogenic oocytes.

Besides, it is important to say that the females in this experiment were at 1st ovarian maturation. According to Romagosa et al. (1990), the number of released oocytes per female (297,308 and 377,643 oocytes) increased with age (3 and 4 years, 1st and 2nd ovarian maturation, respectively) for *Piaractus mesopotamicus* kept in ponds.

We did not observe effect ($p>0.05$) of lysine on the concentrations of cortisol and estradiol. The cortisol concentrations found ranged from 32.35 to 48.22 ng.mL^{-1} for estradiol, from 0.54 to 0.70 ng.mL^{-1} (Fig. 3).

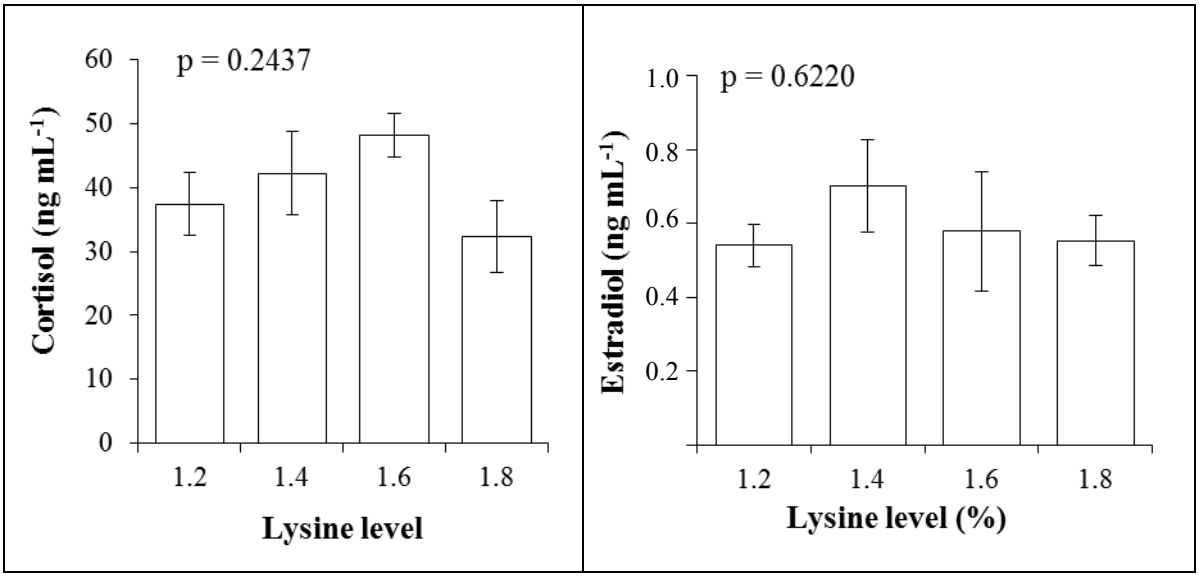

Fig. 3. Effect of lysine in the concentration of cortisol and estradiol of female breeding *Rhamdia voulezi*, fed with diets containing different lysine levels.

One of the undesirable changes during fish farming, mainly due to wrong management and nutrition, is the reproductive stress, which increases the concentration of plasma cortisol, hormone that induces immunosuppression and reduces fish resistance to bacterial and fungal infections Cericato et al. (2008). The concentrations of cortisol obtained were similar to the ones found previously by Barcellos et al. (2004) for *R. quelen*.

In Figure 3 we can see that lysine did not present effect on estradiol. However, the concentrations measured were low, may be due to the first maturation, or the fact that the blood was collected after reproduction. According to Iseki et al. (2008), studying the seasonal variations in the plasma levels of sexual steroids during the reproductive cycle of *Piaractus mesopotamicus* females, the levels of estradiol varied according to gametogenesis, exhibiting the lowest values

at the initial stages of development. At the phase of final maturation, the *Dicentrarchus labraxos* females presented progressively decreasing plasma levels of estradiol, but without effect during spawning (Prat et al., 1999).

Rhamdia voulezi ovaries presented analogous anatomical and morphological archetypes during the differentiation of ovarian follicles for the four treatments (diets) (Figs 1A; 1B and 4A; 4B). Certainly, the ovarian structure variations reported by Reidel et al. (2010) for *R.quelen*, such as paired, elongated and bag-like ovaries located in the area of the abdominal cavity were equivalent to the ones found in *R. voulezi.* In the present study, the ovarian walls are covered by an albugineous tunic that emits septa towards the center, forming the ovuligerous lamellae, where the germinal epithelium houses all the oocyte development (Figs. 1A and B). The analysis of the germinal epithelium associated to the stage of development of the germ cells present during the reproductive cycle of *R. voulezi* adult females allowed us to propose four reproductive phases throughout the year: (1) *Developing*, (2) *Spawning capable*, (3) *Regressing* and (4) *Regenerating*, according to the terminology proposed by Reidel et al. (2010) and Brown-Peterson et al. (2011) and adapted to this species.

R. voulezi developing ovaries, in general, occur from September to February, occupy much of the abdominal cavity (Figure 1A), have light-yellow color, showing they are at the stage of advanced vitellogenesis, together with the previtellogenic oocytes. After that, the females become spawning capable – spawning (November to February), presenting hyperemic urogenital orifice, soft and large abdomen. The ovaries reach their maximum size, with dark-yellow color (Fig. 4B) and vitellogenic oocytes are predominant (Final Maturation or Mature), with relocated germinal vesicle or nucleus (Fig. 1C) towards the micropyle, which is formed by invagination of the zona radiata in its extremity (Fig. 1D). Fewer reserve previtellogenic oocytes are observed. At this phase, some remaining structures called postovulatory follicles (Fig. 1F) can be observed after ovulation (oocyte expulsion) (Fig.1E). Those follicles are formed by the involution of the follicular envelope of the granulosa cells (Romagosa et al. 2005). According to the authors, those postovulatory follicles do not have endocrine function and are quickly absorbed, which involves programmed cell death or apoptosis of the follicular cells.

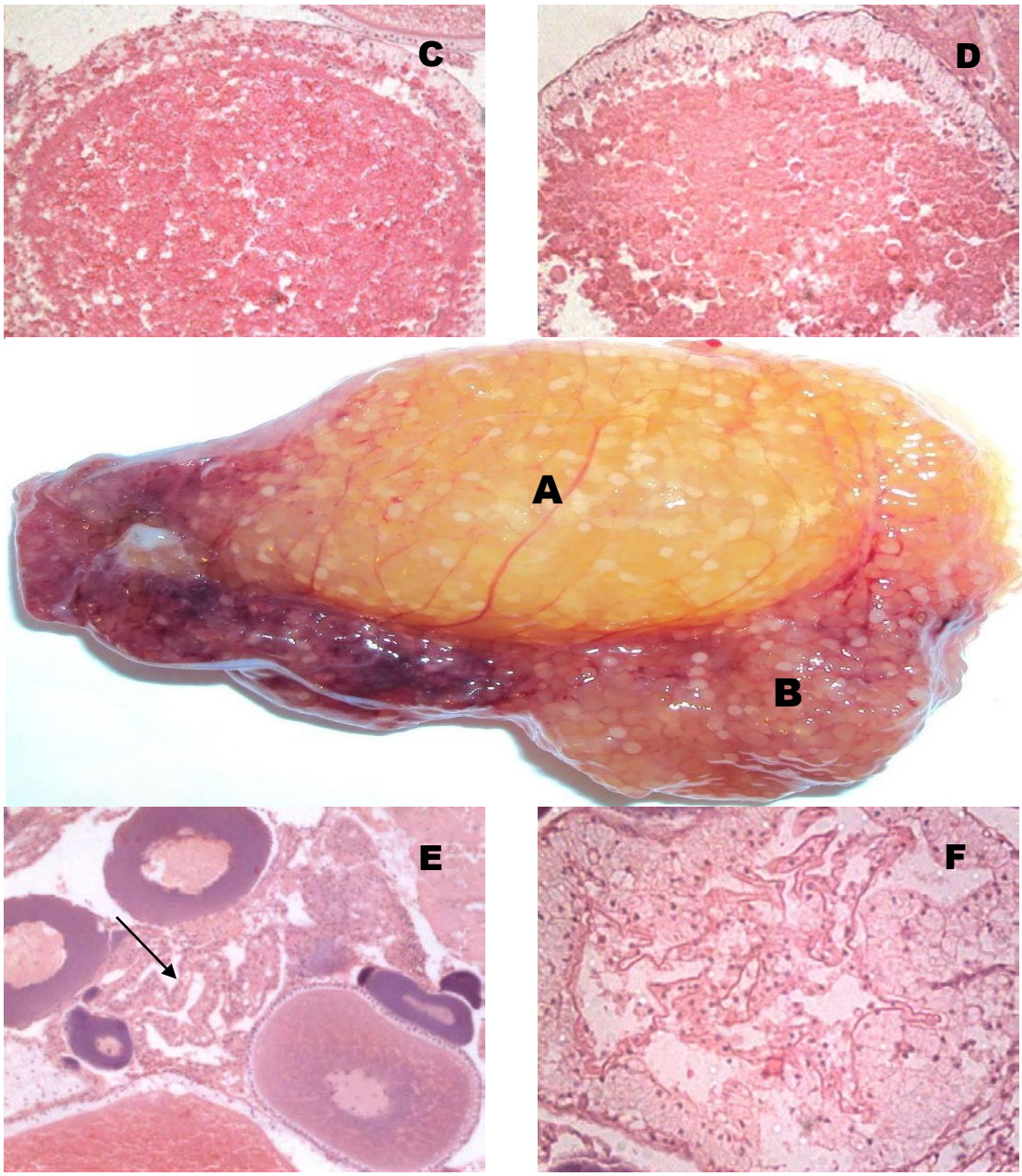

Fig. 4. Ovaries partial spawn (**A**, 10x) and remnants (**B**, 10x) of *Rhamdia voulezi* (in the middle). (**C**, 20x and **D**, 20x) it can be observed a large number of atresics follicles and **(E**, 10x) it is show the post-ovulatory follicle (arrow) and oocytes in another phases; (**F**, 40x) detail of post-ovulatory follicles.

At the stage of final maturation of the *R. voulezi* oocytes, numerous atretic follicles were observed in the four treatments applied (Figures 4C; 4D). However, in the females that had received diet 2, the presence of atretic follicles was more pronounced and could be clearly verified by means of percent distribution of oocyte diameter (Fig. 2B2). That fact is different from the results obtained by Torres (1994), who studied *Piaractus brachypomus* and found that the presence of atretic oocytes varied with growing levels of protein and energy (25.4; 32.8; 38.2%

CP and 2,605; 3,415 and 3,828 Kcal GE). According to Romagosa et al. (2005), the increase in the number of atretic oocytes, especially in previtellogenic follicles, may indicate an adverse physiological condition, reflecting the presence of several stressor agents.

In some of the females used in the present study we could notice that after oocyte release (expulsion), the ovaries remained in the area of the abdominal cavity (Figs 4 A and B), exhibiting two distinct regions: *(i)* left - macroscopically, full of yellow and white oocytes, with quite apparent irrigation (Fig. 4A); microscopically, at first, the atretic follicles mark the fragmentation and absorption of zona radiata and breakup of yolk granules (Fig. 4C). The follicular envelope is then totally broken and the follicular cells invade the ooplasma by phagocytosis (Fig. 4D). Thus, it could be noted that the *R. voulezi* females were at regressing stage (partial and total), signalizing the end of the reproductive cycle; and *(ii)* right - remnants of this region of the ovary are congested and thick (Fig. 4B). Still at this stage it is possible to see the presence of oocytes at different stages of development during the reproductive period, noting that the species presents asynchronous development (Fig. 4E), when more than one group of oocytes follow the same stage of development until the end of the cycle, characterizing them as multiple or parceled spawning (Reidel et al. 2010). This ovarian description is similar to that of *R. quelen* according to Ghiraldelli et al. (2007). At this moment, the postovulatory follicles remain in the lamella after oocyte release and are composed of follicular layer, theca and basal membrane in the process of degeneration and absorption, which corroborates the description given by Romagosa (2010) for the catfish, *Pseudoplatystoma fasciatum* kept in net cages.

The following phase, *Regenerating* is the moment when the ovaries get ready to start a new reproductive cycle. At this phase, the walls of the ovaries become thick and the ovigerous lamellae reorganize, the process of folliculogenesis is active and there is great mitotic proliferation of the germ cells, and nests of oogonia.

Concomitantly, the highest values of GSI were obtained at the phase *spawning capable* (4.528), present in the females sampled in September, November and February, followed by females at the end of *developing* (1.391), *regenerating* (0.901), *regressing* (0.754), and beginning of *developing* (0.531). The lowest mean values were observed in immature animals (0.502).

Nevertheless, the different levels of lysine evaluated did not influence ($p>0.05$) the GSI, whose mean values were 11.86±5.31, 11.29±3.79, 11.92±3.65 and 14.08±5.08% for the treatments with 1.20, 1.40, 1.60 and 1.80% lysine, respectively. The same happened with regard to HSI and VSI ($p>0.05$). However, the visceral fat was different ($p<0.05$) between the treatments, with the lowest values observed in the treatment with 1.20 and 1.80% lysine, with mean values of 1.08 and 1.07%, respectively. The viscerosomatic index ranged between 18.18 and 22.35%, gonadosomatic between 11.86 and 14.08%, and hepatosomatic between 1.84 and 2.43%.

Other studies reported less accumulation of fat in fish fed diets with different levels of lysine in *Oncorhynchus mykiss* (Encarnação et al., 2004); *Cyprinus carpio* (Zhou et al., 2008); *Piaractus mesopotamicus* (Abimorad et al., 2010); *Oreochromis niloticus* (Furuya et al., 2013). The reduction in visceral fat may have occurred due to the fact that lysine acts as a precursor of carnitine, which is involved in the transport of long-chain fatty acids to the mitochondria (Zhou et al., 2010).

The concentrations of total amino acids in the oocytes were not affected ($p>0.05$) by the different levels of lysine in the diet (Table 5). Therefore, the profile of amino acids found in the oocytes of the females remained constant, even with the variation of lysine.

Table 5. Composition of essential amino acids in oocytes on wet basis of females breeding *Rhamdia voulezi*, fed with diets containing different lysine levels.

Total amino acids (%)	Lysine total level (%)				p
	1.20	1.40	1.60	1.80	
Aspartic acid	1.71±0.34	1.60±0.78	1.67±0.45	1.46±0.06	0.922
Glutamic acid	2.40±0.41	2.23±1.10	2.32±0.63	2.05±0.08	0.922
Serine	1.28±0.27	1.23±0.53	1.26±0.31	1.11±0.04	0.921
Glycine	0.72±0.12	0.70±0.34	0.73±0.20	0.64±0.03	0.944
Histidine	0.49±0.04	0.46±0.23	0.48±0.12	0.43±0.01	0.925
Arginine	1.25±0.25	1.19±0.60	1.16±0.37	1.08±0.03	0.925
Threonine	0.90±0.11	0.80±0.37	0.83±0.21	0.72±0.02	0.791
Alanine	1.54±0.29	1.49±0.69	1.56±0.43	1.43±0.06	0.981
Proline	0.95±0.20	0.96±0.44	1.01±0.28	0.90±0.05	0.970
Tirosine	0.63±0.11	0.60±0.31	0.63±0.17	0.55±0.05	0.953
Valine	1.19±0.18	1.03±0.52	1.03±0.31	0.94±0.06	0.795
Methionine	0.45±0.05	0.40±0.22	0.38±0.14	0.38±0.03	0.910
Cistine	0.33±0.07	0.34±0.15	0.39±0.14	0.30±0.03	0.792
Isoleucine	1.16±0.16	1.02±0.51	1.11±0.30	0.97±0.06	0.875
Leucine	1.91±0.30	1.81±0.88	1.91±0.52	1.70±0.09	0.954
Phenylalanine	0.68±0.10	0.62±0.33	0.65±0.18	0.56±0.03	0.899
Lysine	1.33±0.26	1.27±0.66	1.33±0.34	1.18±0.06	0.959
Taurine	0.03±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.480

Values did not differ ($p>0.05$) statistically.

Since there was no difference in the amino acid composition of the oocytes, it is assumed that the diets were sufficient for the development of the oocytes. Similarly, according to Gunasekera et al. (1997), the profile of amino acids found in the oocytes of *Oreochromis niloticus* females remains constant, even if the level of protein in the diet is below the nutritional demand of the species. Besides, Khan et al. (2005) stated that the influence of diets with different levels of protein on the composition of fish oocytes has not been totally clarified yet. On the other hand, Kabir et al. (2013) suggest that the levels of protein in the muscle, liver and oocytes might be related to weight gain, although the protein content in the oocyte is influenced by the diet, and the nutritional quality of the protein affects the reproductive development and quality of the *Pangasianodon hypophthalmus* eggs.

The composition of non-essential amino acids in whole carcass: aspartic acid, glutamic acid, serine, alanine, and the essential ones: arginine, threonine, valine, methionine and phenylalanine was affected ($p<0.05$) by the different levels of lysine in the diet. However, the other amino acids were not influenced ($p>0.05$) (Table 6).

Table 6. Composition of essential amino acids of whole body of female breeding *Rhamdia voulezi*, fed with diets containing different lysine levels.

Total amino acids (%)	Lysine total level (%)				P
	1.20	1.40	1.60	1.80	
Aspartic acid	5.26±1.02^{a}	3.62±0.56^{b}	4.10±0.07^{ab}	4.82±0.43^{ab}	0.047*
Glutamic acid	7.94±1.16^{a}	6.20±0.27^{b}	6.21±0.09^{b}	7.59±0.65^{a}	0.025*
Serine	2.46±0.20^{a}	2.18±0.04^{ab}	1.94±0.05^{b}	2.33±0.21^{a}	0.013*
Glycine	4.86±0.80	5.99±0.63	4.23±0.48	4.93±1.31	0.173
Histidine	0.98±0.16	0.77±0.06	0.74±0.02	0.86±0.10	0.058
Arginine	3.87±0.26^{a}	3.53±0.03^{ab}	3.09±0.11^{b}	3.68±0.28^{a}	0.007*
Threonine	2.19±0.34^{ab}	1.81±0.16^{ab}	1.65±0.02^{b}	2.22±0.21^{a}	0.025*
Alanine	3.61±0.12^{a}	3.45±0.06^{ab}	2.97±0.19^{b}	3.48±0.35^{ab}	0.027*
Proline	2.99±0.32	3.48±0.27	2.61±0.24	2.90±0.62	0.130
Tirosine	1.82±0.35	1.29±0.08	1.34±0.08	1.54±0.23	0.062
Valine	2.45±0.46^{ab}	1.92±0.10^{ab}	1.84±0.05^{b}	2.62±0.27^{a}	0.019*
Methionine	1.56±0.25^{a}	1.17±0.06^{ab}	1.12±0.09^{b}	1.46±0.16^{ab}	0.021*
Cistine	0.44±0.09	0.33±0.10	0.43±0.01	0.37±0.04	0.279
Isoleucine	2.49±0.48	1.90±0.13	1.89±0.04	2.42±0.28	0.055
Leucine	4.24±0.81	3.24±0.28	3.31±0.09	3.68±0.43	0.117
Phenylalanine	2.11±0.35^{a}	1.63±0.11^{b}	1.61±0.05^{b}	1.97±0.19^{ab}	0.048*
Lysine	4.57±0.94	3.48±0.33	3.54±0.13	4.18±0.45	0.111
Taurine	0.48±0.33	0.25±0.05	0.28±0.10	0.22±0.01	0.306

*Different small letters in the line indicates significative difference, ANOVA followed by Tukey test ($p<0.05$).

The alterations detected must be related to the different requirements of amino acids by the organs, mainly by the ovaries. According to Kabir et al. (2013), the relationship between the content of amino acids in the liver and the oocytes is due to the fact that during oocyte maturation, essential nutrients are transferred from the liver to the oocytes by the blood, and in general, greater protein deposition is observed in the muscles, followed by oocytes and liver. Assem et al. (2005) reported that seven amino acids (proline, alanine, valine, methionine, isoleucine, leucine and histidine) exhibited significant increase in mature ovaries followed by reduction in spawning for *Trachinotus ovatus*. Thus, new studies are necessary to help us elucidate the processes involved with nutrition and reproduction in order to define the metabolic importance of amino acids, both for oocyte development, and embryonic and larval nutritional intake.

We can conclude that the level of 1.80% lysine promoted greater growth and consequently higher amount of oocytes produced in the in relation to the other treatments.

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8. CONSIDERAÇÕES FINAIS

No Brasil, ainda, são escassas as informações relacionadas à nutrição *versus* reprodução, conhecimentos, que podem trazer benefícios significativos para a aquicultura comercial. Neste mesmo cenário, são praticamente inexistentes pesquisas sobre a avaliação de aminoácidos na fase reprodutiva e larval dos peixes nativos.

As investigações sobre os efeitos dos aminoácidos são necessárias para a elaboração de dietas ambiental e economicamente sustentáveis que promovam adequado desenvolvimento reprodutivo aos peixes. Dessa forma, estudos nesse sentido devem ser aprimorados para o desenvolvimento de um pacote tecnológico adequado à cadeia produtiva das espécies nativas.

Durante o período exterimental várias dificuldades foram encontradas, impossibilitanto a avaliação de outros parâmetros que poderiam reforçar os resultados observados, todavia, os resultados avaliados demostram a importância da continuidade dos estudos.