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ESCOLA DE VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

ESTRESSE DO TRANSPORTE EM RÃ-TOURO: RESPOSTAS HEMATOLÓGICAS E BIOQUÍMICAS

BRUNO DIAS DOS SANTOS

BELO HORIZONTE – MG
2020

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ESTRESSE DO TRANSPORTE EM RÃ-TOURO: RESPOSTAS HEMATOLÓGICAS E BIOQUÍMICAS

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

Área de Concentração: Produção animal.

Prof. Orientador: Dr. Galileu Crovatto Veras
Co-orientador: Dr. Renan Rosa Paulino

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ESCOLA DE VETERINÁRIA DA UFMG
COLEGIADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA
Av. Antônio Carlos 6627 - CP 567 - CEP 30123-970 - Belo Horizonte- MG
TELEFONE: (31) - 3409 2173

www.vet.ufmg.br/academicos/pos-graduacao
E-mail cpgzootec@vet.ufmg.br

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Às 14:00h do dia 20 de fevereiro de 2020, reuniu-se, na Escola de Veterinária da UFMG a Comissão Examinadora de Dissertação, indicada pelo Colegiado na reunião do dia 05/12/2019 para julgar, em exame final, a defesa da dissertação intitulada.

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_____, como requisito final para a obtenção do Grau de **Mestre em Zootecnia, área de Concentração em Produção Animal Aquacultura.**

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

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Belo Horizonte, 20 de fevereiro de 2020.

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“Nunca é tarde demais pra começar tudo de novo...”

Raul Santos Seixas

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LISTA DE SIGLAS, ABREVIATURAS E SÍMBOLOS

ALT	Alanina-aminotransferase.
AST	Aspartato-aminotransferase.
ANOVA	Análise de Variância.
ºC	Graus Celsius.
CEUA	Comissão de Ética no Uso de Animais – UFMG.
CHCM	concentração de hemoglobina corpuscular média.
cm	Centímetro.
dL	Decilitro.
g	Grama.
HCM	Hemoglobina corpuscular média.
L	Litro.
LAQUA	Laboratório de Aquacultura da UFMG.
µL	Microlitro.
m	Metros.
m^2	Metros quadrados.
mg	Miligrama.
ml	Mililitro.
mm	Milímetro.
ng	Nanograma.
PB	Proteína Bruta.
UFMG	Universidade Federal de Minas Gerais.
U/L	Unidade Internacional.
VCM	Volume corpuscular médio.

RESUMO

Com o estudo objetivou-se avaliar a recuperação da homeostase de rã-touro (*Lithobates catesbeianus*) após o transporte. O experimento foi realizado em um delineamento inteiramente casualizado em esquema fatorial com um tratamento adicional [5 x 2 + (1)]. Foram avaliados cinco tempos após o transporte (0 – imediatamente após o transporte, 6, 12, 24 e 48 horas), em ambos os gêneros e um tratamento controle (condição antes do transporte). Os tratamentos foram constituídos de cinco repetições, sendo o animal a unidade experimental. Setenta rãs, 35 machos (352,12 ± 34,67 g) e 35 fêmeas (375,87 ± 48,10 g) foram submetidas a um jejum de 42 horas antes do transporte. Destas, 10 (cinco machos e cinco fêmeas) foram utilizadas para coleta de sangue antes do transporte (controle). As outras 60 foram transportadas por nove horas em cinco canos de PVC de 1 m e 100 mm de diâmetro, na densidade de 12 animais/ cano. Ao final do transporte, as rãs foram distribuídas em cinco baias alagadas (1,15 x 1,06 m) na densidade de 12 rãs/ baia (seis machos e seis fêmeas), até os tempos de avaliação pré-estabelecidos. Para cada horário de avaliação foi coletado o sangue de 10 rãs (cinco machos e cinco fêmeas). Com as amostras de sangue foram realizadas as seguintes análises: glicemia, lactato, proteínas totais, albumina, globulinas, relação albumina/ globulina (A:G), triglicerídeos, colesterol total, AST, ALT, hemoglobina, hematócrito, contagem total de eritrócitos e índices hematimétricos absolutos (VCM, HCM e CHCM). Não houve interação significativa dos horários de coleta e o gênero das rãs. A glicemia e o lactato aumentaram nas rãs do tempo 0 h, mas restabeleceram nos demais horários. A proteína total aumentou nas rãs do tempo 0 h, mas diminuiu nos tempos 24 e 48 h. A albumina aumentou nas rãs dos tempos 0 e 6 h, mas reduziu na coleta de 48 h. As globulinas aumentaram nas rãs do tempo 0 h, mas reduziram nos tempos 24 e 48 h. Os triglicerídeos aumentaram nas rãs do tempo 0 h, mas diminuíram nos demais horários. O colesterol total reduziu progressivamente nas rãs do tempo 0 h e foi maior nos machos. A AST aumentou nas rãs dos tempos 0 e 6 h, mas reduziu a partir do tempo 12 h. A ALT aumentou nas rãs do tempo 0 h, mas reduziu nos tempos 24 e 48 h. O hematócrito reduziu nas rãs do tempo 48 h. O número de eritrócitos aumentou nas rãs do tempo 0 h, mas reduziu no tempo 48 h. A hemoglobina diminuiu nas rãs do tempo 48 h. O VCM e a HCM diminuíram nos animais do tempo 0 h. A CHCM aumentou nas rãs avaliadas no tempo 24 h. As variáveis respondem de forma diferente ao estresse. No entanto, entre 6 e 12 horas, a maioria das variáveis avaliadas já retornaram a mesma condição do tratamento controle. A redução de algumas variáveis após 24 h pode ser em consequência do déficit energético promovido pelo jejum prolongado.

Palavras chave: anfíbio, anuro, ranicultura, hematologia, estresse.

ABSTRACT

The study aimed to evaluate the recovery of bullfrog (*Lithobates catesbeianus*) homeostasis after transport. The experiment was carried out in a completely randomized design in a factorial scheme with an additional treatment [5 x 2 + (1)]. Five times after transport were evaluated (0 - immediately after transport, 6, 12, 24 and 48 hours), in both genders and a control treatment (condition before transport). The treatments consisted of five repetitions, the animal being the experimental unit. Seventy frogs, 35 males (352.12 ± 34.67 g) and 35 females (375.87 ± 48.10 g) were fasted for 42 hours before transport. Of these, 10 (five males and five females) were used to collect blood before transportation (control). The other 60 were transported for nine hours in five PVC pipes of 1 m and 100 mm in diameter, in the density of 12 animals / pipe. At the end of the transport, the frogs were distributed in five flooded bays (1.15 x 1.06 m) in the density of 12 frogs / bay (six males and six females), until the pre-established evaluation times. For each evaluation time, blood was collected from 10 frogs (five males and five females). With blood samples, the following analyzes were performed: blood glucose, lactate, total proteins, albumin, globulins, albumin / globulin (A:G) ratio, triglycerides, total cholesterol, AST, ALT, hemoglobin, hematocrit, total erythrocyte count and absolute hematimetric indices (VCM, HCM and CHCM). There was no significant interaction between the collection times and the gender of the frogs. Glycemia and lactate increased in frogs from time 0 h, but reestablished at other times. The total protein increased in frogs from time 0 h, but decreased in time 24 and 48 h. Albumin increased in frogs from time 0 and 6 h, but decreased in the collection of 48 h. Globulins increased in frogs from time 0 h, but decreased in time 24 and 48 h. Triglycerides increased in frogs from time 0 h, but decreased in other hours. Total cholesterol decreased progressively in frogs from time 0 h and was higher in male. AST increased in frogs from time 0 and 6 h, but decreased from time 12 h. ALT increased in frogs from time 0 h, but decreased in time 24 and 48 h. The hematocrit reduced in the time frogs 48 h. The number of erythrocytes increased in frogs from time 0 h, but decreased in time 48 h. Hemoglobin decreased in frogs over 48 h. VCM and HCM decreased in animals from time 0 h. CHCM increased in the frogs evaluated at 24 h. The variables respond differently to stress. However, between 6 and 12 hours, most of the variables evaluated have already returned to the same condition as the control treatment. The reduction of some variables after 24 h may be a consequence of the energy deficit caused by prolonged fasting.

Keywords: amphibian, anuran, raniculture, hematology, stress.

1. INTRODUÇÃO

A rã-touro (*Lithobates catesbeianus*) é nativa da América do Norte (Estados Unidos e Canadá), sendo a única espécie permitida de ser criada em cativeiro no Brasil. Apresenta carne com baixo teor de gordura, proteína de boa qualidade e alta taxa de digestibilidade. É vendida principalmente congelada e inteira, podendo também ser comercializada apenas as coxas, parte mais nobre do animal e que, portanto, possui maior valor. No entanto, apesar dos altos preços praticados na comercialização da carne de rã, ainda é difícil encontrar o produto nas gôndolas dos mercados. Isso porque é baixa a quantidade de produtores inseridos na atividade, o que gera um ciclo vicioso, no qual a criação em pequena escala, aumenta o custo de produção, que por sua vez eleva o preço do produto, levando a um consumo restrito a apenas uma parcela da população.

Dentre outros motivos, o baixo número de ranicultores pode ser atribuído à carência de informações sobre o cultivo do animal, principalmente as que priorizam o desenvolvimento de novas tecnologias, como técnicas de manejo que minimizam o estresse dos animais durante as rotinas realizadas no ranário. Nos sistemas de criação, uma das práticas de manejo mais comuns, e que causam um elevado grau de estresse nos animais, é o transporte de animais vivos. Esta prática é considerada um procedimento traumático por expor os animais a uma série de manejos como captura, confinamento, manuseio, adensamento e o transporte propriamente dito.

Em situação de estresse, ocorre a ativação de dois eixos neuroendócrinos. O primeiro é o Hipotálamo-Sistema Nervoso Simpático-Células Cromafins, que resulta na liberação das catecolaminas (adrenalina e noradrenalina). O segundo é o eixo Hipotálamo-Hipófise-Glândula Suprarrenal, que culmina na liberação dos corticosteroides (cortisol e cortisona). Assim, a ação desses hormônios em diversos órgãos-alvos resulta em modificações bioquímicas e fisiológicas (Perry e Laurent, 1993; Wendelaar Bonga, 1997) responsáveis pela regulação da homeostase. Desta forma, mesmo em situações em que não há mortalidade, o monitoramento de variáveis fisiológicas durante operações estressantes, como o transporte, pode trazer resultados importantes para o estabelecimento de práticas adequadas de manejo. Portanto, com o presente estudo, objetiva-se avaliar a recuperação da homeostase de adultos de rã-touro após o transporte.

2. OBJETIVOS

2.1. Geral

Avaliar a recuperação da homeostase de rã-touro (*Lithobates catesbeianus*) após o transporte e entre os sexos.

2.2. Objetivos específicos

- Avaliar o eritrograma de rã-touro em diferentes tempos após o transporte;
- Averiguar o comportamento de algumas variáveis bioquímicas (glicose, proteína total, globulina, albumina, relação A:G, colesterol, triglicerídeos, lactato, ALT e AST) relacionadas ao estresse em diferentes tempos após o transporte.

CAPÍTULO I

3. REVISÃO BIBLIOGRÁFICA

Taxonomia da rã-touro *Lithobates catesbeianus* (Shaw, 1802);

Classe: Amphibia;

Ordem: Anura;

Família: Ranidae;

Gênero: *Lithobates*;

Espécie: *Lithobates catesbeianus.*

Figura A: Rã-touro, *Lithobates catesbeianus* (Shaw, 1802),
Fonte: Setor de ranicultura – UFMG.

A rã-touro, *Lithobates catesbeianus* (Shaw, 1802), é um ranídeo naturalmente distribuído no leste da América do Norte (Frost, 2008). Por se tratar de um anfíbio apresenta duas fases de vida, uma exclusivamente aquática e outra terrestre. Na fase inicial de vida, conhecida com girino, possui respiração branquial e cauda utilizada na natação. Durante o processo de metamorfose desenvolve membros posteriores e anteriores e absorve a cauda e as brânquias, passando a habitar ambientes úmidos (Lima e Agostinho, 1992).

A espécie adaptou-se bem ao clima tropical, apresentando bom desempenho zootécnico na criação quando comparada com as espécies nativas do Brasil, rã-manteiga

(*Leptodactylus latrans* Linnaeus, 1758) e rã-pimenta (*Leptodactylus labyrinthicus* Spix, 1824) (Figueiredo, 2005). Apresenta alta rusticidade, número elevado de ovos por postura, precocidade e carne com bom valor nutricional. Diversos são os meios de produção de rã-touro existentes, sendo alguns já consolidados, como o sistema anfigranja, confinamento e inundado, assim como suas variações.

A ranicultura iniciou no Brasil no inicio em 1935. Há época o Canadense Tom Cyrril Harrison trouxe 300 exemplares de rã-touro americana (*Lithobates catesbeianus*) do Canada para a Baixada Fluminense, no estado do Rio de Janeiro. A partir desses animais originou -se o primeiro ranário brasileiro, o Ranário Aurora, situado nas proximidades da Rodovia Presidente Dutra, Município de Itaguaí, no Estado do Rio de Janeiro (Vizotto, 1975; Lima e Agostinho, 1992; Ferreira et al., 2002).

No entanto, apesar da ranicultura ser uma atividade antiga no Brasil, a maioria das fazendas comerciais de rãs observam uma significativa mortalidade de indivíduos devido à ação de agentes estressores como deficiência alimentar, baixa qualidade da água, instalações inadequadas e má gestão do manejo (Rocha et al*.,* 2010; Teixeira et al., 2012), que podem promover a incidência de doenças e a consequente mortalidade dos animais. Dentre as práticas de manejo mais comum nos sistemas de criação, destaca-se o transporte de animais vivos, que por ser um procedimento traumático por expor os animais a uma série de manejos como captura, confinamento, manuseio, adensamento e o transporte propriamente dito, causam um elevado grau de estresse nos animais.

O estresse é uma resposta não específica do ser vivo a uma situação a qual passou, podendo ou não acarretar em um gasto extra de energia para a adequação à condição submetida para o restabelecimento da homeostase (Martínez-porchas et al*.,* 2009). Porém, quando o estresse é intenso e duradouro, a homeostasia pode não ser reestabelecida, acarretando prejuízos ao seu estado de saúde (Oliveira e Galhardo, 2007).

As diversas rotinas de manejo da aquicultura como despesca, embalagem, vacinação e transporte sujeitam os animais ao estresse, que em demasia pode acarretar redução de crescimento, doenças e mortalidade (Bendhack, 2004). Brandão et al*.,* (2006), mostram que as respostas ao estresse são divididas em respostas primárias, secundárias e terciárias, apresentando como sintomas alterações hormonais; mudanças de parâmetros bioquímicos e fisiológicos; comprometimento do desempenho, mudanças no comportamento e aumento da susceptibilidade a doenças, respectivamente.

As respostas primárias são responsáveis pela ativação dos centros cerebrais que resultam na liberação de catecolaminas e corticosteroides. A resposta primária ao estresse está

relacionada inicialmente à percepção do estímulo estressor pelo sistema nervoso central. Após esta constatação, o sistema nervoso simpático, por nervos eferentes, estimula as células do tecido cromafim na glândula suprarrenal a sintetizar as catecolaminas: noradrenalina e adrenalina. Ocorre ainda a liberação do hormônio fator liberador de corticotrofina (CRF) pelo hipotálamo, que por sua vez estimula a hipófise a liberar o hormônio adrenocorticotrófico (ACTH). O ACTH estimula as células do córtex da adrenal da glândula suprarrenal para a produção de cortisol e de hormônios corticosteróides (Duellman e Trueb, 1986). Estes hormônios, por sua vez, iniciam uma série de mudanças bioquímicas e fisiológicas, conhecidas como respostas secundárias ao estresse.

Os efeitos metabólicos das respostas secundárias incluem ativação do sistema cardiovascular, hiperglicemia, hiperlactatemia, depleção das reservas glicogênicas, lipólise e inibição da síntese proteica. Ocorre ainda, aumento do catabolismo de proteínas musculares e alterações nos níveis plasmáticos de aminoácidos, ácidos graxos livres e colesterol (Milligan, 2003; Rocha et al., 2004). Além destas respostas, há elevação dos valores de hematócrito e aumento do volume dos eritrócitos e número de eritrócitos circulantes, com consequente elevação da concentração de hemoglobina (Soldatov, 1996; Mariano, 2006).

Após esse conjunto de modificações, controlado pelo sistema neuroendócrino na tentativa de recuperação da homeostase, é conduzida a chamada resposta terciária ao estresse. Esse estágio é caracterizado pela quebra total da homeostase e consequente redução do crescimento, sucesso reprodutivo, resistência do organismo frente a infecções, doenças e sobrevivência (Pankhust e Van Der Kraak, 1997). Portanto, neste período de resposta ao estresse há uma redução na imunidade para direcionar os recursos afim de desenvolver atividades mais importantes para a sobrevivência (Sapolsky et al*.,* 2000).

Parâmetros hematológicos demonstram ser um método alternativo na análise dos efeitos do estresse que causam alterações qualitativas e quantitativas na estrutura celular do sangue (Allender e Fry, 2008). Os manejos inadequados na ranicultura acarretam estresse que podem influenciar o aparecimento de alterações no hemograma em animais da espécie *Lithobates catesbeianus* (Rocha et al*.,* 2010, Teixeira et al*.,* 2012). Geralmente os vertebrados respondem aos agentes estressores em seus ambientes elevando os níveis de glucocorticosteróides supra-renais, corticosterona ou cortisol, dependendo da espécie (Romero, 2002).

A corticosterona, o cortisol e a glicose, juntos com constituintes sanguíneos adicionais, geralmente são usados para avaliar o estresse. Porém, a corticosterona parece ser mais expressiva em anfíbios (Belden et al., 2003, 2010). No entanto, o cortisol também foi

encontrado em girinos de *Rana catesbeiana* e em plasma de animais adultos (Krug et al*.,* 1983; Wright et al., 2003). Desta forma, a opção por utilizar o cortisol como um biomarcador é justificada, já que análise de corticosterona é expressivamente mais difícil e onerosa.

Após a exposição ao agente estressor, o balanço energético é normalizado através do aumento da concentração plasmática de glicocorticóides, que diminui a expressão de CRF via feedback negativo voltando a estimular o apetite (Sapolsky et al*.,* 2000). Neste caso, em estudos com anfíbios a glicose aparece comumente na regulação do balanço energético (Coppo et al*.,* 2005). Outra variável utilizada na mensuração de estresse para anfíbios são os padrões de leucócitos (Davis et al*.,* 2008; Shutler et al., 2009). Portanto, é aconselhável considerar a hematologia no diagnóstico para avaliação de qualquer anfíbio doente (Teixeira et al*.,* 2012) ou para mensuração de uma possível atuação de um agente estressor.

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CAPÍTULO II

Artigo: Transport stress in bullfrog: Hematological and plasma biochemical responses

Artigo preparado para submissão na revista Aquaculture Research

Transport stress in bullfrog: Hematological and plasma biochemical responses

Abstract

This work aims to evaluate the effect of transport stress on the hematological and biochemical responses in males and females of bullfrogs (*Lithobates catesbeianus*). Frog conditions were evaluated before and at five times after transport (0, 6, 12, 24, and 48 h) for both genders. Glycemia increased in the frogs evaluated at time 0 h and recovered 24 h after transport. Lactate increased in the animals evaluated at time 0 h, recovered at time 6 h and reduced 48 h after transport. Aspartate-aminotransferase (AST) increased in animals analysed 0, 6, and 12 h and recovered 24 h after transport. Alanine-aminotransferase (ALT) increased in the frogs analysed at time 0 h, recovered at time 6 h and decrease 48 h after transport. Total proteins, albumin and globulins decreased 48 h after transport. Triglycerides and total cholesterol decrease in the animals evaluated 24 and 48 h after transport. Males had high levels of total cholesterol. Number of erythrocytes increased in the animals evaluated 0 and 6 h after transport and recovered 12 h after transport. Mean corpuscular volume (MCV) of the frogs evaluated 0 and 6 h after transport were lower and recovered 12 h after transport. Hematocrit and hemoglobin decrease 48 h after transport. Mean corpuscular hemoglobin (MCH) decreased in the animals evaluated 0, 6, and 12 h and recovered 24 h after transport. Frogs demonstrated rapid recovery of homeostasis after transport, between 6 and 24 h. Between 24 and 48 h after transport, a decrease in energy reserves and red blood series is observed, caused by prolonged fasting.

Keywords: Bullfrog farming, hematology, *Lithobates catesbeianus,* stress management.

1. Introduction

Native to North America, the United States and Canada (Frost, 2008), the bullfrog (*Lithobates catesbeianus*) has a meat of excellent nutritional quality, with adequate balance of amino acids and low levels of triglycerides and cholesterol (Casali et al., 2005; Pires et al., 2006). Frog meat is appreciated in several countries (Hsu et al., 2011) where it is sold whole or only the legs, the most noble part of the animal.

Most of the literature available on the raising of frogs in captivity is about the bullfrog, as it is the most cultivated species (Alvarez and Real, 2006). However, there is still a lack of

studies on the cultivation of frogs, especially those that prioritize the development of new technologies, such as management techniques that prioritize the welfare of animals during the routines performed in the bullfrog farming. Among the management techniques for raising frogs, the transport of live animals is a quite common practice. Frogs must be transported in closed containers that allow only small movements (Cribb et al., 2013) to avoid injuries during transport. These containers must have small holes that allow air to enter, thus preventing the animals from suffocation and death. The frogs normally are transported inside puncture polyvinyl chloride (pvc) tubes of 100 mm diameter, nylon bags and punctured plastic boxes with the frogs free or inside nylon bags.

Transport can be considered stressful procedure, since it exposes the frogs to a series of management such as the capturing of the frogs in the pens, the handling animals, densification, and transport itself. In response to the stressing agent, catecholamines are secreted by the chromaffin cells of the interrenal gland of amphibians after activation of the sympathetic nervous system, which promotes rapid increase of these hormones (Herman, 1977). Amphibians also activate the hypothalamus-pituitary-interrenal axis in a stressful situation and this axis make mediation of the animal's response to its environment (Moore and Jessop, 2003; Rollins-Smith, 2017). After the perception of stressor agent, the hypothalamus produces the corticotrophin-releasing hormone (CRH), which stimulates the pituitary to produce the adrenocor-ticotrophic hormone (ACTH). In turn, ACTH acts on cortex cells of interrenal gland, where it stimulates steroidogenic cells in the production of corticosteroid hormones (Rollins-Smith, 2017).

In response to the stressor, a series of rapid structural, metabolic and hematological physiological adaptations is necessary for the restoration of the organism's homeostasis and its survival. In these circumstances, there is an increase in energy demand, with catecholamines and glucocorticoids acting in the mobilization of energy reserves. This mobilization occurs mainly in hepatic glycogen (Mbangkollo and deRoos, 1983; Broughton and deRoss, 1984; Rosenthal and deRoos, 1985) and muscular (Mbangkollo and deRoos, 1983), as well as in the tissues that store lipids (Farkas, 1969; Harri and Puuska, 1973; Migliorini et al., 1992; Sheridan and Kao, 1998) to supply the energy demand due to the attempt to restore the organism's homeostasis. With the increase in energy demand, there is also an increase in oxygen consumption by the tissues. Therefore, hematological changes, such as changes in hemoglobin concentration (Palenske and Saunders, 2003), the number of erythrocytes, as well as the volume of these cells (Boutilier and Shelton, 1986; Peng et al., 2016) are necessary to increase efficiency transport of oxygen to the tissues.

Thus, the knowledge of physiological changes during the handling, such as transport, can bring important results for the establishment of appropriate management practices for these animals, since this type of works is rare in literature. Therefore, the aim of the present study was to evaluate the hematological and biochemical responses of bullfrogs, males and females, after transportation.

2. **Material and methods**

2.1. *Pre-experimental condition and design experimental*

The research was carried out according to protocol nº132/2018, approved by the Ethics Committee on the Use of Animals (CEUA) of the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil.

Seventy bullfrogs (*Lithobates catesbeianus*), 35 males (352.12 ±34.67 g) and 35 females (375.87 ±48.10 g), were used. The frogs were from at bullfrog farming at Universidade Federal de Uberlândia (UFU), Uberlândia, Minas Gerais, Brazil. The animals were kept in fattening pens of 10 m^2 (2.5 ×4.0 m), in a flooded system with a stocking density of 50 frogs m^{-2}. The fattening pens are inside a covered shed. Natural light illuminates this shed, as it contains areas with transparent roof tiles. The frogs were transferred to these fattening stalls when they still had 50 g and remained there for approximately 4 months until they reached the average weight of the experiment.

The frogs were fed at a feed rate of 1.5 % of body weight, divided into three daily feeds. An extruded feed for carnivorous fish of 8 – 10 mm was used. The feed contained 42 % crude protein, 9 % ethereal extract, and 4 % crude fiber. The experiment was performed in a completely randomised design in a factorial scheme with an additional treatment [5 ×2 +(1)]. Frog conditions were evaluated before (control treatment) and at five times after transport (0, 6, 12, 24, and 48 h) for both genders. The treatments were consisted of five repetitions, the frog being the experimental unit.

2.2. *Transport*

After a 42 h fast (Seixas Filho et al., 2017), 10 frogs (five males and five females) were randomly captured for blood collection before transportation (control treatment). The other 60 specimens were placed in five polyvinyl chloride (pvc) tubes of 1.00 m length and

100.00 mm diameter at a density of 12 frogs per tube (six males and six females). To prevent frogs from escaping, the tubes were closed with pvc connections of the same diameter. Throughout the length of the tube, holes were made to allow air to enter and prevent the frogs from dying by asphyxiation.

The frogs were transported by closed car, from the bullfrog farming at UFU to the bullfrog farming sector at the UFMG Veterinary School, Belo Horizonte, Minas Gerais, Brazil. The total route was 535.7 km, lasting approximately nine hours. The animals were transported throughout the day and part of the night. On the day of transport, the minimum temperature recorded was 18º C and the maximum 28º C.

2.3. *Blood collections, erythrogram and plasma biochemical analyses*

Upon arrival, immediately after transportation (time 0 h), blood was collected from 10 animals from one of the tubes. The remaining frogs were removed from the tubes and housed in flooded pens of 1.22 m^2 (1.15 ×1.06 m) in the density of 12 frogs / pen (six males and six females) until the pre-established evaluation times. For up to 48 h after transport, the animals were not fed.

Blood collection was performed through the posterior limb vessel, by puncture with 3 mL syringes and needles previously moistened in 10 % EDTA. Lidocaine 4 % was used as local external anaesthesia. For each evaluation time, blood was collected from 10 frogs (five males and five females), with 2.0 mL of blood/ frog. With an aliquot of 10 µL of blood, the glycemia of the animals was evaluated using a digital glucometer (ACON, On-Call® Plus, San Diego, USA). The remaining blood was stored in 2 mL microtubes under 4º C refrigeration for further analysis. The hematocrit (Ht) was determined by the microhematocrit method, by means of centrifugation at 19319 g for five minutes (12000 rpm and centrifuge radius – 12 cm) of the microcapillaries containing the blood samples. The rate of total hemoglobin (Hb) was determined by the cyanmethemoglobin method, with a reading on a spectrophotometer with a wavelength of 540 nm. The erythrocyte count (Er) was performed in a Neubauer chamber with the aid of a light microscope, with 400 X magnification. Then, the following absolute hematimetric indices were calculated: mean corpuscular volume – MCV (fL) = Ht x 10 / Er; mean corpuscular hemoglobin – MCH (pg) = Hb x 10 / Er and mean corpuscular hemoglobin concentration – MCHC (g dL^{-1}) = Hb x 100 / Ht. All erythrogram variables were evaluated using the methodology described by Ranzani-Paiva et al. (2013).

The rest of the collected blood was centrifuged at 1006 g for 15 min (3000 rpm and centrifuge radius – 10 cm) in a microtube centrifuge (Biovera® model RB1), the plasma was collected and stored at – 80º C. Subsequently, at the Clinical Pathology Laboratory of the Clinical and Veterinary Surgery Department of the Veterinary School of UFMG, Belo Horizonte, Minas Gerais, Brazil, using the biochemical analyser COBAS MIRA PLUS®, cholesterol, triglycerides, albumin, lactate, total proteins, alanine-aminotransferase (ALT), and aspartate-aminotransferase (AST) were analysed by commercial kits (Biotécnica®). Globulins were determined by the difference between total proteins and albumin. The albumin/ globulin ratio (A/G) was also determined. All plasma biochemical variables were evaluated using the methodology described by Burtis and Ashwod (1998).

2.4. *Survival*

Survival was observed immediately after transport and at others blood collection times, ending 48 h after transport.

2.5. *Statistical analysis*

At the end, the data were analysed in Software R 3.5.3, being submitted to Shapiro Wilk and Bartlett's tests to assess the normality and homoscedasticity of the variances, respectively. An ANVOA – two way (5 × 2) was performed to verify the existence of interaction between the factors, collection time and gender, or the isolated effect of each factor. Subsequently, according to the result of the factor analysis, Dunnett's test was used to compare the additional treatment (control) with all the collection times. Significant differences were considered when $P < 0.05$. To meet normality and or homoscedasticity, the variables lactate, triglyceride, AST, and ALT were transformed into natural logarithm (ln), while the variables glucose, MCV, and MCH were transformed into exponential $(x)^{-0,5}$.

3. **Results**

3.1. *Survival*

There was no mortality during transport and 48 h after transport management.

3.2. *Biochemical variables*

There was no interaction ($P > 0.05$) between the blood collection times of the frogs after transport and the gender of the animals on the plasma biochemical variables evaluated. The blood collection times did not influence ($P > 0.05$) only the albumin / globulin ratio (A/G), demonstrating an effect ($P < 0.05$) on all other measured plasma biochemical. The gender of the animals influenced ($P < 0.05$) only the total cholesterol, demonstrating no effect ($P < 0.05$) on all other variables.

Glycemia increased in the frogs evaluated immediately after transport (time 0 h) and remained high in animals measured at 6 and 12 h after transport. Glucose recovered in the frogs analysed at times 24 and 48 h after transport, not differing from the animals of the control treatment (Fig. 1).

Plasma lactate increased in the animals evaluated immediately after transport in relation to the levels of the frogs of the control treatment. The lactate values of the specimens evaluated at times 6, 12, and 24 h after transport did not differ from levels of the animals of the control treatment. On the other hand, the specimens evaluated at time 48 h after transport showed a reduction in lactate levels compared to the frogs measured before transport (Fig. 1).

The values of total proteins, albumin, and globulins in the frogs evaluated at times 0, 6, 12, and 24 h after transport did not differ from the levels of the animals of control treatment. However, the levels of these variables decreased in the frogs evaluated 48 h after transport in relation to the values of the animals belonging to the control treatment (Fig. 1).

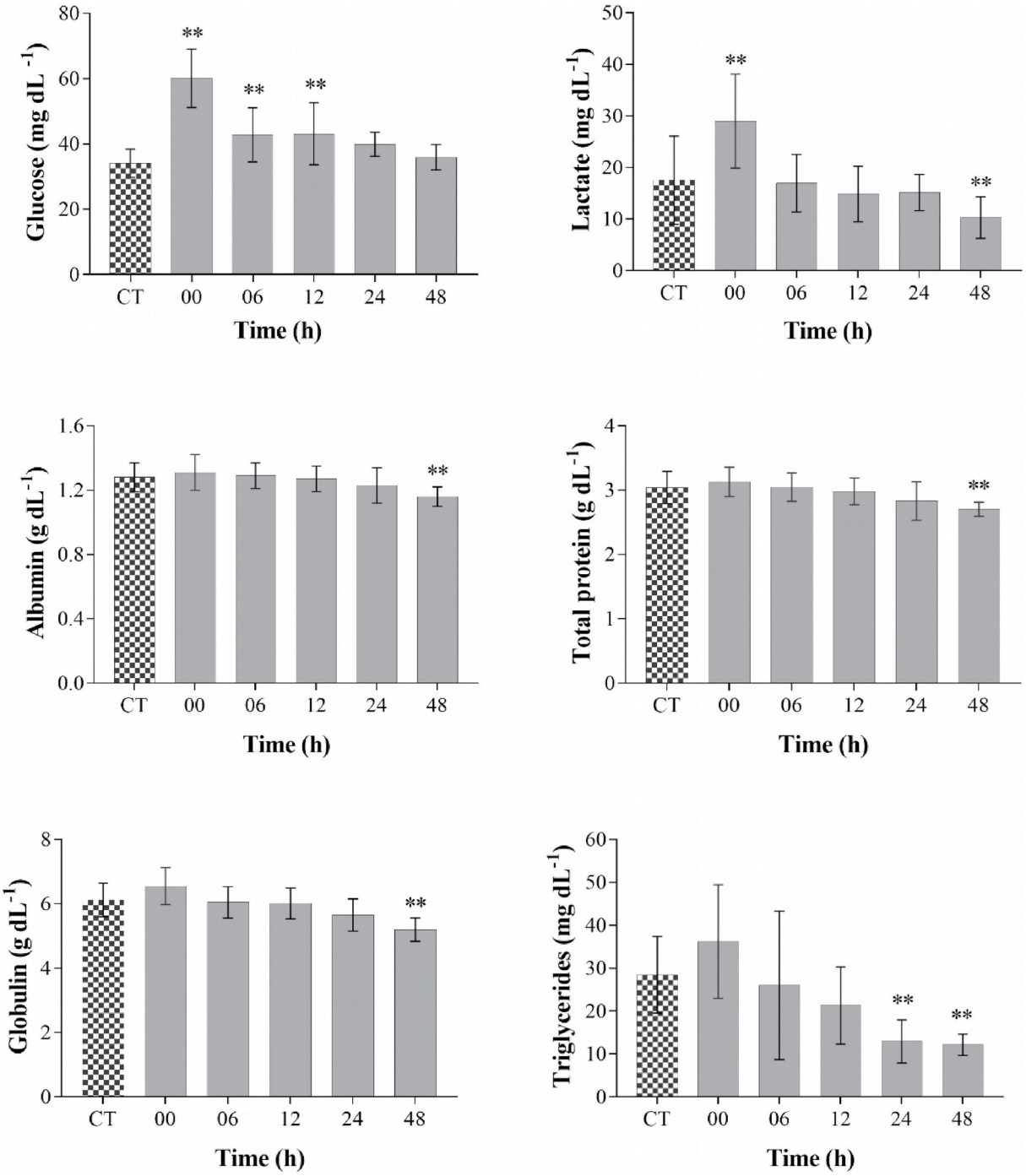

Fig. 1.Bars represent mean ±standard deviation of the variables glucose, lactate, total proteins, albumin, globulins, and triglycerides of bullfrogs before and after transport. CT – control treatment (animals in homeostasis sampled before transport); animals sampled immediately after transport; 06 – animals sampled 6 h after transport; 12 – animals sampled 12 h after transport; 24 – animals sampled 24 h after transport; 48 – animals sampled 48 h after transport. ** Significant difference ($P < 0.01$) in relation to the control treatment by the Dunnett's test.

Triglycerides levels and total cholesterol of the frogs evaluated at times 0, 6, and 12 h after transport did not differ from the values of the animals of the control treatment. However,

there was a decrease in the levels of these variables in the frogs evaluated at 24 and 48 h after transport in relation to the animals of the control treatment (Figs. 1 and 2A). Bullfrog males had higher total cholesterol values (Fig. 2B).

The plasma aspartate-aminotransferase enzyme (AST) increased in the animals evaluated immediately after transport and remained elevated in the frogs analysed at times 6 and 12 h after transport. AST re-established in the animals evaluated at times 24 and 48 h after transport, not differing from the frogs of the control treatment (Fig. 3).

The plasmatic alanine-aminotransferase enzyme (ALT) increased in the animals evaluated immediately after transport. In the frogs analysed at times 6, 12, and 24 h after transport, there was a restoration of the levels of this variable in relation to the animals of the control treatment. However, in the frogs evaluated at time 48 h after transport, there was a reduction in ALT levels in relation from control treatment specimens (Fig. 3).

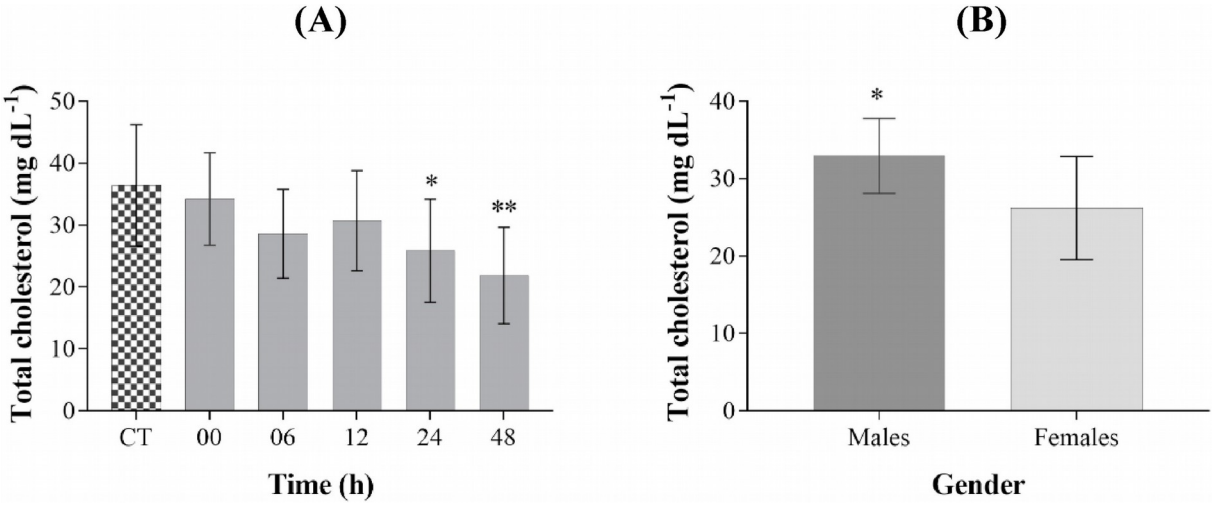

Fig. 2.(A) Bars represent mean ±standard deviation of the variable total cholesterol of bullfrogs before and after transport. CT – control treatment (animals in homeostasis sampled before transport); animals sampled immediately after transport; 06 – animals sampled 6 h after transport; 12 – animals sampled 12 h after transport; 24 – animals sampled 24 h after transport; 48 – animals sampled 48 h after transport. ** Significant difference ($P < 0.01$) in relation to the control treatment by the Dunnett's test. * Significant difference ($P < 0.05$) in relation to the control treatment by the Dunnett's test. (B) The bars represent mean ±standard deviation of the total cholesterol variable in bullfrogs males and females. * Significant difference ($P < 0.05$) by the ANOVA.

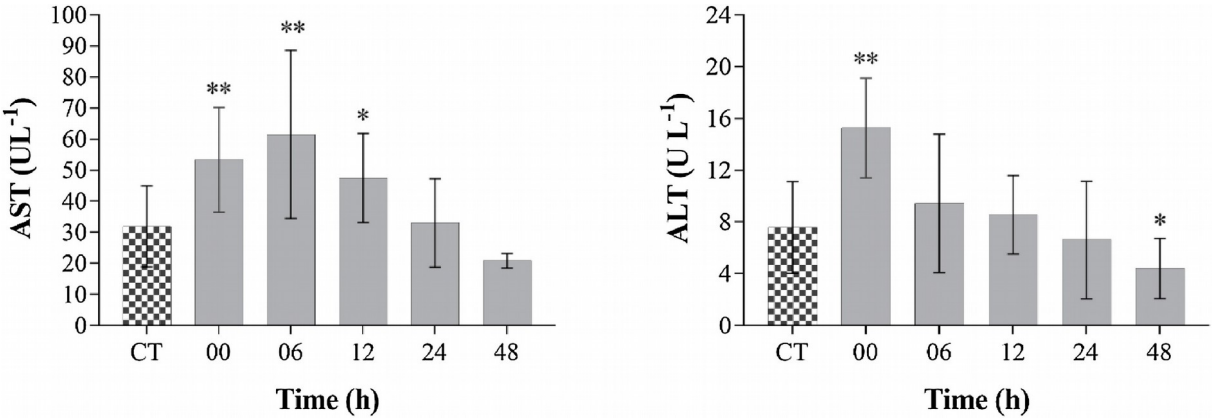

Fig. 3.Bars represent mean ±standard deviation of the plasma variables aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) of bullfrogs before and after transport. CT – control treatment (animals in homeostasis sampled before transport); animals sampled immediately after transport; 06 – animals sampled 6 h after transport; 12 – animals sampled 12 h after transport; 24 – animals sampled 24 h after transport; 48 – animals sampled 48 h after transport. ** Significant difference ($P < 0.01$) in relation to the control treatment by the Dunnett's test. * Significant difference ($P < 0.05$) in relation to the control treatment by the Dunnett's test.

3.3.*Erythrogram variables*

There was no significant interaction ($P > 0.05$) between the blood collection times of the frogs after transport and the gender of the animals on the erythrogram variables. Likewise, the gender of the frogs did not influence ($P > 0.05$) these variables. Blood collection times of the animals did not influence ($P > 0.05$) only the mean corpuscular hemoglobin concentration (MCHC), showing an effect ($P < 0.05$) on all the other variables in the erythrogram.

The number of erythrocytes of the animals evaluated at times 0 and 6 h after transport increased in relation to that of the frogs of the control treatment. The number of erythrocytes recovered in the animals evaluated at times 12, 24, and 48 h after transport, not differing from the frogs of the control treatment (Fig. 4).

The mean corpuscula volume (MCV) values of the frogs evaluated at times 0 and 6 h after transport were lower in relation to the animals of the control treatment. The MCV re-established in the frogs evaluated at times 12, 24, and 48 h after transport, not differing from the animals of control treatment (Fig. 4).

There was a reduction in the hematocrit and hemoglobin of the frogs evaluated at time 48 h after transport in relation to the animals of the control treatment. The values of this

variable in the animals of the other collection times did not differ from the frogs of control treatment (Fig. 4).

The mean corpuscular hemoglobin (MCH) of the animals evaluated at times 0, 6, and 12 h after transport showed lower values than that of the frogs of the control treatment. MCH recovered in the animals evaluated at times 24 and 48 h after transport, not differing from the values of the frogs of the control treatment (Fig. 4).

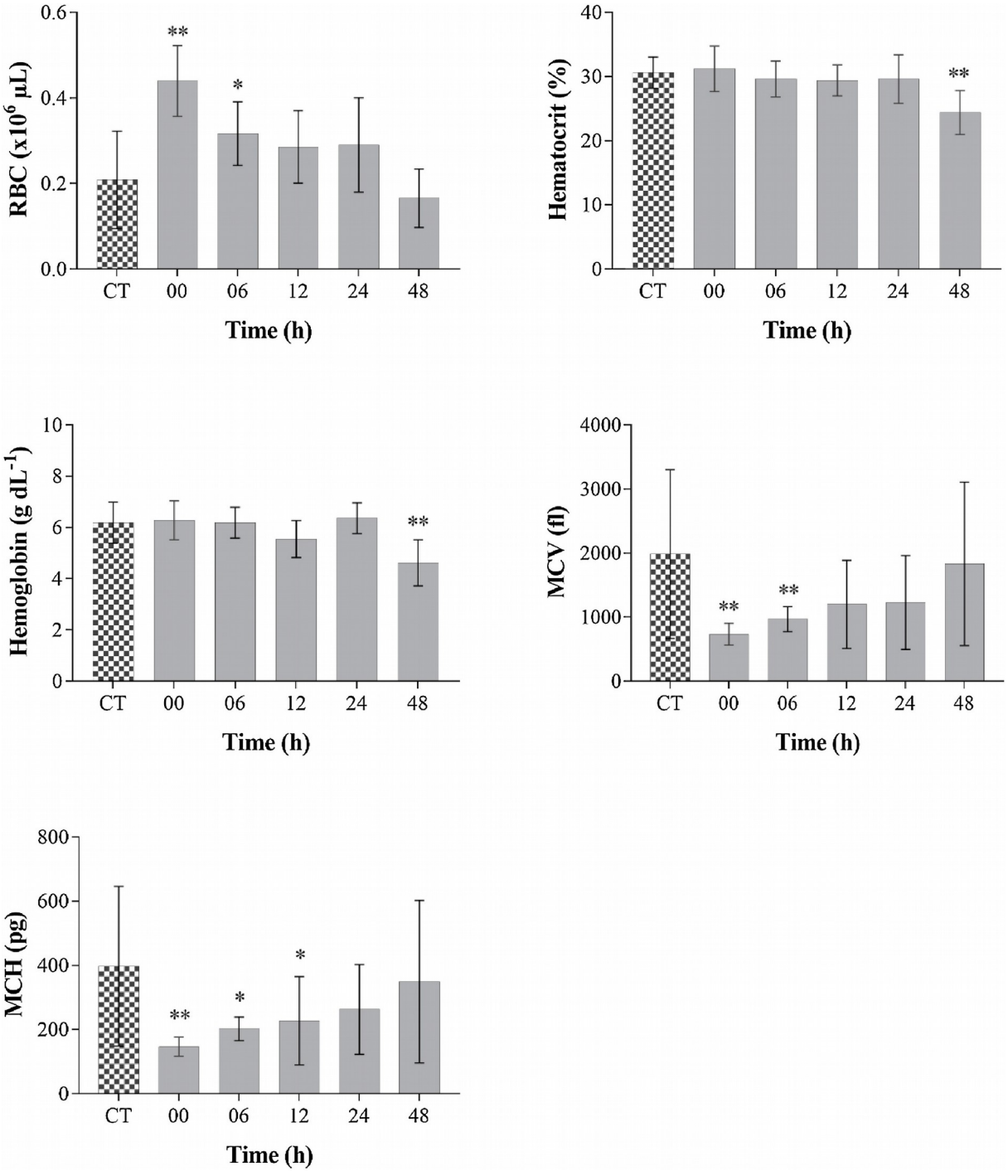

Fig. 4.Bars represent mean ±standard deviation of the variables number of erythrocytes (RBC), hematocrit, hemoglobin, medium corpuscular volume (MCV), and medium corpuscular hemoglobin (MCH) of bullfrogs before and after transport. CT – control treatment (animals in homeostasis sampled before transport); animals sampled immediately after transport; 06 – animals sampled 6 h after transport; 12 – animals sampled 12 h after transport; 24 – animals sampled 24 h after transport; 48 – animals sampled 48 h after transport. ** Significant difference ($P < 0.01$) in relation to the control treatment by the Dunnett's test. * Significant difference ($P < 0.05$) in relation to the control treatment by the Dunnett's test.

4. **Discussion**

A long time ago glucose has been an excellent indicator of stress in studies with amphibians, showing an increase in plasma levels after the action of a stressor agent. (Herman, 1977; Broughton and deRoss, 1984; Rosenthal and deRoos, 1985). This increase is one of the secondary responses to stress and is necessary to meet the body's energy demand in these circumstances. According to Herman (1977), in addition to the probable role of adrenaline in the fast increase in plasma glucose levels in response to the stressor agent, hormones of the pituitary-adrenocortical axis may also be involved. Thus, both adreno-corticotropic hormone (ACTH) as well as glucocorticoids cause an increase in plasma glucose levels in bullfrogs as result of the breakdown of hepatic glycogen (Broughton and deRoss, 1984; Rosenthal and deRoos, 1985). In the present study, a situation of hyperglycemia was observed in the animals evaluated immediately after stress, returning to baseline levels 24 h after the end of transport, indicating a rapid recovery of homeostasis in relation to this variable. The increase in glycemia was also verified by Sounderraj et al. (2011) when individuals from *Rana tigrina* were subjected to poisoning by the organophosphate insecticide phosphamidone; by Mbangkollo and deRoos (1983) when bullfrog specimens were subjected to different dosages of adrenaline and noradrenaline and by Harri (1981) when specimens of *Rana temporaria* were subjected to a stirring process, using an automatic stirrer (60 cps min^{-1}) for 1 h.

Lactate is also an important physiological indicator for evaluation stress in amphibians (Bennett and Licht, 1974; Christiansen and Penney, 1973; Fournier and Guderley, 1992; Harri, 1981; Mbangkollo and deRoos, 1983; Fournier and Guderley, 1992). "Vigorous muscle activity" (Fournier and Guderley, 1992) and stress, such as hypoxia and low temperatures (Christiansen and Penney, 1973), have been shown to promote glycogen mobilization through

anaerobe glycolysis, with lactate dehydrogenase (LDH) being the final enzyme that catalyses the reduction of pyruvate to lactate, promoting a rapid increase in lactate concentrations. Therefore, probably the densification associated with the stuffy container in which the frogs were transported induced the performance of anaerobic glycolysis by the muscles, resulting in elevated plasmatic lactate concentrations. Elevation of lactate concentrations was also observed 15 min after the brief manipulation of bull-frog adults for two minutes (Mbangkollo and deRoos, 1983). Increased lactate levels were also observed in *Rana temporaria* specimens recently captured and transported by car 200 km away and when these animals were subjected to a stirring process in the laboratory, using an automatic stirrer (60 cps min^{-1}) for 1 h (Harri, 1981).

In the present study, 6 h after transport, the frogs had already restored baseline plasma lactate standards. According to Hutchison and Turney (1975), lactate levels return to baseline levels when oxygen consumption returns to normal. According to the same authors, specimens of *Rana pipiens* re-established the baseline lactate pattern 4 h after the electric shock stimulus. The decrease in plasma lactate in frogs 48 h after transport was probably due to energy demand since these animals were fasting for a long period. However, it is also important for anurans to recover glycogen stores after a period of activity to serve as a substrate for a future need for anaerobic energy production (Withers et al., 1988). A study with *Rana pipiens*, hepatectomized and normal, showed that muscle is the main tissue responsible for recycling lactate after performing intense exercise (Fournier and Guderley, 1992). According to these authors, the liver plays an insignificant role in the reuse of this metabolite, not having the capacity to transform lactate into glucose. According to the authors, this inability to transform is not related to the absence of enzymes active in gluconeogenesis, but due to the liver's limitations in metabolizing lactate and/or its limited permeability in this organ. This ability to recycle lactate promotes a muscle glycogen sparing effect that may be related to the evolution of the species and its survival in dangerous circumstances.

In the present study, one cannot fail to take into account the possible interference of the circadian cycle on the biochemical and hematological variables analyzed. Hutchison and Turney (1975) were the first to demonstrate the presence of a daily rhythm in plasma lactate levels in amphibians. According to the authors, there is an increase in the concentration of this variable in *Rana pipens* at the beginning of the night, reaching its maximum level at 19:00, at which time the species has greater activity. Then, there is a decrease in plasma lactate concentrations until the end of the night period. Thus, the increase in lactate levels in bullfrog

samples evaluated immediately after transport (time 0 h) could also be attributed to this daily rhythmicity, since these frogs were evaluated at 20:00 and the treatment frogs control at 08:00. However, when comparing the control (evaluation at 08:00) with times 6 (evaluation at 02:00), 12 (evaluation at 08:00) and 24 h after transportation (evaluation at 20:00) it was found there were no differences in plasma lactate levels, even though the collection occurred at different times. In addition, there was a significant difference in the lactate concentration in the animals evaluated immediately after transport in relation to the control group, and this difference was not observed in the animals evaluated 24 h after transport, and the collections of times 0 and 24 were performed at the same time (at 20:00). Hutchison and Turney (1975) also demonstrated that there is no daily glycemic rhythm in *Rana pipens*. Therefore, it is very likely that the elevated glucose levels in the present study with bullfrogs were influenced by transport stress. In addition, for lactate and glucose levels, the action of the stressor is likely to have greater interference than the circadian cycle, requiring a study with a bullfrog to assess the interference of the circadian cycle and the seasons on the other variables hematological and biochemical.

According to Dornelles and Oliveira (2014), the decrease in proteins may indicate a physiological adaptation to compensate for the high energy demand caused by stress. Therefore, the decrease in total proteins, albumin and globulins of animals evaluated 48 h after transport may be related to a physiological regulation due to high energy demands, which stimulates the mobilization of amino acid reserves for gluconeogenesis. The decrease in the levels of this variable can also be related to the long period of fasting that these animals were submitted before transport until the end of blood collections, 99 h (42 h of fasting before transport plus, 9 h of transport and 48 h referring to the time of the last collection after transport). According to Coppo et al. (2005) albumin is an excellent indicator of protein biosynthesis, in addition to functioning as a reserve of amino acids. The albumin/ globulin ratio (A / G) of the frogs did not change in any evaluation time after transport. According to Gras (1983), the maintenance of the A / G ratio indicates a balance between plasma protein fractions, and this balance is maintained until the compensation mechanisms of stressor agent fail. The decrease in temperature also did not influence the A / G ratio of adults of the salamander *Batrachupems tibetanus* (Xia and Li, 2010).

Triglycerides represent most body lipids in anurans (Brown, 1964; Ryuzaki and Oonuki, 1990), with fatty bodies being the most abundant structure in this type of lipid (Brown, 1964). In the present study, the animals evaluated 24 and 48 h after transport showed triglyceride values below half in relation to the frogs evaluated in the control treatment.

During prolonged fasting, lower insulin levels and, consequently, there is a lower insulin/ glucagon ratio. McGarry et al. (1973) showed that liver isolates, when exposed to increasing concentrations of glucagon, increase ketogenesis and triglycerides production decreases. Therefore, the higher the rate of hepatic ketogenesis, the lower the proportion of free fatty acids available to be esterified in triglycerides.

In situations of increased energy demand, animals can mobilize energy through these lipids stored as triglycerides (Byrne and White, 1975). Stored triglycerides can be rapidly mobilized by various hormonal changes. Pituitary hormones, such as prolactin and ACTH, as well as those produced by the adrenal cortex, cortisol and corticosterone, are generally lipolytic in anurans (Sheridan and Kao, 1998). On the other hand, in these animals, catecholamines have not been shown to affect lipolysis, these hormones being responsible only for decreasing the levels of circulating fatty acids (Farkas, 1969; Harri and Puuska, 1973; Migliorini et al., 1992). Under stress, the liver increases triglyceride production, making this metabolite available in the bloodstream. Cortisol, catecholamines and glucagon stimulate phosphatidate-phospho-hydrolase, leading to increased hepatic triglyceride synthesis. In order to be transported in the blood to the target tissues, triglycerides are transported by the very-low-density lipoprotein (VLDL). When VLDL-linked triglycerides are released into the circulation, they undergo the action of lipoprotein lipase present in blood capillaries (Brindley et al., 1993). Norepinephrine also decreases hepatic lipase activity, which could increase plasma levels of VLDL and low-density-lipoprotein (LDL) (Niaura et al., 1992), triglyceride and cholesterol transporters. In addition, cortisol, norepinephrine and fatty acids can reduce insulin sensitivity (Niaura et al., 1992; Brindley et al., 1993), which is advantageous for the organism during stress, since it favors the use of glucose by the central nervous system. Consequently, insulin resistance causes less activity of lipoprotein lipase. The reduction in the activity of lipoprotein lipase causes less uptake of lipoproteins in peripheral tissues, retaining lipoproteins in the blood, which increases triglyceride and cholesterol levels (Rizza et al., 1982). This increase in triglyceride levels was probably not seen right after transport since the transport was long (approximately 10 h) and the animals were already on a long period of fasting. An increase in triglycerides levels was observed in adults of the frog *Pelophylax ridibundus* exposed to the herbicide glyphosate (Pãunescu and Ponepal, 2011), in adults of the salamander *Batrachupems tibetanus* exposed to low temperatures (Xia and Li, 2010). However, in the present study there was no increase in plasma triglyceride levels after transport of the frogs.

In the present study, cholesterol levels decreased in the 24 and 48 h after transport. In stressful situations, the reduction of cholesterol may be due to an inhibition of the biosynthesis of this compound by the liver or using fatty acid reserves as an energy source under these circumstances (Ganeshwade, 2012). It is likely that the reduction is also due to the prolonged fasting that the frogs were subjected to, before and after transport. On the other hand, as with triglycerides, the increase in cholesterol levels cannot be seen in the first evaluations after transport due to the long time that these animals were transported. Therefore, most likely the peak of glucocorticoids and their effects on triglyceride and cholesterol levels occurred at the beginning of the trip. Normally, under stress, cholesterol levels can increase. According to Cavenee and MeInykovych (1979), glucocorticoids and the presence of free fatty acids in the circulation stimulate the activity of the enzyme 3-hydrox-y-3-methyl-glutaryl coenzyme A reductase (HMG-CoA reductase) in the liver, increasing the synthesis of cholesterol. In addition to its importance in energy metabolism, lipid is involved in gametogenesis and in the formation of cell membranes (Bruscalupi et al., 1989). According to Alyousif (1991), the amount of cholesterol present in the cell membrane is related to the ability of water to diffuse through it, and cholesterol is, therefore, very important for waterproofing tissues that are in direct contact with body fluids. The same author demonstrated in a study with *Rana ridibunda* that the tissues responsible for the loss of water (kidney, lung, skin, ileum, and rectum) contain a large amount of cholesterol, while the tissues that are not responsible for the loss of water present low levels of this compound. In the present study, no increase in plasma cholesterol levels was observed immediately after transport, even though the animals remained approximately nine hours with water restriction. However, the amount of cholesterol in tissues responsible for water control was not measured.

In the present study bullfrog males had a higher concentration of total cholesterol. Bruscalupi et al. (1989), demonstrated in a study with *Rana esculenta* that the levels of total lipids and total cholesterol are also influenced by the time of year and the gender of the animals. According to the same authors, females of *Rana esculenta* had higher levels of total lipid and total cholesterol during the reproductive period. The cholesterol levels of the females in the present study were probably lower, since the transport was carried out after the reproductive period of the animals, in autumn. Therefore, in this circumstance, it is likely that a large part of the female cholesterol may have been used as a precursor to the steroid hormones involved in reproduction and another part directed to the formation of the oocyte.

The evaluation of liver enzymes in the plasma of animals is used to demonstrate hepatocellular damage (Peng et al., 2016) and stress has shown an intimate relationship with

these enzymes. In the present study, aspartate-aminotransferase (AST) levels of bullfrogs increased immediately after transport and were restored to baseline levels within 24 h. According to Coppo et al. (2005), the elevation of plasma AST levels is related to necrosis of liver cells and skeletal or cardiac muscle, lack of vitamin E and hunger. In relation to the alanine-aminotransferase (ALT) assessed in bullfrogs after transport, the levels of this enzyme increased only immediately after transport. The increase in the concentration of ALT in the plasma is already well established as a marker of acute liver injury (Coppo et al., 2005). Janssens (1967) demonstrated that cortisol administered to *Xenopus laevis* increased the rate of urea excretion and the activity of the liver enzymes ALT and AST. Bullfrog adults demonstrated an increase in ALT levels during the hibernation period, which is considered a stressful situation, however the submission of frogs to low temperatures did not influence AST levels (Peng et al., 2016). On the other hand, in the present study there was a decrease in the ALT levels of the frogs evaluated 48 h after transport. It is widely known that during the fasting period, animals use amino acids to carry out gluconeogenesis. Alanine, which comes from muscle, is the main amino acid used, and when it reaches the liver is deaminated to the formation of pyruvate. However, in prolonged fasting the brain decreases the use of glucose, reducing gluconeogenesis in the liver, which stimulates muscle and other peripheral tissues to use energy sources from lipids. Therefore, as the frogs evaluated 48 h after transport were fasting for 99 h, they probably started using lipids as an energy source, as evidenced by the decrease in triglyceride and cholesterol levels at this time. Concomitantly, there was a decrease in the transamination process by ALT, reducing the release of gluconeogenic amino acids by the muscle to save proteins.

Despite the increase in the number of erythrocytes in the first 24 h after transport, this increase was not evident in the hematocrit of the frogs evaluated in the same interval. Under stress conditions, there is a greater recruitment of young erythrocytes from erythropoietic organs, due to the increased demand for oxygen by the tissues (Boutilier and Shelton, 1986). According to the same authors, the increase in erythrocytes can also be caused by dehydration, which makes the blood more viscous. Therefore, because it causes an increase in blood viscosity, the increase in the number of erythrocytes to improve oxygen transport is limited (Carvalho et al., 2017). Immature erythrocytes have a circular shape, high nucleus/ cytoplasm ratio, and distinctly smaller size (Broyles et al., 1981; Allender and Fry, 2008). In this way, the stability demonstrated by the hematocrit can be explained, since the frogs evaluated immediately after transport presented young erythrocytes and of smaller volume, that is, lower mean corpuscular volume (MCV). According to Peng et al. (2016) the reduced

size of erythrocytes during the active period may be a necessary physiological adaptation for the blood to circulate faster due to the increased physical activity of the animal. The increase in the number of erythrocytes at 0, 6, and 12 h after transport is also accompanied by a decrease in mean corpuscular hemoglobin (MCH), demonstrating a direct relationship between the size of the erythrocyte (MCV) and the amount of hemoglobin present in it. An increase in the number of erythrocytes and a decrease in MCV has also been demonstrated in bullfrog froglets submitted to management stress (Teixeira et al., 2012) and in adults of the same species submitted to low temperatures (Palenske and Saunders, 2003). On the other hand, 48 h after transport there was a decrease in the number of erythrocytes, hematocrit and hemoglobin, compared to the control treatment. The decrease in these red series variables may be related to a condition of hemodilution promoted by the rehydration of these animals after transport, or due to a possible condition of anaemia, since the total fasting time was very prolonged. The loss of water and the decrease in weight gain, as well as the weight gain due to the reintroduction of the frog in the water after transport, could elucidate the hydration status of the animals and a possible hemoconcentration and hemodilution, respectively. However, weighing the animals, especially after transport, could be an additional stress effect on the animals and interfere with the variables evaluated.

5. **Conclusions**

Transport promotes stress in adult bullfrogs. However, bullfrogs have shown to be very resistant to transport and have demonstrated a rapid recovery of homeostasis after transport, between 6 and 24 h depending on the variable evaluated. Between 24 and 48 h after transport, a decrease in energy reserves and red blood series is observed, probably caused by prolonged fasting.

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