UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA, EVOLUÇÃO E BIODIVERSIDADE

ESTADO REDOX E MOBILIZAÇÃO ENERGÉTICA DURANTE RESPOSTA DE FASE AGUDA EM MORCEGOS DE DIFERENTES HÁBITOS ALIMENTARES

LUCIA VELARDE CABRERA MARTINEZ

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Coorientador: Dra. Mariella Bontempo Duca de Freitas

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AUTORA: LUCÍA VELARDE CABRERA MARTINEZ
ORIENTADOR: ARIOVALDO PEREIRA DA CRUZ NETO
COORIENTADORA: MARIELLA BONTEMPO DUCA DE FREITAS

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Prof. Dr. ARIOVALDO PEREIRA DA CRUZ NETO (Participaçao Virtual)
Departamento de Biodiversidade / Unesp - IB Rio Claro

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Profa. Dra. JERUSA MARIA DE OLIVEIRA (Participaçao Virtual)
Instituto de Química e Biotecnologia / Universidade Federal de Alagoas

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Prof. Dr. FERNANDO RIBEIRO GOMES (Participaçao Virtual)
Departamento Fisiologia / Universidade de São Paulo

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Dedico esta tese ao meu filho, Iberê. Com a intenção de ser um exemplo de persistência e dedicação, mas também de respeito e amor pela ciência e toda forma de vida.

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RESUMO GERAL

A indução da resposta de fase aguda (RFA) por meio de lipopolissacarídeo (LPS) de *Escherichia coli* desencadeia aumento na taxa metabólica e, consequentemente, no gasto calórico em várias espécies de morcegos. Nesta tese, apresentamos uma introdução geral destacando os principais indicadores fisiológicos associados à RFA em morcegos e suas relações com o equilíbrio oxidativo e a mobilização de reservas energéticas — temas centrais de ambos os capítulos experimentais. No primeiro capítulo, simulamos a RFA utilizando LPS em morcegos insetívoros (*Molossus molossus*) e frugívoros (*Artibeus lituratus*), e avaliamos seus efeitos na variação da massa corporal, ingestão alimentar, atividade de leucócitos e mobilização de proteínas, ácidos graxos e glicogênio. Esperávamos observar maior variação de massa corporal, redução da ingestão alimentar, aumento na razão neutrófilo-linfócito e menores concentrações de substratos energéticos em animais desafiados com LPS em comparação aos animais do controle. No entanto, nossos resultados sugerem que a ativação da RFA não compromete as reservas energéticas dos morcegos — ao menos sob condições experimentais e em um curto período de tempo. Em *M. molossus*, não observamos efeito da RFA na variação da massa corporal nem na contagem diferencial de leucócitos. Em *A. lituratus*, os animais tratados com LPS ganharam massa apenas na noite anterior à injeção; após a injeção, não houve ganho de massa, e a ingestão alimentar diminuiu progressivamente até o final do experimento. Além disso, a razão neutrófilo-linfócito aumentou 24 horas após a injeção de LPS, mas não apresentou variação após 12 horas, nem após a injeção de PBS ao longo do período experimental. No segundo capítulo, analisamos a biologia redox do fígado e do músculo peitoral durante a ativação da RFA em morcegos com diferentes dietas. Espécies reativas são liberadas durante a RFA, e esperávamos detectar maiores concentrações de antioxidantes ou marcadores de oxidação em animais desafiados em comparação aos animais do controle — indicando desequilíbrio oxidativo ou aumento na produção de antioxidantes em resposta a danos oxidativos. Contrariamente às expectativas, não observamos efeito do tratamento no fígado ou no músculo peitoral. Esses resultados sugerem que, independentemente do hábito alimentar, a RFA não compromete o equilíbrio oxidativo nos tecidos analisados durante as horas que seguem o desafio imune. Com esta tese, concluímos que, embora a RFA retenha características conservadas dentro de grupos taxonômicos, ela pode variar entre espécies. No entanto, são necessários mais estudos para determinar se tais variações são atribuídas exclusivamente aos hábitos alimentares. Além disso, nossos achados sustentam a noção de que a dieta influencia as concentrações de antioxidantes endógenos em morcegos; ainda assim, nossos dados não indicam desequilíbrio oxidativo como resultado da ativação da RFA com LPS. Isso sugere que a suscetibilidade a danos oxidativos causados pela ativação da imunidade inata nas espécies estudadas não está diretamente associada ao hábito alimentar.

Palavras-chave: resposta imune inata, LPS, reservas energéticas, antioxidantes, estresse oxidativo, biologia redox.

GENERAL ABSTRACT

The induction of the acute phase response (APR) using lipopolysaccharide (LPS) from *Escherichia coli* triggers an increase in metabolic rate and, consequently, caloric expenditure in several bat species. In this thesis, we present a general introduction highlighting the main physiological indicators associated with the APR in bats, and their relationships with oxidative balance and the mobilization of energy reserves—central themes of both experimental chapters. In the first chapter, we simulated the APR using LPS in insectivorous (*Molossus molossus*) and frugivorous (*Artibeus lituratus*) bats, and evaluated its effects on body mass variation, food intake, leukocyte activity, and the mobilization of protein, fatty acids, and glycogen. We expected to observe greater variation in body mass, reduced food intake, increased neutrophil-to-lymphocyte ratio, and lower concentrations of energy substrates in LPS-challenged animals compared to control animals. However, our results suggest that activation of the APR does not compromise bats' energy reserves—at least under experimental conditions and over a short period. In *M. molossus*, we found no effect of the APR on body mass variation or leukocyte differential count. In *A. lituratus*, LPS-treated animals only gained body mass on the night preceding the injection; following the injection, no mass gain was observed, and food intake progressively decreased until the end of the experiment. Additionally, the neutrophil-to-lymphocyte ratio increased 24 hours after LPS injection but showed no variation 12 hours post-injection or following PBS injection throughout the experimental period. In the second chapter, we analyzed the redox biology of the liver and pectoral muscle during APR activation in bats with different diets. Reactive species are released during the APR, and we expected to detect higher concentrations of antioxidants or oxidative markers in challenged animals compared to controls—indicating either oxidative imbalance or an upregulation of antioxidant production in response to oxidative damage. Contrary to our expectations, we observed no treatment effect in either the liver or pectoral muscle. These results suggest that, regardless of dietary habit, the APR does not compromise oxidative balance in the analyzed tissues during the hours following immune challenge. With this thesis, we conclude that while the APR retains conserved characteristics within taxonomic groups, it may vary between species. However, further studies are necessary to determine whether such variations are solely attributable to dietary habits. Additionally, our findings support the notion that diet influences the concentrations of endogenous antioxidants in bats; nonetheless, our data do not indicate oxidative imbalance as a result of APR activation with LPS. This suggests that susceptibility to oxidative damage caused by innate immune activation in the studied species is not directly associated with dietary habit.

Keywords: innate immune response, LPS, energy reserves, antioxidants, oxidative stress, redox biology.

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GENERAL INTRODUCTION

The animal immune capacity is an important factor for survival and, consequently, for the success of populations. The acute phase response (APR) is the first line of defense and consists of a complex reaction to inflammatory or infectious stimuli, involving metabolic, immunological, and biochemical changes to restore homeostasis and fight pathogens. It is triggered by the action of innate immune system cells, such as macrophages, dendritic cells, and mast cells, which, upon recognizing infections or tissue injuries, release pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). (CRAY; ZAIAS; ALTMAN, 2009; JANEWAY; MEDZHITOV, 2002; MURPHY et al., 2012). These cytokines reach the liver through the bloodstream, stimulating the production of acute phase proteins (APPs), which play key roles in innate immunity modulation, acting in inflammation control, and tissue protection (KNOLLE; GERKEN, 2000; LEMASTERS; JAESCHKE, 2020).

Bats are particularly interesting models to study immune function. They are reservoirs of diseases that are potentially fatal to other organisms. Thanks to the combination of evolutionary adaptations in their immune system, metabolism, and physiology, they can tolerate various infections without developing clinical symptoms. Besides their flight-related metabolic adaptations, they show a dampened inflammatory response, constitutively active interferon pathways, reduced virus-induced cell death, high baseline body temperature and positive selection in genes involved in immunity, inflammation, and DNA repair as IFN-α, STING, NLRP3 and P53. (ANINDITA et al., 2015; BROOK; DOBSON, 2015; BRUNET-ROSSINNI; AUSTAD, 2004; FIELD, 2009; GONZALEZ; BANERJEE, 2022; LUIS et al., 2013; WIBBELT et al., 2010; WILKINSON; SOUTH, 2002). However, the magnitude of the physiological and behavioral changes triggered by immune system activation varies considerably among bat species. In general, it is assumed that the APR imposes a significant energy cost on organisms. Macrophages, like other immune cells, are nutrient demanding cells as evidenced by their hypermetabolic state and significant rates of glucose and glutamine utilization, relative to other cells in the host (NEWSHOLME; NEWSHOLME, 1989). The increase in resting metabolic rate (RMR) following the experimental induction of the APR has been reported for some bat species (CABRERA-MARTINEZ; HERRERA; CRUZ-NETO, 2018; CABRERA-MARTINEZ; HERRERA M.; CRUZ-NETO, 2019; GUERRERO-CHACÓN et al., 2018; OTÁLORA-ARDILA et al., 2016, 2017; TRIANA-

LLANOS et al., 2019). However, some differences in physiological responses associated with the APR, such as body mass loss, food ingestion, fever and variation in leucocyte concentrations vary between species (VIOLA; HERRERA; DA CRUZ-NETO, 2022), and may be attributed to their life-history. These discrepancies highlight the complexity of the physiological adjustments triggered by immune activation. Here we aim to explore the primary physiological indicators associated with the APR in bats experimental physiology, and their relationship with oxidative balance and energy reserve mobilization—two subjects that will be discus in this study.

1. *Sickness anorexia*

During the APR, complex alterations occur in the central nervous system (CNS), which acts as a central regulator of the inflammatory response, coordinating behavioral and physiological responses to optimize immune function and conserve energy, thereby promoting survival (LOCHMILLER; DEERENBERG, 2000). Infection induces a hypermetabolic state to support the heightened activity of the immune system. This metabolic shift is driven by the increased demand for glucose and glutamine, essential for sustaining immune function (CROUSER; DORINSKY, 1996; MICHIE, 1996). As a result, catabolic processes are activated to supply the additional fuel needed by immune cells and for protein synthesis, making energy reserves crucial for survival (EXTON, 1997; KLASING; JOHNSTONE, 1991; LEGRAND, 2000; TSIGOS et al., 1997; VAN NIEKERK et al., 2016). In this context, there is growing interest in understanding how energy availability influences the modulation of immune function. Researchers have increasingly focused on how the immune system communicates with the CNS to elucidate why sick animals lose their appetite, since food ingestion in crucial for energy resources availability. A central focus has been on the cytokines released by activated monocytes and macrophages, such as IL-1β, IL-6, and TNF-α (EXTON, 1997; JOHNSON, 1998; LEGRAND, 2000; VAN NIEKERK et al., 2016). These cytokines act on the hypothalamus, the brain's primary appetite-regulating center, altering the production and activity of appetite-related neuropeptides, thereby suppressing hunger (CARLTON; DEMAS; FRENCH, 2012; INGVARTSEN; BOISCLAIR, 2001). In addition to their direct effects on the hypothalamus, the release of cytokines and other immune mediators impacts the gut-brain axis. Altered signaling from the gastrointestinal tract, mediated by

vagal nerve stimulation, sends satiety signals to the brain, further reducing the desire to eat (AVIELLO et al., 2021).

While anorexia during the APR may seem counterproductive given the immune response heightened energy demands, it is considered an adaptive response, designed to balance the needs of immune function with resource conservation (EXTON, 1997; JOHNSON, 1998; VAN NIEKERK et al., 2016). By limiting nutrient availability, anorexia may reduce the resources accessible to pathogens, as well as conserving energy that would otherwise be allocated to food search and the digestive process (ADAMO et al., 2010). Nevertheless, the magnitude of food intake reduction should depend on each species' capacity for glucose regulation and ability to stock and mobilize energy substrates, which may be associated to their dietary habits, as has been documented in some bat species (AMARAL et al., 2019; FREITAS et al., 2003, 2005, 2013, 2010; FREITAS; WELKER; PINHEIRO, 2006).

One common technique used to experimentally induce a bacterial APR is the administration of LPS (lipopolysaccharide of *Escherichia coli*), an immunogenic component of the outer membrane of gram-negative bacteria that causes the release of proinflammatory cytokines (CRAY, 2012; CRAY; ZAIAS; ALTMAN, 2009). Reduced food intake during the induction of the APR with LPS has been recorded in some bat species. A greater decrease in food intake was reported for a fruit-eating bat (*Carollia perspicillata*) challenged with LPS, in relation to control animals (LPS: 85% decrease, PBS: 30% decrease) (MELHADO; HERRERA M.; DA CRUZ-NETO, 2020). The effect of LPS on energy balance was considerable since mean food intake when bats were injected LPS provided 8.85 kJ in contrast to 42.03 kJ obtained after the control injection. Similar results were reported for the same species in VIOLA et al. (2022).

In a previous work we stimulated an APR with LPS to measure metabolic changes in *Carollia perspicillata* (CABRERA-MARTINEZ; HERRERA M.; CRUZ-NETO, 2019). Bats were divided in fed and unfed (24h fasted) groups. Unfed bats started the experiments with lower body mass than fed bats, independently of season and treatment (LPS or control), and such difference was maintained until the end of the experiment. Contrary to our expectations, unfed LPS-treated bats showed similar increases in body temperature and peak RMR compared to fed LPS-treated bats, despite having started the experiments with a lower RMR. Therefore, unfed bats exhibited higher metabolic scope

and caloric costs, which was likely compensated by the mobilization of energy reserves. Additionally, food deprivation led to a delayed metabolic response, as indicated by the longer time required to reach peak RMR values. In another bat species (*Myotis vivesi*), fed and food restricted animals showed comparable RMR, total caloric expenditure, and skin temperature after the LPS challenge, but the metabolic and fever responses were also delayed in bats on the restricted diet. Additionally, body mass loss after LPS injection was 1.4 times in fed bats compared to the restricted bats (OTÁLORA-ARDILA et al., 2017). Therefore, the ability of animals to balance energy demands with immune challenges appears to be influenced by dietary status, species-specific metabolic strategies, and the severity of the immune stimulus. Further research is needed to better understand how these mechanisms vary across taxa and ecological contexts, shedding light on the evolutionary trade-offs that shape immune responses in energy-limited conditions.

2. *Pyrogenic response*

Fever is an important defense mechanism against pathogens. It consists in a controlled body temperature increase, initiated by the hypothalamus in response to pyrogens. By increasing body temperature, fever enhances the effectiveness of neutrophils and lymphocytes while inhibiting the growth of temperature-sensitive pathogens (BILBO; NELSON, 2002; EVANS; REPASKY; FISHER, 2015; MARAIS; MALONEY; GRAY, 2011; MARTIN; WEIL; NELSON, 2008). However, this elevation in body temperature also accelerates metabolic activity, leading to an increase in the production of reactive oxygen species (ROS) (GOMES et al., 2018; HOU et al., 2011). A higher metabolic rate results in greater mitochondrial activity, where ROS are byproducts of aerobic respiration. As mitochondrial oxygen consumption rises, so does ROS production. While oxidative stress is often viewed negatively, the ROS produced during fever play critical roles in host defense, since these reactive molecules are toxic to many pathogens, aiding in their destruction (HOU et al., 2011). Additionally, ROS act as messengers, enhancing immune cell recruitment and promoting cytokine production (LAURIDSEN, 2019). Despite these beneficial roles, a careful balance is necessary, as excessive ROS can damage host tissues. Lipid peroxidation caused by ROS can compromise cell membrane integrity, leading to further tissue damage. Moreover, fever and ROS production may deplete energy reserves, leaving fewer resources available for the repair of oxidative damage (EL-BELTAGI; MOHAMED, 2013; SU et al., 2019). The

increase in body temperature during the APR, however, varies across species and remains a topic of ongoing research. In bats, unique adaptations are believed to help mitigate the costs associated with fever. Bats possess elevated levels of antioxidants to counteract the high ROS production associated with flight, and they exhibit reduced production of pro-oxidants relative to other animals of similar size and RMR (AUSTAD; FISCHER, 1991; BRUNET-ROSSINNI, 2004; WILHELM FILHO et al., 2007). Studies have reported the increase in body temperature following LPS inoculation in the species *Myotis vivesi*, *Carollia perspicillata*, and *Rousettus aegyptiacus* when challenged during the resting period (CABRERA-MARTINEZ; HERRERA M.; CRUZ-NETO, 2019; GUERRERO-CHACÓN et al., 2018; MORENO et al., 2021; OTÁLORA-ARDILA et al., 2016, 2017). However, in *Molossus molossus* challenged with LPS at the beginning of the active period, body temperature did not increase in relation to the control group, but diurnal body temperature decreased considerably in LPS and control animals (STOCKMAIER et al., 2015). Bats possess a remarkable ability to enter torpor even in tropical habitats, a state of controlled hypothermia, which allows them to conserve energy during periods of food scarcity or unfavorable environmental conditions (AUDET; FENTON, 1988; BARTELS; LAW; GEISER, 1998; STAWSKI; GEISER, 2010; TEAGUE O'MARA et al., 2017). In torpor, their body temperature drops significantly, sometimes approaching ambient environmental temperatures, thereby reducing the energy required to maintain homeostasis. Additionally, bats can modulate the production of antioxidants to minimize the return from torpor to normal metabolic state, reducing the oxidative damage caused by the increase in RMR (WILHELM FILHO et al., 2007). Therefore, the relationship between fever and hypothermia in bats highlights the complex physiological adaptations that allow these animals to balance immune responses, energy conservation, and survival in various environmental contexts. While fever plays a critical role in pathogen defense, it demands high energy and can lead to oxidative stress. Hypothermia, by contrast, is a strategy for energy conservation and survival during harsh conditions, but it may reduce immune function, since many immune reactions, including cytokine production, antibody synthesis, and pathogen destruction, are temperature-sensitive and function optimally at normal or slightly elevated body temperatures (BROESSNER et al., 2012; LUO et al., 2021; PIKULA et al., 2020a). More research is need to understand how bats manage these contrasting physiological responses provides valuable insight into their ecology and

survival strategies, especially as they are exposed to a variety of environmental and pathogenic challenges.

3. Leukocyte migration

During the APR, the hematopoietic system is activated to meet immunological demands and repair tissue damage. One key alteration is leukocytosis, characterized by an increase in white blood cell count, particularly neutrophils (CRAY, 2012; CRAY; ZAIAS; ALTMAN, 2009). This response is modulated by pro-inflammatory cytokines that stimulate the production of neutrophils in the bone marrow, and cortisol, which is released through the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol not only promotes the release of neutrophils from the bone marrow but also inhibits their migration to tissues, thereby temporarily increasing neutrophil count in the blood (MURPHY et al., 2012). Neutrophils play a crucial role in tissue injury response. They migrate to the site of damage in response to chemotactic signals, where they engage in phagocytosis of invading microorganisms and release lytic enzymes and ROS that aid in pathogen destruction. A commonly used indicator of the activation of the APR and HPA axis involvement is the neutrophil/lymphocyte ratio (NLR) (CHESLEY; IV, 2011; SOCORRO FARIA et al., 2016). An increased NLR reflects either an increase in neutrophils or a reduction in lymphocytes, often attributed to the immunosuppressive effects of cortisol, which promotes lymphocyte apoptosis and reduces their proliferation in the bone marrow.

Bats of the species *Molossus molossus, Myotis vivesi, Rousettus aegyptiacus, Carollia perspicillata* and *Desmodus rotundus* treated with LPS, did not show a significant increase in total white blood cell counts in relation to control animals. Nevertheless, the variation in NLR differs among bat species. An increase in NLR following APR activation was reported in fed *Myotis myotis* (SELTMANN et al., 2022) and *Rousettus aegyptiacus* (MORENO et al., 2021); during the pre-migration period in *Pipistrellus nathusii* (VOIGT et al., 2020); and in food-deprived *Carollia perspicillata* challenged during the resting period (CABRERA-MARTINEZ; HERRERA M.; CRUZ-NETO, 2019). No effect of LPS on NLR was observed in fed *Carollia perspicillata* (CABRERA-MARTINEZ; HERRERA M.; CRUZ-NETO, 2019) or migratory *Pipistrellus nathusii* (VOIGT et al., 2020). Additionally, a decrease in NLR was reported for *Carollia perspicillata* challenged during the active period (MELHADO; HERRERA

M.; DA CRUZ-NETO, 2020). This broad variation in hematological parameters observed during the inflammatory response has not yet been fully elucidated. However, it can be inferred that the exposure period (resting or active) to the inflammatory agent, the dose of LPS, and other factors related to the species' life history may significantly influence the bacterial response in bats (VIOLA; HERRERA; DA CRUZ-NETO, 2022; VIOLA; HERRERA M.; CRUZ-NETO, 2024). A common feature across the before mentioned studies, is the significant individual variation in bat immune responses to LPS injection, with some individuals showing an increase, decrease, or no change in white blood cell counts. The increase in neutrophils, is thought to be initially mediated by cortisol, which mobilizes marginal pool cells from tissues into the bloodstream as part of an immediate stress response (CRAY, 2012). The dose-dependent increase in the NLR may be attributed to a corresponding increase in glucocorticoid levels, which modulate neutrophil and lymphocyte trafficking (DHABHAR, 2002). Additionally, the robust rise in the NLR during the active phase could be linked to higher baseline glucocorticoid levels during this period, enhancing immune function when pathogen exposure is expected to peak (GONG et al., 2015; MARKOWSKA; MAJEWSKI; SKWARŁO-SOŃTA, 2017; SCHEIERMANN; KUNISAKI; FRENETTE, 2013).

The relationship between leukocytosis and oxidative stress is crucial in understanding the dynamics of the immune response during the APR. Particularly the activation of neutrophils, is associated with the production of ROS as part of the immune system's defense mechanisms. Neutrophils generate ROS to neutralize pathogens, but excessive or prolonged ROS production can lead to oxidative stress, which in turn may cause tissue damage and contribute to chronic inflammation (CHELOMBITKO, 2018; YU et al., 2022). This dual role of neutrophils—defending against pathogens while potentially causing collateral damage through ROS production—highlights the fine balance required for effective immune responses without inducing excessive oxidative damage. In this context, the dynamic changes in the NLR observed during the APR may serve as a marker for both the immune response and oxidative stress levels, with a high NLR potentially indicating an elevated risk of oxidative damage. Thus, understanding the relationship between leukocytosis, oxidative stress, and glucocorticoid signaling is essential for gaining insights into the immune responses of bats and other species during inflammatory events.

4. *Metabolic adjustments*

Inflammatory processes are also accompanied by significant metabolic alterations aimed at meeting the energy and biosynthetic demands of immune cells and regenerating tissues (SUN et al., 2020). These metabolic changes are regulated by the interplay of hormones such as cortisol, insulin, and glucagon, along with pro-inflammatory cytokines, which collectively enhance gluconeogenesis and mobilize fatty acids and amino acids (BROSNAN, 1999; KRAUS-FRIEDMANN, 1984). Gluconeogenesis is the process by which glucose is synthesized from non-carbohydrate precursors such as lactate, amino acids, and glycerol. During the APR, this process is upregulated to supply glucose, the primary energy source for immune cells. The release of cortisol increases the expression of gluconeogenic enzymes in the liver (BOLLEN; KEPPENS; STALMANS, 1998; KNOLLE; GERKEN, 2000; SOON; TORBENSON, 2023). Pro-inflammatory cytokines also indirectly stimulate gluconeogenesis by promoting the release of amino acids and glycerol from peripheral tissues, including muscle and adipose tissue (BROSNAN, 1999; KRAUS-FRIEDMANN, 1984). To conserve glucose for immune cells and the brain, other tissues utilize fatty acids as their primary energy source (ZHANG et al., 2020). Fatty acid mobilization occurs through processes like lipolysis and beta-oxidation (POND, 2005; SCHREIBER; ZECHNER, 2014). During lipolysis, hormones such as cortisol and epinephrine activate hormone-sensitive lipase in adipose tissue, breaking down triglycerides into glycerol (used in gluconeogenesis) and free fatty acids. Beta-oxidation occurs in the liver, where free fatty acids are oxidized in mitochondria to generate ATP, providing metabolic support for the synthesis of APPs (HOUTEN; WANDERS, 2010). This process also produces ketone bodies, which serve as an alternative energy source for other tissues. Additionally, proteolysis, primarily occurring in muscle tissue, is stimulated by cortisol and pro-inflammatory cytokines (SIMMONS et al., 1984). This process releases amino acids, such as alanine, which are used by the liver as substrates for gluconeogenesis and the synthesis of APPs (GABAY; KUSHNER, 1999; GRUYS et al., 2005). These coordinated metabolic adaptations enable the organism to prioritize immune defense and tissue repair, efficiently redirecting metabolic resources to support the inflammatory response and recovery processes.

Understanding the immune response in bats is crucial, given their unique ecological roles and diverse physiological adaptations. Despite the growing interest in bat immunology, we still have many gaps about the physiological mechanisms underlying their immune response, especially regarding the mobilization of energy substrates during immune response activation in bats. It is commonly assumed that bats, like other vertebrates, rely on stored energy reserves when faced with increased energy demands during immune activation, especially in situations that demand the allocation of energy to maintain other important physiological functions. Investigating how bats mobilize and utilize energy substrates in response to immune challenges could provide valuable insights into their resilience to infection and their ability to cope with metabolic demands. Filling these knowledge gaps is crucial not only for understanding bat biology but also for broader ecological and evolutionary implications. Future studies are needed to investigate the energetic dynamics of immune activation in bats, which could lead to a more comprehensive understanding of their immune physiology and help inform conservation efforts, especially as bats face increasing environmental challenges.

Chapter I:

The energetic demand of a simulated bacterial infection does not affect the energy reserves in insectivorous and frugivorous bats

ABSTRACT

The acute phase response is the first line of defense of animals against pathogens. It consists of an inflammatory response activated through the action of macrophages that release cytokines and chemokines, triggering a series of events that culminate, if successful, in the elimination of the infectious agent. Previous studies have recorded the increase in the metabolic rate, and consequently the increase in energy expenditure, in several bat species challenged with LPS (lipopolysaccharide of *Escherichia coli*). In this study, we evaluated the degree of mobilization of energy reserves (protein, carbohydrates and fats) in liver and pectoral muscle, during the APR induced by LPS in two bat species with different feeding habits (insectivorous and frugivorous). We expected that animal challenged with LPS would mobilize more energy substrates when compared with control animals, in order to balance the energetic demands of the immune system activation. However, the concentration of energy reserves in both groups was similar, suggesting that a bacterial APR does not compromises bats glycogen and fat reserves. Additionally, we did not identify differences in body mass variation, total and differential leucocyte count in the insectivorous bat *Molossus molossus* challenged with LPS in contrast to those of the control group, but frugivorous bats of the species *Artibeus lituratus* challenged with LPS showed less body mass gain and food intake than control animals, as well as an increase in the neutrophil/lymphocyte ratio.

Keywords: energy substrates, acute phase response, LPS, immune challenge, glycogen, fatty acids, diet, bats.

INTRODUCTION

The acute phase response (APR) is an inflammatory response that occurs within the first hours after the contact with a pathogen, and lasts nearly for 48 hours. It is common to experimentally induce the APR with LPS (lipopolysaccharide of *Escherichia coli*) administration, an immunogenic component of the outer membrane of gram-negative bacteria. The administration of LPS increases plasma levels cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) and interleukin-6 (IL-6) (HOCKE, 1998; MARSHALL et al., 1994; SCHWARTZ, 2002; WEINGARTEN, 1996), which cause a decrease in food intake (ADAMO et al., 2010; CHUNG et al., 2002; LEGRAND, 2000; LOCHMILLER; DEERENBERG, 2000; MARTIN et al., 2007). Anorexia has been interpreted as a defense mechanism, since the reduction in food search engages in less muscular activity, reduces the chance of predation, decrease heat loss, and the energetic cost associated with digestion, saving energy during illness (HART, 1988).

While reduced food intake and body mass loss appear to be common reactions following LPS administration across several taxa, the extent of these responses varies significantly both within a single species and between different species (VIOLA; HERRERA; DA CRUZ-NETO, 2022). Adult animals maintain stable body weight through mechanisms that regulate energy reserves. The central nervous system (CNS) controls this balance, adjusting behavioral responses to restore weight, if necessary, with the hypothalamus playing a key role. It integrates signals related to metabolism, temperature, body weight, and meal cues (like smell, taste and stomach distension) received via the vagus nerve (TIZARD, 2008). These signals influence appetite reduction and energy use. In short terms, carbohydrates might be a rapid source of energy. The liver is crucial for regulating carbohydrate metabolism by absorbing and storing glucose after meals, and by producing and releasing glucose into the bloodstream during periods of fasting to maintain stable blood sugar levels. The physiologic regulation of hepatic glycogen synthesis, glycogenolysis, gluconeogenesis, glycolysis, and other enzymatic reactions is mediated by mechanisms like the balance of insulin and glucagon, the availability of substrates, and the cellular redox state (PETERSEN; VATNER; SHULMAN, 2017). It is expected that in periods of low energy resource availability, animals will mobilize their glycogen reserves to maintain glycemic homeostasis (BOLLEN; KEPPENS; STALMANS, 1998). Besides the liver, the muscular tissues also

serve as a critical energy reserve (MA et al., 2020; SOON; TORBENSON, 2023) . Glycogen is synthesized in muscle cells through glycogenesis. This process is primarily driven by insulin, which promotes glucose uptake and storage after carbohydrate consumption. When energy demands are low, muscles replenish their glycogen stores, ensuring an available energy supply for future needs, when muscle cells can rapidly break down glycogen to produce glucose for ATP synthesis. Prolonged high-energy demanding activity or fasting can deplete glycogen reserves. Once glycogen stores are exhausted, muscle fatigue sets in, and the body may shift to using fat or protein for energy, which is less efficient (BOLLEN; KEPPENS; STALMANS, 1998; MA et al., 2020; SOON; TORBENSON, 2023; ZHANG et al., 2021). Additionally, sick animals may experience a greater reduction in body mass. The weight loss associated with inflammation is mediated by TNF-α, which affects lipid metabolism. Lipid mobilization directly interferes with triglycerides and cholesterol metabolism, making lipids available for immune function activity and also affecting the regulation of inflammatory responses, since adipocytes and other cells located within the adipose tissue appear to be a source of inflammatory cytokines (FAIN, 2006; GABAY; KUSHNER, 1999; TIZARD, 2008).

In the past years, bacterial APR has been induced in bats under different conditions (CABRERA-MARTINEZ; HERRERA; CRUZ-NETO, 2018; CABRERA-MARTINEZ; HERRERA M.; CRUZ-NETO, 2019; GUERRERO-CHACÓN et al., 2018; HERNÁNDEZ-ARCIGA et al., 2020; MELHADO; HERRERA M.; DA CRUZ-NETO, 2020; MORENO et al., 2021; OTÁLORA-ARDILA et al., 2016, 2017; SCHNEEBERGER; CZIRJÁK; VOIGT, 2013; SELTMANN et al., 2022; STOCKMAIER et al., 2015; TRIANA-LLANOS et al., 2019; VIOLA; HERRERA; DA CRUZ-NETO, 2022; VIOLA; HERRERA M.; CRUZ-NETO, 2024). During the APR, the increase of metabolic rate have been reported in the piscivorous species *Myotis vivesi* (OTÁLORA-ARDILA et al., 2016, 2017), frugivorous species *Carollia perspicillata* (CABRERA-MARTINEZ; HERRERA M.; CRUZ-NETO, 2019), *Artibeus lituratus* (GUERRERO-CHACÓN et al., 2018), *Rousettus aegyptiacus* (MORENO et al., 2021), and the nectarivorous species *Glossophaga soricina* (CABRERA-MARTINEZ; HERRERA; CRUZ-NETO, 2018). To our knowledge, no study measuring metabolic rate in bats during the APR, failed to detect a significant increase after LPS injection (VIOLA; HERRERA; DA CRUZ-NETO, 2022), despite variation in doses (from 1.75 to 4 mg/kg of LPS. Besides, studies suggest that feeding habits influence how bats accumulate and

mobilize energy reserves (AMARAL et al., 2019; FREITAS et al., 2003, 2005, 2013, 2010; FREITAS; WELKER; PINHEIRO, 2006; MCNAB, 1976). The energy demand required to maintain metabolic homeostasis comes from nutrient intake, which provides direct energy for the organisms to use, or the exceeded energy can be stored as carbohydrate or lipid reserves. Animals that consume carbohydrate-rich diets exhibit high concentrations of liver glycogen and fat reserves under normal nutritional conditions. During short fasting periods, they are able to maintain glycemic homeostasis despite the decrease in plasma glucose levels, mainly through glycogenolysis (the breakdown of glycogen reserves to release glucose) and gluconeogenesis (the production of glucose from non-carbohydrate compounds) (BROSNAN, 1999; KETTELHUT; FOSS; MIGLIORINI, 1980; SARTORI et al., 1995; TIRONE; BRUNICARDI, 2001; TURNER; APPLEGATE; LILBURN, 1999). In contrast, in animals with protein-rich diets, plasma glucose levels tend to remain stable after short fasting periods, possibly due to the constant activity of gluconeogenesis. Therefore, it can be inferred that animals that primarily consume protein tend to be more resistant to food restriction or deprivation (DA SILVA; MIGLIORTNI, 1990; KETTELHUT; FOSS; MIGLIORINI, 1980).

In this study, we induced an APR using LPS in two bat species with different diets, *Molossus molossus* (insectivorous) and *Artibeus lituratus* (frugivorous), and analyzed its consequences on energy reserve concentration. Addicionally, we measured changes in body mass, food intake (in *A. lituratus* only) and the neutrophil/lymphocyte ratio (NLR) as indicators of the APR activation. The NLR is also associated with the HPA axis activation since it triggers the release of glucocorticoids, which can subsequently cause the redistribution of immune cells (BORNSTEIN et al., 2006; CAIN; CIDLOWSKI, 2017; DAVIS; MANEY; MAERZ, 2008). We determined the concentrations of glycogen and protein (in liver and pectoral muscle) and total fatty acids (in carcass). We expected that, compared with control bats, challenged bats would show a decrease in food intake (*A. lituratus*) and loss of body mass after LPS application. We also expected a significant increase in NLR after LPS injection, according to previous results for other bats species (CABRERA-MARTINEZ; HERRERA M.; CRUZ-NETO, 2019; MELHADO; HERRERA M.; DA CRUZ-NETO, 2020; MORENO et al., 2021; SELTMANN et al., 2022; VIOLA; HERRERA; DA CRUZ-NETO, 2022; VIOLA; HERRERA M.; CRUZ-NETO, 2024; WEISE et al., 2017). Moreover, along with the reduction in food intake, we expect frugivorous bats injected with LPS to exhibit less concentrations of glycogen than

control animals, while insectivorous bats injected with LPS should have lower fat concentrations, in relation to control and frugivorous animals.

MATERIAL AND METHODS

Animal Capture and Maintenance

Non-reproductive adults (males with apparent testicles, lactating or pregnant females) of *M. molossus* (n = 9 males, 4 females), weighting 12.8 ± 2.3 g (males) and 10.9 ± 1.8 g (females) and *A. lituratus* (n = 18 males) weighing 65.7 ± 8.2 g were captured in Araçoiaba da Serra - SP, in a private area near the Ipanema National Forest (23° 27' 23.57" S, 47° 35' 32.93" W), between September 2023 and February 2024, and immediately transported to a climate chamber at Universidade Estadual Paulista (Rio Claro, São Paulo). The bats were housed in individual cages and maintained within the thermoneutral zone (28°C – 29°C), in a photoperiod of 12h:12h until the end of the experiments. Lights were switched on at 06:00 h and switched off at 18:00 h. The acclimatation period lasts for two nights since capture. Animals were fed every night according to their respective diets. *M. molossus* was manually fed with mealworm larvae until they refused, while *A. lituratus* received drinking containers with industrial mango juice (> 95% of the composition in the form of simple sugars - Serigy®) to maintain a consistent caloric intake and the same composition and proportion of micro and macronutrients. Ethical permits for this study were issued by the animal ethics committee of the Universidade Estadual Paulista at Rio Claro (Authorization no. 09/2021) and the Sistema de Autorização e Informação em Biodiversidade - SISBIO (Authorization no. 77445-1).

Immune Challenge

The immune challenge started in the third night after capture, and it was conducted in the same climatic chamber where bats were acclimatized. Bats were weighed to the nearest 0.1 g (Ohaus Precision Balance, USA) every 12 hours (18:00 and 07:00). Nocturnal body mass change was calculated subtracting the morning body mass from the nocturnal body mass of the immediately previous night, in order to obtain the magnitude of mass change during each experimental night. Food intake was only quantified in *A.*

lituratus (as described in VIOLA; HERRERA M.; CRUZ-NETO (2024)) due to the imprecision in measuring the consumption of larvae by individuals of *M. molossus,* and the need of manual feeding. The drinking containers offered to *A. lituratus* were weighed immediately before being provided to the bats (18:00) and in the following morning (07:00), allowing for the calculation of each individual ingested volume per night. Animals were randomly assigned to a single experimental group, either the control (injected with phosphate-buffered saline - PBS; P4417, Sigma-Aldrich) (n = 4 *M. molossus* ; n = 6 *A. lituratus*) or the treatment (injected with LPS; L2630, Sigma-Aldrich) (n = 9 *M. molossus* ; n = 12 *A. lituratus*) group. Injections were administered subcutaneously in the scapular region, 24 hours after the beginning of the experiments. Individuals in the control group were injected with 50 µL of PBS solution, while individuals in the treatment group received an LPS dose of 4 mg/kg, diluted in 50 µL of PBS. Thus, *M. molossus* and *A. lituratus* received on average 0.047 ± 0.007 mg and 0.24 ± 0.04 mg of LPS, respectively. Blood smears for NLR were performed immediately before the injections and 12 and 24 hours after the injections. Cell count was performed following the protocol reported in VIOLA; HERRERA; DA CRUZ-NETO (2022). The experimental design is presented in Figure 1.

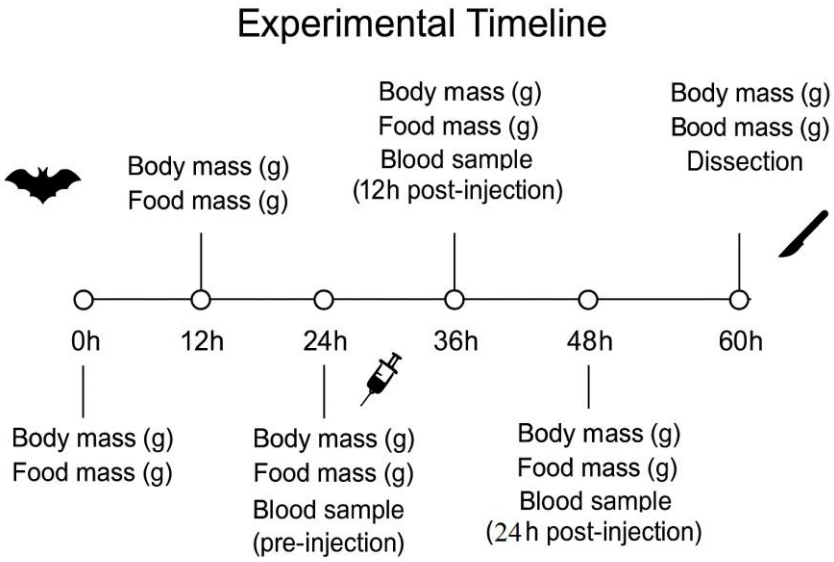

Figure 1. Timeline indicating the time of each sample and experimental procedure during the immune challenge.

Determination of Glycogen, Protein and Total Fatty Acids

The animals were euthanized by decapitation 36 hours after the injections. Portions of 296 ± 0.06 mg of liver and 301 ± 0.05 mg of pectoral muscle from *A. lituratus* were immersed in 2 mL of KOH (30%) for 24 hours for subsequent extraction and determination of glycogen concentrations, according to the method proposed by SJORGREN er al. (1938). The volume of these tissues in *M. molossus* was not enough to determine glycogen for this species, as both were completely used for redox state analyses (Chapter II). Total protein content of liver and pectoral muscle was determined by the protocol described by (LOWRY et al. (1951). The carcasses, excluding the head and digestive tract, were used to determine total fatty acids, being completely digested for 30 days in 100 (*M. molossus*) and 200 (*A. lituratus*) mL of KOH (6N) and subsequently filtered. After extraction with chloroform, the samples were maintained in previously weighted Petri dishes at room temperature until complete evaporation of the liquid. After evaporation, the dishes were weighed to determine the concentration of fatty acids per volume of sample, as reported in FREITAS; WELKER; PINHEIRO (2006).

Data Analysis

Statistical analyses were conducted in RStudio (2024.04.2). The differences in initial body mass and NLR between sexes were tested for *M. molossus* using a Student's t-test (*car* package). The variation in nocturnal body mass for each species was analyzed with a permutation test for mixed linear models (*lme4* and *permuco* packages) to detect possible effects of treatment and time, and their interaction. We used permutation tests because of their increased power in small, unbalance and non-parametric samples. Food intake of *A. lituratus* during the pre-injection night was compared between treatments using a Student's t-test, and a permutation test for mixed linear models was used to test the effect of treatment, time, and their interaction. Permutation tests for linear mixed models were also used to analyze the effect of treatment, time (after 12h and 24h) and their interaction on the relative variation (Δ) in NLR (Δ = (post-injection – pre-injection)/pre-injection). The hepatic and muscle glycogen concentrations of *A. lituratus* were compared between treatments using Student's t-tests (parametric), and the concentrations of carcass fatty acids in both species were compared between treatments using Mann-Whitney tests (non-parametric). Post-hoc Tukey tests (*emmeans* package) were applied when the permutation tests detected an effect of time (which have three

levels), in order to identify pair-wise differences. The rejection criterion for all analyses was $P < 0.05$.

RESULTS

We found no significant differences between sexes in *M. molossus* for initial body mass ($F = 2.07$, $P = 0.06$) and NLR ($F = 1.72$, $P = 0.12$), so we did not consider sex as a variable. Other statistical results are reported in Table 1.

Body mass lost

LPS did not affect the nocturnal body mass change of *M. molossus*. No effect of treatment, time, or interaction was detected (Table 1). Bats in both treatments lost body mass 24h after the injections. In control group the body mass loss was on average 0.28g, while in LPS group was about 0.32g, representing 2.3% and 2.7% of body mass loss respectively. Body mass loss across the hole experimental period was 0.6g for both treatments, representing 5% of total body mass loss (Figure 2A).

Table 1. Results of statistical tests for acute phase response indicators and energy metabolism in both species. Asterisks (*) indicate statistical significance (P<0.05).

	Molossus molossus		
	Treatment	**Time**	**Interaction**
Nocturnal body mass change	(F<0,001 ; P=0,98)	(F=0,68 ; P=0,52)	(F=0,31 ; P=0,73)
Δ Leucocyte count	(F=2,27 ; P=0,16)	(F=1,24 ; P=0,29)	(F=0,84 ; P=0,38)
Δ NLR	(F<0,001 ; P=0,98)	(F=1,27 ; P=0,28)	(F=1,06 ; P=0,32)
Liver total protein	(t=1,21; P=0,25)		
Pectoral muscle total protein	(t=0,92; P=0,37)		
Total carcass fatty acids	(W=18; P=1,0)		
	Artibeus lituratus		
	Treatment	**Time**	**Interaction**
Nocturnal body mass change	(F=0,996, P=0,32)	(F=4,13, P=0,03) *	(F=0,51, P=0,61)
Food intake	(F=8,51; P=0,01)*	(F=0,34; P=0,71)	(F=2,15; P=0,13)
Δ Leucocyte count	(F=1,87 ; P=0,19)	(F=0,02 ; P=0,9)	(F=3,47 ; P=0,08)
Δ NLR	(F=5,61 ; P=0,03)*	(F=7,52 ; P=0,02)*	(F=2,34 ; P=0,15)
Hepatic glycogen	(t=0,17; P=0,87)		
Muscular glycogen	(t=1,0; P=0,34)		
Liver total protein	(t=0,8; P=0,43)		
Pectoral muscle total protein	(t=0,24; P=0,81)		
Total carcass fatty acids	(W=42; P=0,4)		

In *A. lituratus*, the permutation test identified an isolated effect of time (Table 1). Since this variable has three levels, we applied a post-hoc Tukey test, which detected statistical difference in nocturnal body mass change only of LPS-treated bats, between the pre-injection night and the night immediately after the injection (Figure 2B), indicating lower body mass gain immediately after LPS challenge, but not 24h after that. Such difference was not detected in control animals. Additionally, 24h after the injections, the control group lost about 3.2% of body mass (2.3 ± 1.8g) in relation to pre-injection night, while the body mass loss of LPS-treated animals was about 5.3% (3.4 ± 4.9g). In contrast to *M. molossus*, when considering the hole experimental period, *A. lituratus* did not loss body mass, nevertheless, the percentage of body mass gain of control individuals was twice the body mass gain of LPS injected bats (1.8% and 0.9%, respectively).

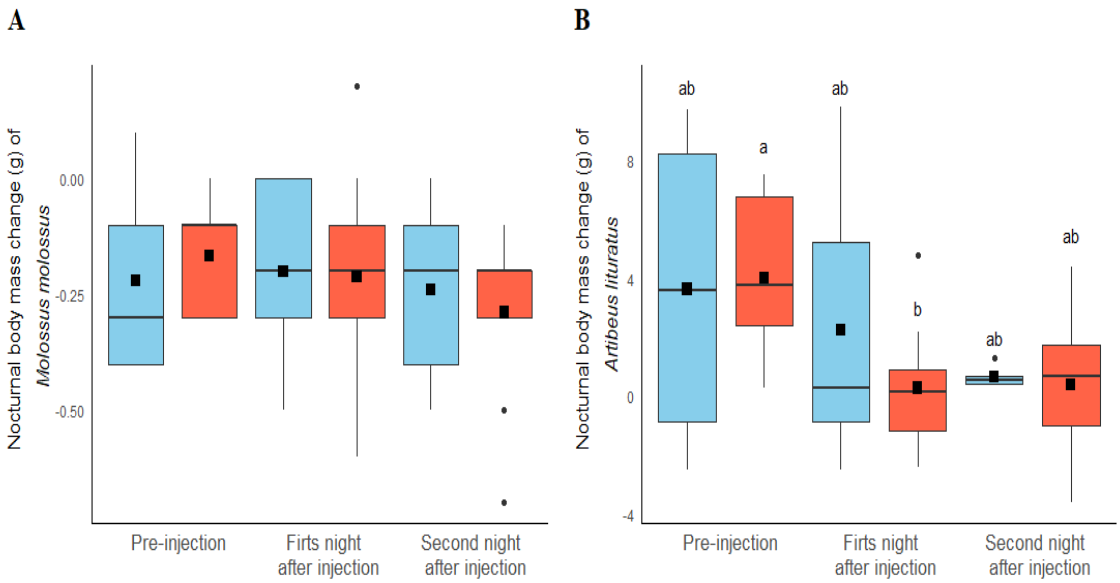

Figure 2. Nocturnal body mass change of **A.** *M. molossus* and **B.** *A. lituratus*, before and after PBS (blue) and LPS (red) injections. Black squares represent the mean, horizontal lines represent the medians and black dots indicate the outliers. Letters indicate statistical differences.

Food Intake

Pre-injection food intake of *A. lituratus* was similar between LPS and PBS treated animals (t = 1.03 ; P = 0.33). However, the permutation test detected an isolated effect of treatment when considering the entire experimental period (Table 1).

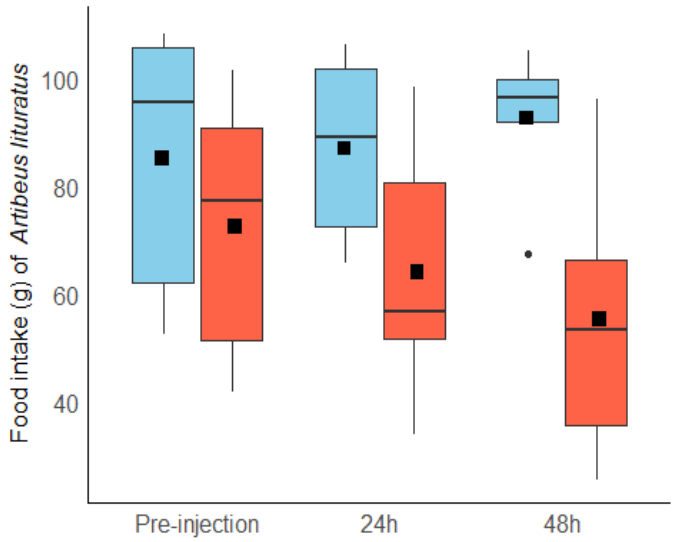

Figure 3. Variation in food intake of *A. lituratus*, before and after PBS (blue) and LPS (red) injections. Black squares indicate the means and horizontal lines indicate the medians. Black dots indicate the outliers.

In relation to the pre-injection night, bats of the PBS group increased food intake in 2.1% during the night immediately after the injection, and in 8.8% during the second night after the injection, while bats injected with LPS decreased food intake in 11.7% and 23.5% during the first and second post-injection night respectively (Figure 3). Moreover, despite the initial food intake was similar in both treatments, the control animals ate 1.36 and 1.67 times more than LPS injected animals, during the first and second night after injections.

Neutrophil/Lymphocyte Ratio

No statistical difference was detected in relative variation of the NLR in *M. molossus* (Figure 4A). In relation to the pre-injection values, the control group had an average increase of 348% and a decrease of 5%, 12h and 24h after the injection, while the LPS group had an increase of 251% and 173% respectively. In *A. lituratus* the analysis detected an isolated effect of treatment and time, but no interaction. The post-hoc test showed that the relative variation in the NLR was significantly higher 24h after LPS injection in relation to the previous count for the same LPS-treated animals, and to the control group. The NLR decreased in 8% in animals of the control group, 12h after the

injection, and increased in 40% after 24h. Thus, animals injected with LPS exhibited an increase of 41% and 197% after 12h and 24h respectively (Figure 4B).

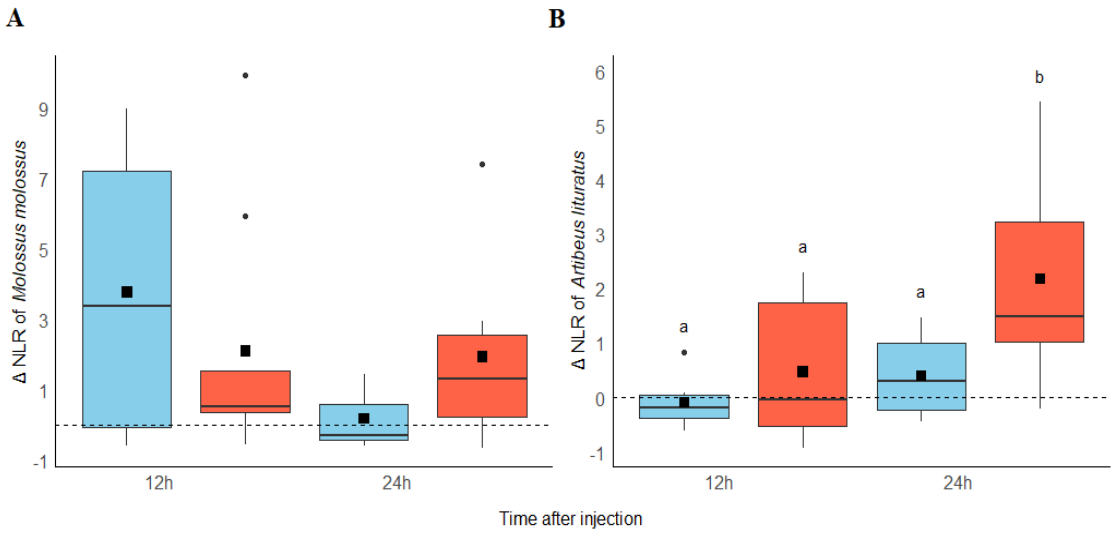

Figure 4. Relative variation in NLR of **A.** *M. molossus* and **B.** *A. lituratus*, after PBS (blue) and LPS (red) injections. The black horizontal lines indicate the median and the black dots the outliers. Letters indicate statistical difference.

Mobilization of Energy Reserves

Contrary to our expectations, any of the studied species showed a significant effect of LPS challenge on energy reserves. Hepatic and muscular glycogen in *A. lituratus*

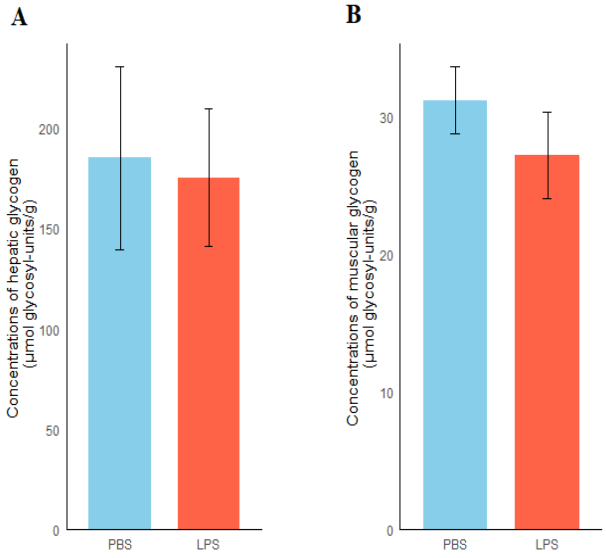

Figure 5. Glycogen concentrations (mean ± SD) in **A.** liver and **B.** pectoral muscle of *A. lituratus* after PBS (blue) and LPS (red) injections.

(Figure 5), total protein content in liver and muscle (Figure 6) and the concentration of total fatty acids of the carcass (Figure 7) in both species, was similar between treatments.

DISCUSSION

Our study suggests that the APR induced with LPS does not compromises the concentrations of energy reserves in *M. molossus* and *A. lituratus*.

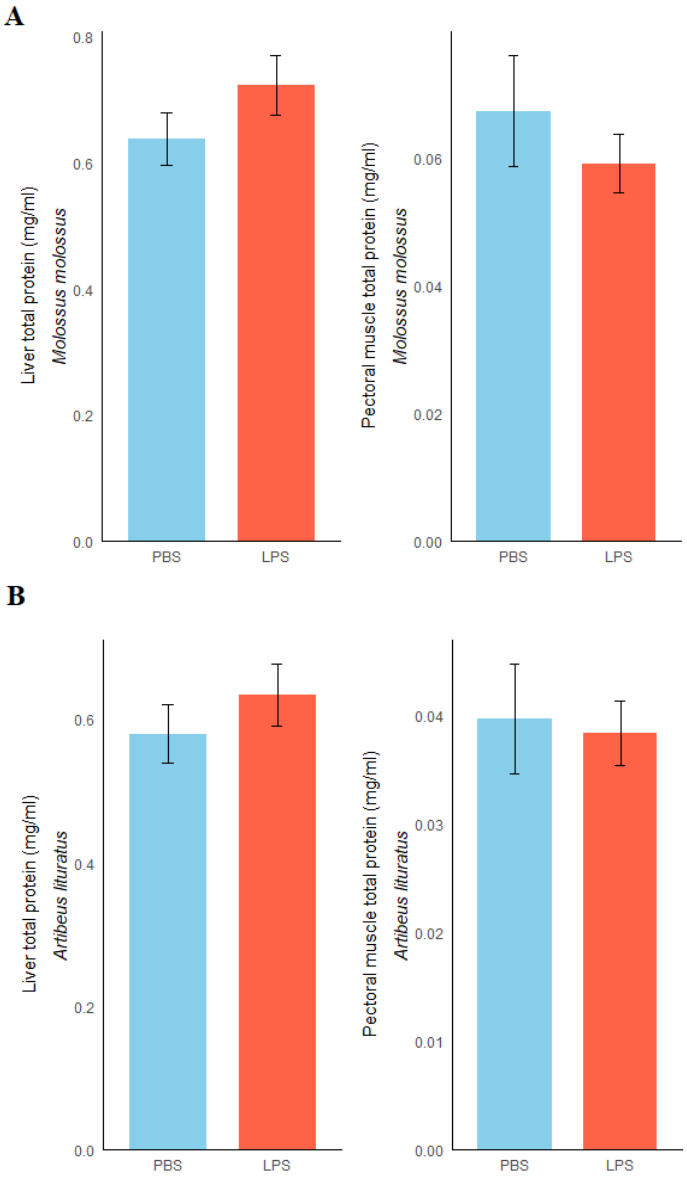

Figure 6. Total protein content (mean ± SD) in liver and pectoral muscle of **A.** *Molossus molossus* and **B.** *Artibeus lituratus* after PBS (blue) and LPS (red) injections.

Body mass seems not to be affected also in *M. molossus*, however *A. lituratus* challenged with LPS only gained body mass during the first experimental nigh, before treatment injection. *M. molossus* body mass loss in both treatments was similar to that reported only for control group animals in STOCKMAIER et al. (2015), representing about 2.3 – 2.7% of body mass loss. Similar to us, they used LPS in a dose of 4.53 ± 0.66 mg/kg, to induce an APR in *M. molossus*. Nevertheless, they reported significant body mass loss 24h after injections, in animals treated with LPS (~0.55g; about 7% of body mass loss), in contrast to animals of the control group (~0.25g). Despite animals were fed similarly in both studies. Additionally, they concluded that the bacterial APR in *M. molossus* does not include fever, nor variation in total leucocyte count. Also, we did not registered variation in NLR. The lack of these immunity markers suggests that the APR in *M. molossus* may not include some conservative mechanisms reported in other mammals. *M. molossus* reduces body temperature nearly to 28°C in daily basis, even after an immune challenge induced with LPS (STOCKMAIER et al., 2015). Such decrease in body temperature reduces the number of circulating leucocytes in other mammals (BOUMA; CAREY; KROESE, 2010), and it could affect the leucocyte profile of species that depends on torpor for manteinign homeostasis (AMARAL-SILVA et al., 2021; BROESSNER et al., 2012; LUO et al., 2021; PIKULA et al., 2020a).

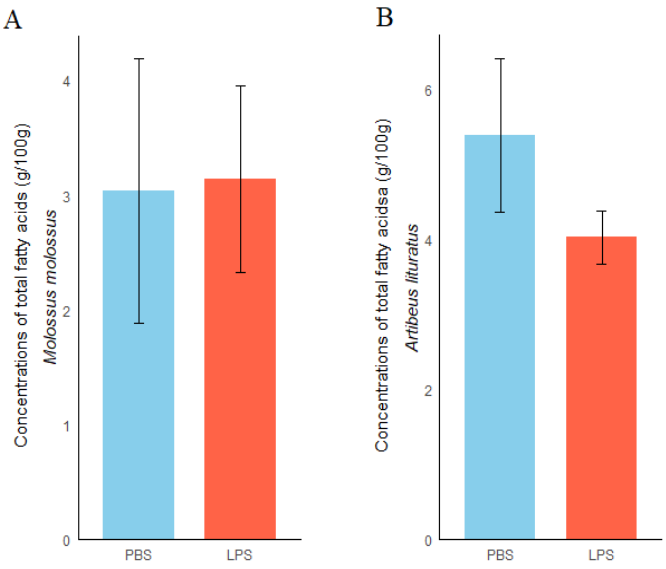

Figure 7. Concentration of carcass total fatty acids (mean ± SD) in **A.** *M. molossus* and **B.** *A. lituratus*, after PBS (blue) and LPS (red) injections.

The APR in *A. lituratus* has been previously induced by GUERRERO-CHACÓN et al. (2018). The authors reported an increase of resting metabolic rate (RMR) of approximately 41 ± 13% in energy expenditure following an LPS challenge. This was associated with a body mass loss in the LPS group (3.3 ± 1.4 g), which was three times greater than that observed in PBS-injected animals (1.3 ± 0.4 g) during the first 6 hours after the injection. Nevertheless, 24h after the injections, animals treated with LPS regain body mass, and they equaled the PBS group body mass. It should be emphasized that, in contrast to our study, GUERRERO-CHACÓN et al. (2018) injected the animals in the morning, in order to measure metabolic rate during the resting period, however, the body mass loss 6h after LPS injection reported by GUERRERO-CHACÓN et al. (2018) was similar to that detected in our LPS-treated animals, though 24h after the injections. We took blood samples 12 and 24 hours after the injections, but *A. lituratus* only exhibit an increase in NLR 24h after LPS application. GUERRERO-CHACÓN et al. (2018) did not measured the NLR, but they reported a lack of variation in total leucocyte count, associated with the APR. The variation in leucocyte count reflects the increase or decrease of total circulating leucocytes. Nevertheless, an increase in NLR reflects the increase of neutrophils in relation to lymphocytes in the bloodstream., and it is used as an inflammatory marker since LPS triggers the activation of the HPA axis, leading to a release of glucocorticoids (CHESLEY; IV, 2011; SOCORRO FARIA et al., 2016). This promotes the migration of neutrophils from the bone marrow into the bloodstream, where they play a critical role in combating infections (BORNSTEIN et al., 2006; CAIN; CIDLOWSKI, 2017). Glucocorticoids also influence lymphocyte movement, directing these cells from the circulation into lymph nodes, the spleen, bone marrow, and skin (DHABHAR, 2002; TAVES; ASHWELL, 2021).

Despite the potential relationship between food intake and body mass variation, few studies quantified food intake in LPS-challenged bats (MELHADO; HERRERA M.; DA CRUZ-NETO, 2020; MORENO et al., 2021; VIOLA; HERRERA; DA CRUZ-NETO, 2022; VIOLA; HERRERA M.; CRUZ-NETO, 2024). In the wild, searching for food often requires significant effort and increases the risk of predation, especially for animals in poor physical condition. Laboratory studies in mice showed increased survival in animals with restricted food intake, and it has been suggested that anorexia may support the immune response to bacteria (EXTON, 1997; MURRAY; MURRAY, 1979; WANG et al., 2016). The APR in *A. lituratus* seems to include sickness anorexia. The bats exhibited a

decrease in food intake after LPS injection, and such decrease was reflected in the low nocturnal body mass gain specially during the night immediately after the injection. Unfortunately, we encountered difficulties in measuring food consumption in *M. molossus*, as the animals needed to be manually fed. Consequently, food intake during the first night—immediately following the injections—would not accurately reflect any treatment effects, and the animals would not voluntarily consume the larvae during the night. Therefore, the lack of difference in body mass variation between treatments in *M. molossus* after the injections, could be attributed to the fact that we provided the same amount of larvae to all individuals at the beginning of the night, and they did not have the opportunity to feed further throughout the night, even if they intended to.

Little is known about the effects of sickness-induced anorexia on the mobilization of energy reserves during the immune response in wild animals. Nevertheless, studies that have observed body mass loss during immune activation often attribute this variation to the mobilization of energy substrates. To our knowledge, this is the first study to assess the mobilization of energy reserves in bats during the APR. High hepatic glycogen concentrations have been reported for *A. lituratus*, which contributes with glycemic homeostasis during periods of food depravation (PINHEIRO et al., 2006). Muscle glycogen reserves may also contribute to glycemic homeostasis by providing lactate for glucose synthesis via gluconeogenesis, given that lactate concentrations significantly decrease after 48 hours of fasting in previous studies with the same species (PINHEIRO et al., 2006). Therefore, glycogen reserves in the liver and muscles participate in maintaining plasma glucose levels when frugivorous bats are subjected to short fasting periods (24 - 48 hours). We found no differences in glycogen concentrations in the liver and pectoral muscle of *A. lituratus* between treatments. The bats in our study did not lose body mass by the end of the study, and, although they reduced food consumption during the experiments, they did not experience complete food depravation. Therefore, even though we euthanized the animals 36 hours after the LPS injection—consistent with the typical duration of the APR in immune challenges induced by LPS—we cannot rule out the possibility of glycogen mobilization during the initial hours of APR activation, especially immediately after the first night after the injection, when we detected a decrease in food intake. As our intention was to determine whether the animals would exhibit sickness anorexia during the APR, we allowed them to feed *ad libitum*, which may have enabled these energy reserves to be replenished before the experiments concluded.

Similarly, we may have influenced the replenishment of substrates in *M. molossus* by offering the same amount of food to individuals in both groups. We expected that if the acute phase response caused a significant increase in the animals' energy demand, the difference between the treatments would be detectable. However, it appears that the energy expenditure induced by the APR may be relatively low for the bats, likely being easily balanced by food intake, even if it is limited or reduced. The lack of significant effect of LPS on liver and pectoral muscle total protein content in this study can be attributed to several factors. The animals did not experience significant body mass loss during the immune challenge, further suggesting that the APR did not induce a substantial catabolic shift in their metabolism. These bats likely prioritize the efficient use of their energy reserves, particularly during immune activation, minimizing protein breakdown. The concentration of total carcass fatty acids did not vary between treatments in either species, suggesting that the energetic demand of the APR is also not high enough to trigger fat mobilization, which is consistent with our body mass results since *M. molossus* showed similar body mass loss across treatments, while *A. lituratus* did not experience body mass loss throughout the experimental period.

STOCKMAIER et al. (2015) reported torpor in resting *M. molossus* during the day. This torpor was not associated with LPS injections, as the control group also showed a significant decrease in body temperature. Nevertheless, lowering metabolic rate may represent a strategy to favor energy balance, even during an APR. Additionally, animals on protein-rich diets are capable of maintaining blood glucose levels during short periods of food restriction through gluconeogenesis, avoiding the need to mobilize fat stores (DA SILVA; MIGLIORTNI, 1990; KETTELHUT; FOSS; MIGLIORINI, 1980). On the other hand, frugivorous species primarily rely on glycogenolysis to sustain glycemic homeostasis. Fat mobilization would likely only occur in cases where glycogen stores were depleted. So, we assume that *A. lituratus* they did not rely on fat mobilization since they maintained normal concentrations of liver and muscle glycogen by the end of the experiments.

CONCLUSSIONS

In this study, we hypothesized that the APR cost, combined with sickness-induced anorexia, would lead to the mobilization of glycogen or fatty acids reserves in bats, with such mobilization differing between species with distinct dietary habits. However, our findings suggest that a simulated bacterial immune challenge does not compromise energy balance in *M. molossus* and *A. lituratus*, as the bats did not need to rely on glycogen or fat mobilization during the immune challenge. Food was offered to the animals during the immune challenge to allow them the possibility of eating if it aligned with their natural behavior. Nonetheless, an immune challenge combined with complete food deprivation over a shorter period could potentially reveal some mechanisms of glycemic homeostasis that we were unable to detect due to our experimental design. Moreover, it has been proposed that a trade-off exists between gene families that mitigate the physiological demands of flight and aspects of the immune system in bats, potentially reducing their sensitivity to bacterial challenges (ZHANG et al., 2013). Therefore, a clear understanding of the immune response in bats, including their reactions to viral and fungal antigens, is essential to elucidate the unique features of their immune function.

REFERENCES

ADAMO, S. A. et al. Illness-induced anorexia may reduce trade-offs between digestion and immune function. **Animal Behaviour**, v. 79, n. 1, p. 3–10, jan. 2010.

AMARAL, T. S. et al. Low energy reserves are associated with fasting susceptibility in Neotropical nectar bats Glossophaga soricina. **Brazilian Journal of Biology**, v. 79, n. 2, p. 165–168, 1 abr. 2019.

AMARAL-SILVA, L. DO et al. Regulated hypothermia in response to endotoxin in birds. **The Journal of Physiology**, v. 599, n. 11, p. 2969–2986, 3 jun. 2021.

ANINDITA, P. D. et al. Detection of coronavirus genomes in Moluccan naked-backed fruit bats in Indonesia. **Archives of Virology**, v. 160, n. 4, p. 1113–1118, 4 abr. 2015.

AUDET, D.; FENTON, M. B. Heterothermy and the Use of Torpor by the Bat Eptesicus fuscus (Chiroptera: Vespertilionidae): A Field Study. **Physiological Zoology**, v. 61, n. 3, p. 197–204, maio 1988.

AUSTAD, S. N.; FISCHER, K. E. **Mammalian Aging, Metabolism, and Ecology: Evidence From the Bats and MarsupialsJournal of Gerontology: BIOLOGICAL SCIENCES**. [s.l: s.n.]. Disponível em: <http://geronj.oxfordjournals.org/>.

AVIELLO, G. et al. Brain control of appetite during sickness. **British Journal of Pharmacology**, v. 178, n. 10, p. 2096–2110, 2 maio 2021.

BALABAN, R. S.; NEMOTO, S.; FINKEL, T. Mitochondria, Oxidants, and Aging. **Cell**, v. 120, n. 4, p. 483–495, 25 fev. 2005.

BARJA, G. et al. A decrease of free radical production near critical targets as a cause of maximum longevity in animals. **Comparative Biochemistry and Physiology Part B: Comparative Biochemistry**, v. 108, n. 4, p. 501–512, ago. 1994.

BARJA, G. Endogenous oxidative stress: relationship to aging, longevity and caloric restriction. **Ageing Research Reviews**, v. 1, n. 3, p. 397–411, jun. 2002.

BARTELS, W.; LAW, B. S.; GEISER, F. Daily torpor and energetics in a tropical mammal, the northern blossom-bat Macroglossus minimus (Megachiroptera). **Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology**, v. 168, n. 3, p. 233–239, 7 abr. 1998.

BAUD, V.; KARIN, M. Signal transduction by tumor necrosis factor and its relatives. **Trends in Cell Biology**, v. 11, n. 9, p. 372–377, set. 2001.

BECKMAN, K. B.; AMES, B. N. The Free Radical Theory of Aging Matures. **Physiological Reviews**, v. 78, n. 2, p. 547–581, 1 abr. 1998.

BILBO, S. D.; NELSON, R. J. **Melatonin Regulates Energy Balance and Attenuates Fever in Siberian Hamsters**. [s.l: s.n.].

BOLLEN, M.; KEPPENS, S.; STALMANS, W. **Specific features of glycogen metabolism in the liverBiochem. J**. [s.l: s.n.].

BORNSTEIN, S. R. et al. The Role of Toll-like Receptors in the Immune–Adrenal Crosstalk. **Annals of the New York Academy of Sciences**, v. 1088, n. 1, p. 307–318, 8 nov. 2006.

BOUMA, H. R.; CAREY, H. V; KROESE, F. G. M. Hibernation: the immune system at rest? **Journal of Leukocyte Biology**, v. 88, n. 4, p. 619–624, 2 jun. 2010.

BROESSNER, G. et al. Update on therapeutic temperature management. **Critical Care**, v. 16, n. S2, p. A1, 7 jun. 2012.

BROOK, C. E.; DOBSON, A. P. Bats as 'special' reservoirs for emerging zoonotic pathogens. **Trends in Microbiology**, v. 23, n. 3, p. 172–180, mar. 2015.

BROSNAN, J. Comments on metabolic needs for glucose and the role of gluconeogenesis. **European Journal of Clinical Nutrition**, v. 53, n. S1, p. s107–s111, 1 abr. 1999.

BROWN, J. C. L. et al. Examining the mechanisms responsible for lower ROS release rates in liver mitochondria from the long-lived house sparrow (Passer domesticus) and big brown bat (Eptesicus fuscus) compared to the short-lived mouse (Mus musculus). **Mechanisms of Ageing and Development**, v. 130, n. 8, p. 467–476, ago. 2009.

BRUNET-ROSSINNI, A. K. Reduced free-radical production and extreme longevity in the little brown bat (Myotis lucifugus) versus two non-flying mammals. **Mechanisms of Ageing and Development**, v. 125, n. 1, p. 11–20, jan. 2004.

BRUNET-ROSSINNI, A. K.; AUSTAD, S. N. Ageing Studies on Bats: A Review. **Biogerontology**, v. 5, n. 4, p. 211–222, ago. 2004.

BUEGE, J. A.; AUST, S. D. Microsomal lipid peroxidation. Em: [s.l: s.n.]. p. 302–310.

CABRERA-MARTINEZ, L. V.; HERRERA, M. L. G.; CRUZ-NETO, A. P. The energetic cost of mounting an immune response for Pallas's longtongued bat (Glossophaga soricina). **PeerJ**, v. 2018, n. 6, 2018.

CABRERA-MARTINEZ, L. V.; HERRERA M., L. G.; CRUZ-NETO, A. P. Food restriction, but not seasonality, modulates the acute phase response of a Neotropical bat. **Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology**, v. 229, p. 93–100, 1 mar. 2019.

CAIN, D. W.; CIDLOWSKI, J. A. Immune regulation by glucocorticoids. **Nature Reviews Immunology**, v. 17, n. 4, p. 233–247, 13 abr. 2017.

CARLTON, E. D.; DEMAS, G. E.; FRENCH, S. S. **Leptin, a neuroendocrine mediator of immune responses, inflammation, and sickness behaviors**. **Hormones and Behavior**, ago. 2012.

CHATTERJEE, S. Oxidative Stress, Inflammation, and Disease. Em: **Oxidative Stress and Biomaterials**. [s.l.] Elsevier, 2016. p. 35–58.

CHELOMBITKO, M. A. Role of Reactive Oxygen Species in Inflammation: A Minireview. **Moscow University Biological Sciences Bulletin**, v. 73, n. 4, p. 199–202, 12 out. 2018.

CHEN, Y.; JUNGER, W. G. Measurement of Oxidative Burst in Neutrophils. Em: **Methods in Molecular Biology**. [s.l: s.n.]. v. 844p. 115–124.

CHESLEY, L.; IV, S. **Neutrophil:Lymphocyte Ratio as a Possible Indicator of Chronic Anthropogenic Stress in Bats (Mammalia: Chiroptera)**. [s.l: s.n.]. Disponível em: <http://hdl.handle.net/10415/2683>. Acesso em: 23 set. 2024.

CHUNG, H. Y. et al. Molecular inflammation hypothesis of aging based on the anti-aging mechanism of calorie restriction. **Microscopy Research and Technique**, v. 59, n. 4, p. 264–272, 15 nov. 2002.

CHUNG, H. Y. et al. The Molecular Inflammatory Process in Aging. **Antioxidants & Redox Signaling**, v. 8, n. 3–4, p. 572–581, mar. 2006.

COSTANTINI, D. Oxidative stress in ecology and evolution: lessons from avian studies. **Ecology Letters**, v. 11, n. 11, p. 1238–1251, 2 nov. 2008.

COSTANTINI, D. et al. From molecules to living systems: historical and contemporary issues in oxidative stress and antioxidant ecology. **Functional Ecology**, v. 24, n. 5, p. 950–959, 16 out. 2010.

COSTANTINI, D. et al. Induced bacterial sickness causes inflammation but not blood oxidative stress in Egyptian fruit bats (Rousettus aegyptiacus). **Conservation Physiology**, v. 10, n. 1, 2022.

COSTANTINI, D. et al. Oxidative damage varies in response to bacterial, fungal and viral antigen challenges in bats. **Journal of Experimental Biology**, v. 226, n. 21, 1 nov. 2023.

COSTANTINI, D.; MØLLER, A. P. Carotenoids are minor antioxidants for birds. **Functional Ecology**, v. 22, n. 2, p. 367–370, 28 abr. 2008.

COSTANTINI, D.; MØLLER, A. P. Does immune response cause oxidative stress in birds? A meta-analysis. **Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology**, v. 153, n. 3, p. 339–344, 2009.

CRAY, C. Acute Phase Proteins in Animals. Em: **Progress in Molecular Biology and Translational Science**. [s.l.] Elsevier B.V., 2012. v. 105p. 113–150.

CRAY, C.; ZAIAS, J.; ALTMAN, N. H. Acute phase response in animals: a review. **Comparative medicine**, v. 59, n. 6, p. 517–26, dez. 2009.

DA SILVA, R. S. M.; MIGLIORTNI, R. H. Effects of starvation and refeeding on energy-linked metabolic processes in the turtle (iTphrynops hilarii). **Comparative Biochemistry and Physiology Part A: Physiology**, v. 96, n. 3, p. 415–419, jan. 1990.

DAVIS, A. K.; MANEY, D. L.; MAERZ, J. C. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. **Functional Ecology**, v. 22, n. 5, p. 760–772, 20 out. 2008.

DHABHAR, F. S. Stress-induced augmentation of immune function - The role of stress hormones, leukocyte trafficking, and cytokines. **Brain, Behavior, and Immunity**, v. 16, n. 6, p. 785–798, 2002.

DIETERICH, S. et al. **Gene Expression of Antioxidative Enzymes in the Human Heart Increased Expression of Catalase in the End-Stage Failing HeartCirculation**. [s.l: s.n.]. Disponível em: <http://www.circulationaha.org>.

DRÖGE, W.; SCHIPPER, H. M. Oxidative stress and aberrant signaling in aging and cognitive decline. **Aging Cell**, v. 6, n. 3, p. 361–370, 27 jun. 2007.

EL-BELTAGI, H. S.; MOHAMED, H. I. Reactive Oxygen Species, Lipid Peroxidation and Antioxidative Defense Mechanism. **Notulae Botanicae Horti Agrobotanici Cluj-Napoca**, v. 41, n. 1, p. 44, 28 maio 2013.

EVANS, S. S.; REPASKY, E. A.; FISHER, D. T. Fever and the thermal regulation of immunity: the immune system feels the heat. **Nature Reviews Immunology**, v. 15, n. 6, p. 335–349, 15 jun. 2015.

EXTON, M. S. Infection-Induced Anorexia: Active Host Defence Strategy. **Appetite**, v. 29, n. 3, p. 369–383, dez. 1997.

FAIN, J. N. Release of Interleukins and Other Inflammatory Cytokines by Human Adipose Tissue Is Enhanced in Obesity and Primarily due to the Nonfat Cells. Em: **Vitamins and Hormones**. [s.l: s.n.]. v. 74p. 443–477.

FIELD, H. E. Bats and Emerging Zoonoses: Henipaviruses and SARS. **Zoonoses and Public Health**, v. 56, n. 6–7, p. 278–284, 9 ago. 2009.

FINKEL, T.; HOLBROOK, N. J. Oxidants, oxidative stress and the biology of ageing. **Nature**, v. 408, n. 6809, p. 239–247, nov. 2000.

FREITAS, M. B. et al. Metabolic responses induced by fasting in the common vampire bat Desmodus rotundus. **Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology**, v. 173, n. 8, p. 703–707, 1 nov. 2003.

FREITAS, M. B. et al. Effects of short-term fasting on energy reserves of vampire bats (Desmodus rotundus). **Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology**, v. 140, n. 1, p. 59–62, jan. 2005.

FREITAS, M. B. et al. Reduced insulin secretion and glucose intolerance are involved in the fasting susceptibility of common vampire bats. **General and Comparative Endocrinology**, v. 183, p. 1–6, 1 mar. 2013.

FREITAS, M. B.; WELKER, A. F.; PINHEIRO, E. C. Seasonal variation and food deprivation in common vampire bats (Chiroptera: Phyllostomidae). **Brazilian Journal of Biology**, v. 66, n. 4, p. 1051–1055, nov. 2006.

FREITAS, MB. et al. Energy metabolism and fasting in male and female insectivorous bats Molossus molossus (Chiroptera: Molossidae). **Brazilian Journal of Biology**, v. 70, n. 3, p. 617–621, ago. 2010.

GABAY, C.; KUSHNER, I. Acute-Phase Proteins and Other Systemic Responses to Inflammation. **New England Journal of Medicine**, v. 340, n. 6, p. 448–454, 11 fev. 1999.

GARRATT, M.; BROOKS, R. C. **Oxidative stress and condition-dependent sexual signals: More than just seeing red**. **Proceedings of the Royal Society B: Biological Sciences**Royal Society, , 2012.

GOMES, B. R. B. et al. Increase of reactive oxygen species in different tissues during lipopolysaccharide-induced fever and antipyresis: an electron paramagnetic resonance study. **Free Radical Research**, v. 52, n. 3, p. 351–361, 4 mar. 2018.

GONG, S. et al. Dynamics and Correlation of Serum Cortisol and Corticosterone under Different Physiological or Stressful Conditions in Mice. **PLOS ONE**, v. 10, n. 2, p. e0117503, 20 fev. 2015.

GONZALEZ, V.; BANERJEE, A. Molecular, ecological, and behavioral drivers of the bat-virus relationship. **iScience**, v. 25, n. 8, p. 104779, ago. 2022.

GRUYS, E. et al. Acute phase reaction and acute phase proteins. **Journal of Zhejiang University-SCIENCE B**, v. 6, n. 11, p. 1045–1056, 1 nov. 2005.

GUERRERO-CHACÓN, A. L. et al. Metabolic cost of acute phase response in the frugivorous bat, Artibeus lituratus. **Mammal Research**, v. 63, n. 4, p. 397–404, 30 out. 2018.

HABIG, W. H.; PABST, M. J.; JAKOBY, W. B. Glutathione S-Transferases. **Journal of Biological Chemistry**, v. 249, n. 22, p. 7130–7139, nov. 1974.

HADWAN, M. H.; ABED, H. N. Data supporting the spectrophotometric method for the estimation of catalase activity. **Data in Brief**, v. 6, p. 194–199, 1 mar. 2016.

HANADHITA, D.; SATYANINGTIJAS, A. S.; AGUNGPRIYONO, S. Bats Oxidative Stress Defense. **Jurnal Riset Veteriner Indonesia (Journal of The Indonesian Veterinary Research)**, v. 3, n. 1, 30 jan. 2019.

HART, B. L. Biological basis of the behavior of sick animals. **Neuroscience & Biobehavioral Reviews**, v. 12, n. 2, p. 123–137, jun. 1988.

HERNÁNDEZ-ARCIGA, U. et al. Synergetic effects of immune challenge and stress depress cortisol, inflammatory response and antioxidant activity in fish-eating Myotis. **Journal of Experimental Biology**, v. 223, n. 24, 1 jan. 2020.

HOCKE, G. M. Leukemia Inhibitory Factor. Em: **Encyclopedia of Immunology**. [s.l.] Elsevier, 1998. p. 1560–1562.

HOU, C.-C. et al. Oxidative stress and pyrogenic fever pathogenesis. **European Journal of Pharmacology**, v. 667, n. 1–3, p. 6–12, 30 set. 2011.

HOUTEN, S. M.; WANDERS, R. J. A. A general introduction to the biochemistry of mitochondrial fatty acid β-oxidation. **Journal of Inherited Metabolic Disease**, v. 33, n. 5, p. 469–477, 2 out. 2010.

INGVARTSEN, K. L.; BOISCLAIR, Y. R. **Leptin and the regulation of food intake, energy homeostasis and immunity with special focus on periparturient ruminantsDomestic Animal Endocrinology**. [s.l: s.n.].

JAESCHKE, H. Mechanisms of Liver Injury. II. Mechanisms of neutrophil-induced liver cell injury during hepatic ischemia-reperfusion and other acute inflammatory conditions. **American Journal of Physiology-Gastrointestinal and Liver Physiology**, v. 290, n. 6, p. G1083–G1088, jun. 2006.

JANEWAY, C. A.; MEDZHITOV, R. Innate Immune Recognition. **Annual Review of Immunology**, v. 20, n. 1, p. 197–216, abr. 2002.

JOHNSON, R. W. Immune and endocrine regulation of food intake in sick animals. **Domestic Animal Endocrinology**, v. 15, n. 5, p. 309–319, set. 1998.

KETTELHUT, I. C.; FOSS, M. C.; MIGLIORINI, R. H. Glucose homeostasis in a carnivorous animal (cat) and in rats fed a high-protein diet. **American Journal of Physiology-Regulatory, Integrative and Comparative Physiology**, v. 239, n. 5, p. R437–R444, 1 nov. 1980.

KNOLLE, P. A.; GERKEN, G. Local control of the immune response in the liver. **Immunological Reviews**, v. 174, n. 1, p. 21–34, 19 abr. 2000.

KRAUS-FRIEDMANN, N. Hormonal regulation of hepatic gluconeogenesis. **Physiological Reviews**, v. 64, n. 1, p. 170–259, jan. 1984.

LANGLOIS, M. R.; DELANGHE, J. R. Biological and clinical significance of haptoglobin polymorphism in humans. **Clinical Chemistry**, v. 42, n. 10, p. 1589–1600, 1 out. 1996.

LAURIDSEN, C. From oxidative stress to inflammation: redox balance and immune system. **Poultry Science**, v. 98, n. 10, p. 4240–4246, 1 out. 2019.

LEGRAND, E. K. Why infection-induced anorexia? The case for enhanced apoptosis of infected cells. **Medical Hypotheses**, v. 54, n. 4, p. 597–602, abr. 2000.

LEMASTERS, J. J.; JAESCHKE, H. Oxidative Stress and Inflammation in the Liver. Em: **The Liver**. [s.l.] Wiley, 2020. p. 714–727.

LEVINE, R. L. et al. Carbonyl assays for determination of oxidatively modified proteins. Em: [s.l: s.n.]. p. 346–357.

LOCHMILLER, R. L.; DEERENBERG, C. Trade-offs in evolutionary immunology: just what is the cost of immunity? **Oikos**, v. 88, n. 1, p. 87–98, 24 jan. 2000.

LOWRY, OLIVERH. et al. PROTEIN MEASUREMENT WITH THE FOLIN PHENOL REAGENT. **Journal of Biological Chemistry**, v. 193, n. 1, p. 265–275, nov. 1951.

LUIS, A. D. et al. A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? **Proceedings of the Royal Society B: Biological Sciences**, v. 280, n. 1756, p. 20122753, 7 abr. 2013.

LUO, J. et al. Flight rapidly modulates body temperature in freely behaving bats. **Animal Biotelemetry**, v. 9, n. 1, p. 45, 3 dez. 2021.

MA, J. et al. Glycogen metabolism regulates macrophage-mediated acute inflammatory responses. **Nature Communications**, v. 11, n. 1, p. 1769, 14 abr. 2020.

MACHADO, A. M. et al. Effects of yacon flour associated with an energy restricted diet on intestinal permeability, fecal short chain fatty acids, oxidative stress and inflammation markers levels in adults with obesity or overweight: a randomized, double blind, placebo controlled clinical trial. **Archives of Endocrinology and Metabolism**, v. 64, n. 5, p. 597–607, 30 mar. 2020.

MARAIS, M.; MALONEY, S. K.; GRAY, D. A. The metabolic cost of fever in Pekin ducks. **Journal of Thermal Biology**, v. 36, n. 2, p. 116–120, mar. 2011.

MARKOWSKA, M.; MAJEWSKI, P. M.; SKWARŁO-SOŃTA, K. Avian biological clock – Immune system relationship. **Developmental & Comparative Immunology**, v. 66, p. 130–138, 1 jan. 2017.

MARRI, V.; RICHNER, H. Immune response, oxidative stress and dietary antioxidants in great tit nestlings. **Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology**, v. 179, p. 192–196, 1 jan. 2015.

MARSHALL, M. K. et al. Leukemia inhibitory factor induces changes in lipid metabolism in cultured adipocytes. **Endocrinology**, v. 135, n. 1, p. 141–147, jul. 1994.

MARTIN, C. K. et al. Effect of Calorie Restriction on Resting Metabolic Rate and Spontaneous Physical Activity. **Obesity**, v. 15, n. 12, p. 2964–2973, 6 dez. 2007.

MARTIN, L. B.; WEIL, Z. M.; NELSON, R. J. Fever and sickness behaviour vary among congeneric rodents. **Functional Ecology**, v. 22, n. 1, p. 68–77, 29 fev. 2008.

MCNAB, B. K. **Seasonal Fat Reserves of Bats in Two Tropical Environments SEASONAL FAT RESERVES OF BATS IN TWO TROPICAL ENVIRONMENTS'Source: Ecology**. [s.l: s.n.].

MELHADO, G.; HERRERA M., L. G.; DA CRUZ-NETO, A. P. Bats respond to simulated bacterial infection during the active phase by reducing food intake. **Journal of Experimental Zoology Part A: Ecological and Integrative Physiology**, v. 333, n. 8, p. 536–542, 1 out. 2020.

MORENO, K. R. et al. Sick bats stay home alone: fruit bats practice social distancing when faced with an immunological challenge. **Annals of the New York Academy of Sciences**, v. 1505, n. 1, p. 178–190, 1 dez. 2021.

MURPHY, K. et al. **Janeway´s Immunobiology**. 8th. ed. New York: Garland Science, Taylor & Francis Group, LLC, 2012.

MURRAY, M.; MURRAY, A. Anorexia of infection as a mechanism of host defense. **The American Journal of Clinical Nutrition**, v. 32, n. 3, p. 593–596, mar. 1979.

NATHAN, C.; CUNNINGHAM-BUSSEL, A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. **Nature Reviews Immunology**, v. 13, n. 5, p. 349–361, 25 maio 2013.

NEWSHOLME, P.; NEWSHOLME, E. A. Rates of utilization of glucose, glutamine and oleate and formation of end-products by mouse peritoneal macrophages in culture. **Biochemical Journal**, v. 261, n. 1, p. 211–218, 1 jul. 1989.

OLIVEIRA, J. M. et al. Exposure to the insecticide endosulfan induces liver morphology alterations and oxidative stress in fruit-eating bats (*Artibeus lituratus*). **International Journal of Experimental Pathology**, v. 98, n. 1, p. 17–25, 27 fev. 2017.

OLIVEIRA, J. M. et al. Exposure to deltamethrin induces oxidative stress and decreases of energy reserve in tissues of the Neotropical fruit-eating bat Artibeus lituratus. **Ecotoxicology and Environmental Safety**, v. 148, p. 684–692, 1 fev. 2018.

OTÁLORA-ARDILA, A. et al. Metabolic Cost of the Activation of Immune Response in the Fish-Eating Myotis (Myotis vivesi): The Effects of Inflammation and the Acute Phase Response. **PLOS ONE**, v. 11, n. 10, p. e0164938, 28 out. 2016.

OTÁLORA-ARDILA, A. et al. The effect of short-term food restriction on the metabolic cost of the acute phase response in the fish-eating Myotis (Myotis vivesi). **Mammalian Biology**, v. 82, p. 41–47, 1 jan. 2017.

PEREIRA, F. D. et al. Oxidative Stress and Antioxidant Defense in the Heart, Liver, and Kidney of Bat Species with Different Feeding Habits. **International Journal of Molecular Sciences**, v. 24, n. 22, p. 16369, 15 nov. 2023.

PETERSEN, M. C.; VATNER, D. F.; SHULMAN, G. I. Regulation of hepatic glucose metabolism in health and disease. **Nature Reviews Endocrinology**, v. 13, n. 10, p. 572–587, 21 out. 2017.

PIKULA, J. et al. Phagocyte activity reflects mammalian homeo- and hetero-thermic physiological states. **BMC Veterinary Research**, v. 16, n. 1, p. 232, 6 dez. 2020a.

PIKULA, J. et al. Phagocyte activity reflects mammalian homeo- and hetero-thermic physiological states. **BMC Veterinary Research**, v. 16, n. 1, p. 232, 6 dez. 2020b.

PINHEIRO, E. C. et al. Effect of fasting on carbohydrate metabolism in frugivorous bats (Artibeus lituratus and Artibeus jamaicensis). **Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology**, v. 143, n. 3, p. 279–284, mar. 2006.

POND, C. M. Adipose tissue and the immune system. **Prostaglandins, Leukotrienes and Essential Fatty Acids**, v. 73, n. 1, p. 17–30, jul. 2005.

RAMPELOTTO, P. H. et al. Oxidative Stress and Antioxidant Defense in the Brain of Bat Species with Different Feeding Habits. **International Journal of Molecular Sciences**, v. 24, n. 15, p. 12162, 29 jul. 2023.

SARTORI, D. R. S. et al. Metabolic adaptations induced by long-term fasting in quails. **Comparative Biochemistry and Physiology Part A: Physiology**, v. 111, n. 3, p. 487–493, jul. 1995.

SCHEIERMANN, C.; KUNISAKI, Y.; FRENETTE, P. S. Circadian control of the immune system. **Nature Reviews Immunology**, v. 13, n. 3, p. 190–198, 8 mar. 2013.

SCHNEEBERGER, K.; CZIRJÁK, G. Á.; VOIGT, C. C. Inflammatory challenge increases measures of oxidative stress in a free-ranging, long-lived mammal. **Journal of Experimental Biology**, v. 216, n. 24, p. 4514–4519, 1 jan. 2013.

SCHNEEBERGER, K.; CZIRJÁK, G. Á.; VOIGT, C. C. Frugivory is associated with low measures of plasma oxidative stress and high antioxidant concentration in free-ranging bats. **Naturwissenschaften**, v. 101, n. 4, p. 285–290, 11 abr. 2014.

SCHREIBER, R.; ZECHNER, R. Lipolysis meets inflammation: arachidonic acid mobilization from fat. **Journal of Lipid Research**, v. 55, n. 12, p. 2447–2449, 1 dez. 2014.

SCHWARTZ, G. J. Neural-immune gut-brain communication in the anorexia of disease. **Nutrition**, v. 18, n. 6, p. 528–533, jun. 2002.

SELTMANN, A. et al. Differences in acute phase response to bacterial, fungal and viral antigens in greater mouse-eared bats (Myotis myotis). **Scientific Reports**, v. 12, n. 1, p. 15259, 10 set. 2022.

SIMMONS, P. S. et al. Increased proteolysis. An effect of increases in plasma cortisol within the physiologic range. **Journal of Clinical Investigation**, v. 73, n. 2, p. 412–420, 1 fev. 1984.

SJORGREN, B. et al. Beitrag zur Kenntnis der Leberrhythmik (glykogen, Phosphor und Calcium in der Kaninchenleber). **Pflugers Arch**, p. 240–247, 1938.

SOCORRO FARIA, S. et al. The neutrophil-to-lymphocyte ratio: a narrative review. **ecancermedicalscience**, v. 10, 12 dez. 2016.

SOHAL, R. S.; MOCKETT, R. J.; ORR, W. C. Mechanisms of aging: an appraisal of the oxidative stress hypothesis. **Free Radical Biology and Medicine**, v. 33, n. 5, p. 575–586, set. 2002.

SOHAL, R. S.; ORR, W. C. Relationship between Antioxidants, Prooxidants, and the Aging Process. **Annals of the New York Academy of Sciences**, v. 663, n. 1, p. 74–84, 17 nov. 1992.

SOON, G. S. T.; TORBENSON, M. The Liver and Glycogen: In Sickness and in Health. **International Journal of Molecular Sciences**, v. 24, n. 7, p. 6133, 24 mar. 2023.

STAWSKI, C.; GEISER, F. Fat and fed: frequent use of summer torpor in a subtropical bat. **Naturwissenschaften**, v. 97, n. 1, p. 29–35, 16 jan. 2010.

STOCKMAIER, S. et al. No fever and leucocytosis in response to a lipopolysaccharide challenge in an insectivorous bat. **Biology Letters**, v. 11, n. 9, p. 20150576, set. 2015.

SU, L.-J. et al. Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. **Oxidative Medicine and Cellular Longevity**, v. 2019, p. 1–13, 13 out. 2019.

SUN, L. et al. Metabolic Reprogramming in Immune Response and Tissue Inflammation. **Arteriosclerosis, Thrombosis, and Vascular Biology**, v. 40, n. 9, p. 1990–2001, 1 set. 2020.

TAVES, M. D.; ASHWELL, J. D. Glucocorticoids in T cell development, differentiation and function. **Nature Reviews Immunology**, v. 21, n. 4, p. 233–243, 4 abr. 2021.

TEAGUE O'MARA, M. et al. Heart rate reveals torpor at high body temperatures in lowland tropical free-tailed bats. **Royal Society Open Science**, v. 4, n. 12, 20 dez. 2017.

TIRONE, T. A.; BRUNICARDI, F. C. Overview of Glucose Regulation. **World Journal of Surgery**, v. 25, n. 4, p. 461–467, 20 abr. 2001.

TIZARD, I. **Sickness behavior, its mechanisms and significance. Animal health research reviews / Conference of Research Workers in Animal Diseases**, 2008.

TRIANA-LLANOS, C. et al. The acute phase response elicited by a viral-like molecular pattern increases energy expenditure in Artibeus lituratus. **Biologia**, v. 74, n. 6, p. 667–673, 15 jun. 2019.

TURNER, K.; APPLEGATE, T.; LILBURN, M. Effects of feeding high carbohydrate or high fat diets. 1. Growth and metabolic status of the posthatch poult following immediate or delayed access to feed. **Poultry Science**, v. 78, n. 11, p. 1573–1580, nov. 1999.

VAN NIEKERK, G. et al. Sickness-Associated Anorexia: Mother Nature's Idea of Immunonutrition? **Mediators of Inflammation**, v. 2016, p. 1–12, 2016.

VIOLA, M. F.; HERRERA, L. G.; DA CRUZ-NETO, A. P. The acute phase response in bats (*Carollia perspicillata*) varies with time and dose of the immune challenge. **Journal of Experimental Biology**, v. 225, n. 24, 15 dez. 2022.

VIOLA, M. F.; HERRERA M., L. G.; CRUZ-NETO, A. P. Combined effects of ambient temperature and food availability on induced innate immune response of a fruit-eating bat (Carollia perspicillata). **PLOS ONE**, v. 19, n. 5, p. e0301083, 24 maio 2024.

VOIGT, C. C. et al. The immune response of bats differs between pre-migration and migration seasons. **Scientific Reports**, v. 10, n. 1, p. 17384, 15 out. 2020.

WANG, A. et al. Opposing Effects of Fasting Metabolism on Tissue Tolerance in Bacterial and Viral Inflammation. **Cell**, v. 166, n. 6, p. 1512- 1525.e12, 8 set. 2016.

WEINGARTEN, H. P. Cytokines and food intake: The relevance of the immune system to the student of ingestive behavior. **Neuroscience & Biobehavioral Reviews**, v. 20, n. 1, p. 163–170, jan. 1996.

WEISE, P. et al. Simulated bacterial infection disrupts the circadian fluctuation of immune cells in wrinkle-lipped bats (Chaerephon plicatus). **PeerJ**, v. 2017, n. 8, 2017.

WIBBELT, G. et al. Emerging diseases in Chiroptera: why bats? **Biology Letters**, v. 6, n. 4, p. 438–440, 23 ago. 2010.

WILHELM FILHO, D. et al. Antioxidant defenses, longevity and ecophysiology of South American bats. **Comparative Biochemistry and Physiology - C Toxicology and Pharmacology**, v. 146, n. 1- 2 SPEC. ISS., p. 214–220, 2007.

WILKINSON, G. S.; SOUTH, J. M. Life history, ecology and longevity in bats. **Aging cell**, v. 1, n. 2, p. 124–131, 2002.

YU, W. et al. Reactive Oxygen Species Bridge the Gap between Chronic Inflammation and Tumor Development. **Oxidative Medicine and Cellular Longevity**, v. 2022, p. 1–22, 28 jun. 2022.

ZHANG, G. et al. Comparative Analysis of Bat Genomes Provides Insight into the Evolution of Flight and Immunity. **Science**, v. 339, n. 6118, p. 456–460, 25 jan. 2013.

ZHANG, H. et al. **Beyond energy storage: roles of glycogen metabolism in health and disease**. **FEBS Journal**Blackwell Publishing Ltd, , 1 jun. 2021.

ZHANG, X. et al. Metabolic substrate utilization in stress-induced immune cells. **Intensive Care Medicine Experimental**, v. 8, n. S1, p. 28, 18 dez. 2020.

Chapter II:

A bacterial immune challenge does not affect the redox status of the liver and pectoral muscle in bats (*Molossus molossus* and *Artibeus lituratus*)

ABSTRACT

Reactive species are released during the acute phase response (APR) as a result of processes such as the phagocytic activity of neutrophils and increased mitochondrial metabolism. To counteract the harmful effects of these pro-oxidants, animals depend on an antioxidant defense system, which may be either endogenous or exogenous. Bats, as a unique group of mammals, exhibit a lower production of reactive species compared to non-flying mammals of similar body size, likely due to adaptations associated with their high metabolic demands. Previous studies have suggested that dietary habits, such as insectivory or frugivory, influence the antioxidant capacity of bats. In this study, we simulated a bacterial immune challenge by administering lipopolysaccharide (LPS) from *Escherichia coli* to two bat species with distinct diets: insectivorous *Molossus molossus* and frugivorous *Artibeus lituratus*. We focused on assessing the redox status of two key tissues—the liver, essential for metabolic regulation and detoxification, and the pectoral muscle, critical for flight performance. Our analyses measured oxidative markers and antioxidant levels to determine whether the APR induced by LPS affects oxidative balance in these tissues. We expected to found higher antioxidant concentrations in *M. molossus* tissues, as insectivorous bats depend more on endogenous antioxidant capacity than frugivorous bats. Also, we expected to detect higher concentrations of oxidative markers due to the increase of reactive species caused by the APR. Nevertheless, contrary to previous studies that identified changes in oxidative status in the blood plasma of bats during the APR, we found no significant alterations in the concentration of oxidative markers or antioxidants in the liver or pectoral muscle following LPS administration. These results suggest that the APR does not compromise the redox homeostasis of these important tissues, even under immune activation. Additionally, our findings indicate that dietary habits, such as insectivory or frugivory, do not appear to influence the susceptibility of liver and muscle tissues to oxidative imbalance. This study contributes to understanding the unique physiological resilience of bats to oxidative stress and their potential adaptations to immune challenges.

Key-words: acute phase response, LPS, reactive species, free radicals, antioxidants, oxidative stress, Chiroptera.

INTRODUCTION

The acute phase response (APR) is an inflammatory process triggered by harmful stimuli, such as the presence of pathogenic bacteria. It is commonly characterized by an increased metabolic rate, fever, localized inflammation, proliferation of lymphocytes and macrophages, enhanced neutrophil activity, apoptosis, inhibition of viral replication, and behavioral changes, including lethargy and anorexia (CRAY; ZAIAS; ALTMAN, 2009; VIOLA; HERRERA; DA CRUZ-NETO, 2022). The APR also involves processes that often cause oxidation, which may result in antimicrobial or self-damaging effects. Reactive oxygen species (ROS) are released by many endogenous sources, among them, during the oxidative burst of neutrophils, and by mitochondrial metabolism (NATHAN; CUNNINGHAM-BUSSEL, 2013). In moderate concentrations, ROS play an essential role in various biological processes. Their molecular actions include the inhibition or activation of proteins and the activation of gene transcription. At the cellular level, ROS can either promote or suppress inflammation, immunity, and carcinogenesis (BAUD; KARIN, 2001; CHUNG et al., 2006; NATHAN; CUNNINGHAM-BUSSEL, 2013). Nevertheless, in excess, ROS production may cause an imbalance state known as oxidative stress, which causes damage in cellular structures by the peroxidation of membrane fatty acid chains, loss of sulfhydryls of cellular membrane, carbonylation in proteins, modification of DNA, and also may impose physiological cost on reproduction, immune function, and lifespan (COSTANTINI, 2008; COSTANTINI et al., 2010; SOHAL; ORR, 1992). Besides ROS, there are other reactive signaling molecules, which also play a role in biological processes, including the reactive nitrogen species (nitric oxide, nitrogen dioxide), hydrogen sulfide and carbon monoxide (CHATTERJEE, 2016; FINKEL; HOLBROOK, 2000; LEMASTERS; JAESCHKE, 2020)

To prevent oxidative stress, animals depend on an antioxidant system that can be either exogenous — through the intake of vitamins and micronutrients — or endogenous, comprising enzymatic and non-enzymatic components. Endogenous defenses, such as the superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) enzymes, are highly efficient and constitute a first line of defense against pro-oxidants. SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, reducing their harmful effects. CAT further breaks down hydrogen peroxide into water and oxygen, preventing cellular damage. GST plays a crucial role in detoxification by conjugating

reduced glutathione to reactive molecules, facilitating their elimination. (BALABAN; NEMOTO; FINKEL, 2005; HANADHITA; SATYANINGTIJAS; AGUNGPRIYONO, 2019). However, oxidative damage may become irreparable under certain conditions, leading to physiological deterioration, phenotypic changes, and age-related diseases (BARJA, 2002; CHUNG et al., 2002, 2006; FINKEL; HOLBROOK, 2000). Moreover. the susceptibility to oxidative stress varies among species, and such variation may be associated with multiple factors, including dietary habits (COSTANTINI; MØLLER, 2008; MARRI; RICHNER, 2015; SCHNEEBERGER; CZIRJÁK; VOIGT, 2014). It has been suggested that when endogenous antioxidant levels are insufficient to counteract oxidative stress, dietary antioxidants—such as vitamins A and E, carotenoids, enzyme cofactors like coenzyme Q10, nitrogen compounds, and peptides—may help mitigate the harmful effects of ROS. Although empirical evidence in animals remains inconclusive, it is generally expected that a frugivorous diet may offer more benefits in this context, compared to high-protein diets that are low in antioxidants (COSTANTINI; MØLLER, 2008, 2009; GARRATT; BROOKS, 2012). A study conducted on 33 bat species highlighted the influence of dietary habits on the variation in antioxidant concentrations and the level of reactive oxygen metabolites (ROMs). Frugivorous bats exhibited the highest antioxidant concentrations and the lowest ROM levels, followed by omnivorous bats and species feeding on insects, vertebrates, or blood (SCHNEEBERGER; CZIRJÁK; VOIGT, 2014). The authors proposed that an antioxidant-rich diet may enable frugivorous bats to more efficiently neutralize pro-oxidants than omnivorous or carnivorous bats.

Bats are known for their exceptional longevity among mammals, attributed to their low production of ROS compared with non-flying mammals of similar body size, and high levels of antioxidants (BARJA et al., 1994; BECKMAN; AMES, 1998; BROWN et al., 2009; BRUNET-ROSSINNI, 2004; DRÖGE; SCHIPPER, 2007; HANADHITA; SATYANINGTIJAS; AGUNGPRIYONO, 2019; SOHAL; MOCKETT; ORR, 2002; WILHELM FILHO et al., 2007; WILKINSON; SOUTH, 2002), which protect them from oxidative stress. Bats APR is associated with an increase in metabolic rate (CABRERA-MARTINEZ; HERRERA; CRUZ-NETO, 2018; CABRERA-MARTINEZ; HERRERA M.; CRUZ-NETO, 2019; GUERRERO-CHACÓN et al., 2018; OTÁLORA-ARDILA et al., 2016), consequently, with an increase in mitochondrial activity, which may increase ROS production. Therefore, the activation of the APR should not only increase ROS but also adjust antioxidant levels to reduce potential harm. An immune challenge conducted

in the frugivorous bat *Carollia perspicillata,* challenged with LPS (lipopolysaccharide of *Escherichia coli*) reported increased levels of ROMs but no changes in antioxidant concentrations after the immune challenge (SCHNEEBERGER; CZIRJÁK; VOIGT, 2013). The authors suggested that the immune response can upregulate certain oxidative markers associated with oxidative stress. In contrast, in another frugivorous species, *Rousettus aegyptiacus*, also challenged with LPS, inflammation was induced but with no effect in oxidative markers in the blood (COSTANTINI et al., 2022). Challenged bats exhibited increased levels of haptoglobin and lysozyme, two inflammation markers, but LPS did not affect the concentrations of protein carbonyls, glutathione peroxidase, SOD, or thiols in red blood cells, nor did it alter ROMs levels or non-enzymatic antioxidant capacity in blood serum. These studies highlight the complex interplay between immune response, oxidative stress, and antioxidant defenses in bats. While immune challenges can induce oxidative damage, bats appear to have unique strategies to modulate this stress, potentially contributing to their resilience and long lifespans. The varying effects across species further suggest that diet and ecological context, may influence immune function and redox balance in these animals.

In this study, we induced a bacterial immune response using LPS to determine whether the APR would affect the oxidative status of the liver and pectoral muscle in two bat species with differing diets: the insectivorous *Molossus molossus* and the frugivorous *Artibeus lituratus*. The liver plays a central role in the inflammatory response, as it is involved in the production of APPs and antioxidant enzymes that help counteract oxidative stress (CRAY, 2012; GABAY; KUSHNER, 1999; GRUYS et al., 2005; JAESCHKE, 2006; KNOLLE; GERKEN, 2000; LEMASTERS; JAESCHKE, 2020) This function is crucial during infections or other stressors that trigger inflammation. In contrast, the pectoral muscle of bats, which has a high mitochondrial density due to their need for powered flight, requires an efficient antioxidant defense system to manage the elevated production of ROS during cellular respiration (OHTSU; MŌRI; UCHIDA, 1978). Since this is the first study to assess the redox status in the liver and pectoral muscle during the APR, our hypotheses include the following scenarios: 1) If antioxidant capacity in the tissues is insufficient to counteract the ROS increase, challenged animals will show higher concentrations of pro-oxidant markers compared to controls; 2) Challenged animals will exhibit higher concentrations of antioxidants, indicating an adjustment in antioxidant capacity to combat oxidative stress; 3) If APR activation does not disrupt

oxidative balance, no differences will be observed between challenged and control animals. Regarding dietary influence on susceptibility to oxidative damage, previous research suggests variation in antioxidant concentrations (SCHNEEBERGER; CZIRJÁK; VOIGT, 2014; WILHELM FILHO et al., 2007). Therefore, if dietary habits affect susceptibility to oxidative damage during the APR, with frugivory promoting oxidative balance, we expect to detect the most pronounced effects of LPS on oxidative marker concentrations in *M. molossus*.

MATERIAL AND METHODS

This study was conducted with the same individuals, and after the same immune challenge described in Chapter I.

Tissues sampling and analyses

After the immune challenge, animals were euthanized by decapitation. Portions of the liver and pectoral muscle were extracted, weighed, immediately frozen in liquid nitrogen and finally stored at -80°C until analyses, when portions of 150 mg from each tissue were homogenized in Na phosphate buffer 50 mM pH 7.4 (1500 µl) using a homogenizer (OMNI) and centrifuged (1200 rpm at 4°C for 10 min). The supernatant was used for CAT, SOD, GST and malondialdehyde (MDA - byproduct of lipid peroxidation). The pellets were used to determine protein carbonyl (PC - result from the oxidation of proteins) concentrations. All analyses were determined by duplicate using a spectrophotometer (UV-Mini 1240, Shimadzu) or through an ELISA microplate reader (Multiskan GO, Thermo Scientific). The laboratory protocol used to determine CAT activity is described in (HADWAN; ABED, 2016), the activity of SOD was determined by the method based on the reduction of the superoxide and hydrogen peroxide according to DIETERICH et al. (2000), GST activity was measured using the method of (HABIG; PABST; JAKOBY, 1974), MDA was determined by the method of (BUEGE; AUST, 1978) and PC were measured according to LEVINE et al. (1994).

Statistical Analysis

Statistical analyses were performed in RStudio (2024.04.2). The levels of oxidative markers and antioxidants were analyzed using a *factorial* ANOVA to compare species and

treatments for each tissue (*stats* package). All variables were checked for normality and homogeneity. In cases where one of the assumptions were violated, data were transformed to meet the assumptions of normality and homogeneity. Additionally, we conducted a correlation analysis to evaluate the relationships between all redox state variables We calculated Spearman correlation coefficients for pairs of variables and tested their significance. Statistical significance was set at $P < 0.05$.

RESULTS

The CAT activity in the pectoral muscle of *M. molossus* was excluded from the analyses due to unexpectedly low hydrogen peroxide consumption, which precluded accurate calculation of CAT concentration. Therefore, we are not presenting analysis for this parameter. The results of the statistical tests are reported in Table 1 and Table 2.

Table 1. Results of the *factorial* ANOVA for antioxidant capacity and oxidative markers between PBS and LPS treated *M. molossus* and *A. lituratus.* The asterisk (*) indicate statistical difference.

Liver

	Treatment	Species	Interaction
CAT	(F=1,8 ; P=0,19)	(F=2,72 ; P=0,11)	(F=0,002 ; P=0,97)
SOD	(F=3,57 ; P=0,07)	(F=5,93 ; P=0,02)*	(F=0,09 ; P=0,77)
GST	(F=0,07 ; P=0,8)	(F=147,5 ; P<0,0001)*	(F=2,59 ; P=0,12)
MDA	(F=0,007 ; P=0,93)	(F=19,35 ; P<0,0002)*	(F=0,44 ; P=0,51)
PC	(F=1,47 ; P=0,24)	(F=4.62 ; P=0,04)*	(F=0,25 ; P=0,62)

Pectoral Muscle

	Treatment	Species	Interaction
SOD	(F=1,02 ; P=0,32)	(F=10,04 ; P<0,004)*	(F=0,28 ; P=0,62)
GST	(F=0,29 ; P=0,6)	(F=12,72; P<0,002)*	(F=0,35 ; P=0,56)
MDA	(F=1,97 ; P=0,17)	(F=0,33 ; P=0,57)	(F=1,23 ; P=0,28)
PC	(F=0,06 ; P=0,81)	(F=5,5 ; P<0,03)*	(F=0,09 ; P=0,77)

When comparing between species, we found significant difference in liver content of SOD, GST, MDA and PC, and in pectoral muscle content of SOD, GST and PC (Table 1). However, we did not detect effect of treatment nor interaction in any of the indicators. All the correlations detected between redox status indicators were positive, correlations with significant p-values are indicated for *M. molossus* in Figure 1 for *A. lituratus* in Figure 2.

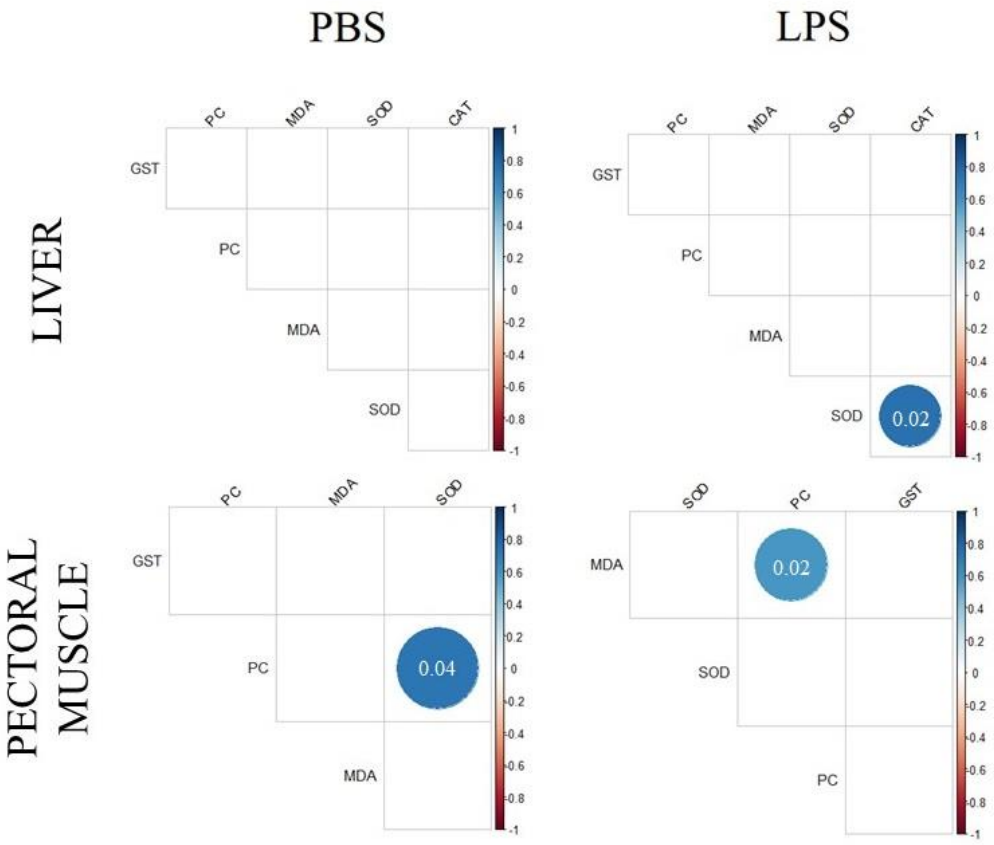

Figure 1. Graphical Spearman's correlation matrix of oxidative markers and antioxidants measured for each treatment in *M. molossus* liver and pectoral muscle. Positive correlation are express from white to blue. Only significant correlations are presented, indicated by the numbers inside the blue circles ($p < 0.05$). The circle size represents the correlation coefficient magnitude.

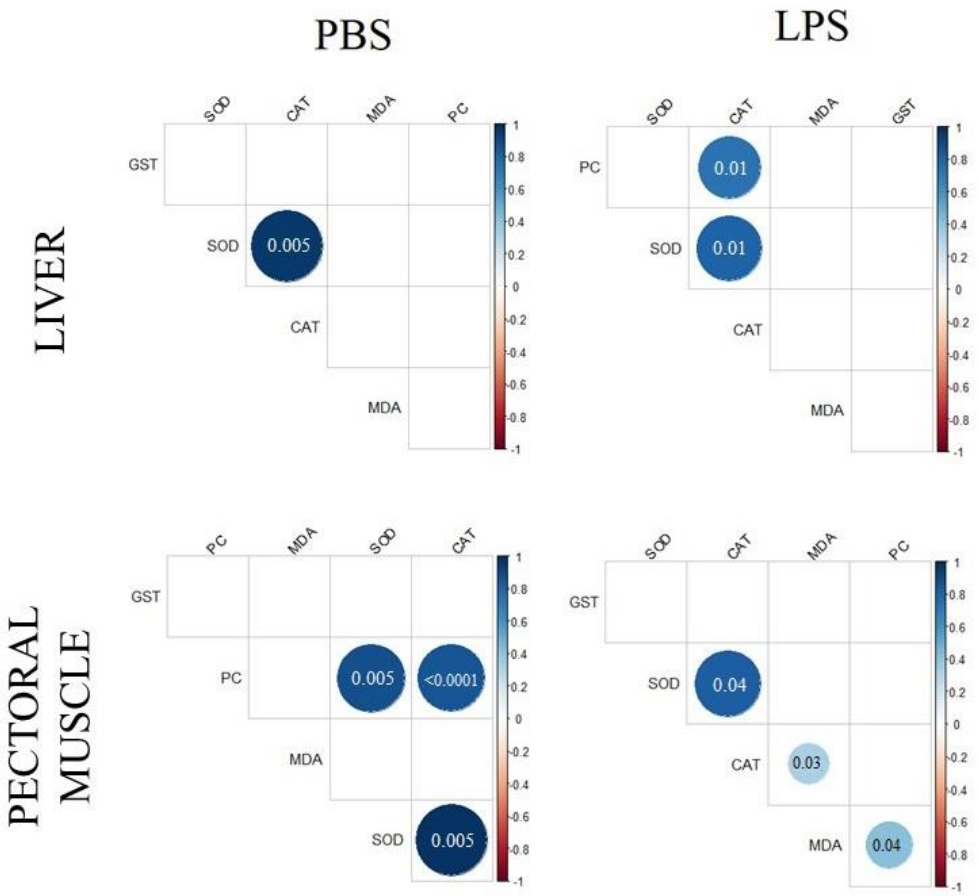

Figure 2. Graphical Spearman's correlation matrix of oxidative markers and antioxidants measured for each treatment in *A. lituratus* liver and pectoral muscle. Positive correlation are express from white to blue. Only significant correlations are presented, indicated by the numbers inside the blue circles ($p < 0.05$). The circle size represents the correlation coefficient magnitude.

DISCUSSION

All detected correlations in this study were positive, suggesting that the concentrations of the oxidative markers and antioxidant may be likely co-regulated under oxidative damage conditions. However, while the correlations suggest a potential effect of LPS treatment, it's important to highlight that the *factorial* ANOVA did not detect a significant treatment effect. This suggests that the observed correlations might not reflect a direct influence of LPS, but rather individual differences between the PBS and LPS treated animals, since bats were not the same between experimental groups. Similar to previous studies, we did not detect any effect of LPS on the redox balance of *M. molossus*

and *A. lituratus*. Both treatment groups displayed comparable levels of oxidative markers and antioxidants. However, as expected, these values varied significantly between the two species. The liver of *M. molossus* exhibited higher levels of both antioxidants (SOD and GST) and oxidative markers (MDA and PC) compared to *A. lituratus*, while in pectoral muscle, *M. molossus* also showed higher contents of GST and PC in relation to *A. lituratus*. Nevertheless, the frugivorous bat presented higher values of SOD. This aligns with previous research suggesting that animals with protein-rich diets, low in exogenous antioxidants, may rely more heavily on endogenous antioxidants—an observation supported by our data (RAMPELOTTO et al., 2023; WILHELM FILHO et al., 2007). One study investigated the oxidative metabolism of four neotropical bat species with different diets, highlighting the relationship between feeding habits, oxidative damage, and antioxidant defenses across specific organs (heart, liver, and kidney)(PEREIRA et al., 2023). Hematophagous bats exhibited lower oxidative damage in the heart, likely due to efficient antioxidant mechanisms, but higher oxidative stress in the liver, attributed to the metabolism of heme-rich blood meals. Nectarivorous bats showed reduced oxidative damage in the kidney, possibly due to diets rich in plant-based antioxidants, while insectivorous and hematophagous bats exhibited higher enzymatic activity in this organ, reflecting increased metabolic demands.

Several studies have evaluated the APR in bats, and it is well-established that during its activation, particularly within the first 24 hours following LPS inoculation, there is an increase in metabolic rate, indicating heightened energy demand and, consequently, increase in mitochondrial metabolism (CABRERA-MARTINEZ; HERRERA; CRUZ-NETO, 2018; CABRERA-MARTINEZ; HERRERA M.; CRUZ-NETO, 2019; GUERRERO-CHACÓN et al., 2018; OTÁLORA-ARDILA et al., 2016, 2017). The physiological effects of the APR extend up to 48 hours, and during this period, studies have recorded several inflammatory markers, though with variation in occurrence and intensity across different species (VIOLA; HERRERA; DA CRUZ-NETO, 2022).

However, few studies have examined the redox state in bats during the APR, and in all cases, markers of oxidative damage and antioxidant capacity were measured exclusively in blood samples. An increase in ROMs but not in antioxidants was observed in the frugivorous species *C. perspicillata* (SCHNEEBERGER; CZIRJÁK; VOIGT, 2013), while no variation was detected on these indicators in *R. aegyptiacus*

(COSTANTINI et al., 2022), also frugivorous. No effect of LPS was reported in pro-oxidant and antioxidant levels in the insectivorous *P. nathusii* (VOIGT et al., 2020). But plasma oxidative damage significantly increased in *M. myotis* challenged with bacterial and fungal antigens, whereas in animals challenged with viral antigen did not (COSTANTINI et al., 2023). This last study is particularly noteworthy due to the contrast in results compared to SELTMANN et al. (2022), in which the same three antigens were used also in *M. myotis*, nevertheless, in this case the authors measured the inflammatory response and it was stronger in bats challenged with viral and bacterial antigens, but not with the fungal antigen. This suggests that, despite bats are capable of mounting a strong inflammatory response for virus, it may not affect their oxidative balance as the other antigens. Moreover, when a fungal response was activated, even with a mild inflammatory response, bats were more susceptible to oxidative stress.

Oxidative stress and inflammation are interrelated, often promoting one another. During inflammation, neutrophils and other activated leucocytes are recruited to the liver, where they produce ROS as part of the defense mechanism. However, the activation and recruitment of leucocytes and pro-inflammatory cells do not necessarily cause toxicity. Neutrophils adhere to target cells, enabling the production of ROS and the release of proteolytic and cytotoxic proteins near these targets. As a result, oxidants like hydrogen peroxide and hypochlorous acid can diffuse into the target cells, leading to elevated intracellular oxidative stress and the formation of hypochlorite-modified proteins (LEMASTERS; JAESCHKE, 2020). However, this neutrophil-generated oxidative stress is typically not strong enough to cause cell death directly through lipid peroxidation. Instead, it induces cellular and mitochondrial dysfunction, which can eventually lead to cell necrosis (JAESCHKE, 2006). Additionally, the oxidative burst is a key mechanism used by neutrophils for effective host defense. It is triggered by the activation of receptors on the neutrophil surface that recognize bacterial peptides or inflammatory signals. Upon activation, these receptors initiate a signaling cascade that causes the rapid assembly of the NADPH oxidase enzyme complex at the cell membrane. This complex catalyzes the conversion of oxygen into superoxide anions and other ROS, such as hydrogen peroxide and hypochlorous acid, which are also toxic to pathogens, so this rapid production of ROS is essential for neutrophils to neutralize invading microorganisms (CHEN; JUNGER, 2012). However, our results do not strongly suggest that a simulated bacterial APR cause oxidative stress in the liver of *M. molossus* and *A. lituratus,* and we also found no effect

in the pectoral muscle, despite its high mitochondrial activity. We decided to euthanize the animals 36 hours after the injections, as studies assessing inflammatory and oxidative markers typically conduct measurements between 24 and 48 hours following the inoculation (COSTANTINI et al., 2022, 2023; SCHNEEBERGER; CZIRJÁK; VOIGT, 2013; VOIGT et al., 2020). Nevertheless, since this is the first time these analyses are being performed in both tissues after inducing an APR in bats, we cannot rule out the possibility that the lack of detection of the LPS effect may be related to the time elapsed after the injections. Another possible explanation for our results may be related to a lower production of ROS or a different regulation of the oxidative status in bats(BRUNET-ROSSINNI, 2004; PIKULA et al., 2020b), which could also contribute to maintaining the redox balance in these tissues during the immune response.

Besides the enzymatic antioxidant defense, animals produce other proteins that may counteract the oxidative damage during the immune response. Haptoglobin is an acute phase protein, primarily produced by the liver during the APR, which is synthesized by hepatocytes and released into the bloodstream. The production of haptoglobin increases significantly due to the influence of pro-inflammatory cytokines, such as IL-6 and TNF-α. The increase in haptoglobin levels helps bind free hemoglobin released from red blood cells, preventing oxidative damage and promoting iron recycling (CRAY; ZAIAS; ALTMAN, 2009; LANGLOIS; DELANGHE, 1996). Previous work has reported the increase of haptoglobin after LPS inoculation in the frugivorous and insectivorous bats *R. aegyptiacus* (COSTANTINI et al., 2022; MORENO et al., 2021), *M. myotis* (SELTMANN et al., 2022) and *P. nathusii* (VOIGT et al., 2020). COSTANTINI et al. (2023) also observed a positive but weak correlation between plasma levels of ROMs and both haptoglobin and bacterial killing ability after inducing an APR with LPS in *M. myotis*. Therefore, considering that haptoglobin is primarily produced in the liver, it is possible that it plays a fundamental role in the oxidative balance of this tissue, especially during the APR.

CONCLUSSIONS

Our results are consistent with those reported by (COSTANTINI et al., 2022; VOIGT et al., 2020), in frugivorous and insectivorous species, where the APR activation for a bacterial antigen does not impose a threat to oxidative balance, although, unlike us,

they analyzed blood samples. Additionally, despite the differences in the antioxidants and oxidative marker levels between the species, it is not possible to conclude that diet determines the susceptibility of bats to oxidative stress, since none of the species was affected by the simulated bacterial immune challenge. The oxidative response in bats has been studied in different contexts (COSTANTINI et al., 2022, 2023; HANADHITA; SATYANINGTIJAS; AGUNGPRIYONO, 2019; MACHADO et al., 2020; OLIVEIRA et al., 2017, 2018; SCHNEEBERGER; CZIRJÁK; VOIGT, 2013, 2014); however, much remains to be understood. Although we know that bats are organisms with greater longevity than expected for their metabolism and body mass, and compared to other animals, they exhibit characteristics that favor a higher tolerance to oxidative damage, much is still unknown about the physiological mechanisms underlying this capacity and how they manage these challenges at the molecular level.

REFERENCES

BALABAN, R. S.; NEMOTO, S.; FINKEL, T. Mitochondria, Oxidants, and Aging. Cell, v. 120, n. 4, p. 483–495, 25 fev. 2005.

BARJA, G. et al. A decrease of free radical production near critical targets as a cause of maximum longevity in animals. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, v. 108, n. 4, p. 501–512, ago. 1994.

BARJA, G. Endogenous oxidative stress: relationship to aging, longevity and caloric restriction. Ageing Research Reviews, v. 1, n. 3, p. 397–411, jun. 2002.

BAUD, V.; KARIN, M. Signal transduction by tumor necrosis factor and its relatives. Trends in Cell Biology, v. 11, n. 9, p. 372–377, set. 2001.

BECKMAN, K. B.; AMES, B. N. The Free Radical Theory of Aging Matures. Physiological Reviews, v. 78, n. 2, p. 547–581, 1 abr. 1998.

BROWN, J. C. L. et al. Examining the mechanisms responsible for lower ROS release rates in liver mitochondria from the long-lived house sparrow (Passer domesticus) and big brown bat (Eptesicus fuscus) compared to the short-lived mouse (Mus musculus). Mechanisms of Ageing and Development, v. 130, n. 8, p. 467–476, ago. 2009.

BRUNET-ROSSINNI, A. K. Reduced free-radical production and extreme longevity in the little brown bat (Myotis lucifugus) versus two non-flying mammals. Mechanisms of Ageing and Development, v. 125, n. 1, p. 11–20, jan. 2004.

BUEGE, J. A.; AUST, S. D. Microsomal lipid peroxidation. Em: [s.l: s.n.]. p. 302–310.

CABRERA-MARTINEZ, L. V.; HERRERA, M. L. G.; CRUZ-NETO, A. P. The energetic cost of mounting an immune response for Pallas's longtongued bat (Glossophaga soricina). PeerJ, v. 2018, n. 6, 2018.

CABRERA-MARTINEZ, L. V.; HERRERA M., L. G.; CRUZ-NETO, A. P. Food restriction, but not seasonality, modulates the acute phase response of a Neotropical bat. Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology, v. 229, p. 93–100, 1 mar. 2019.

CHATTERJEE, S. Oxidative Stress, Inflammation, and Disease. Em: Oxidative Stress and Biomaterials. [s.l.] Elsevier, 2016. p. 35–58.

CHEN, Y.; JUNGER, W. G. Measurement of Oxidative Burst in Neutrophils. Em: Methods in Molecular Biology. [s.l: s.n.]. v. 844p. 115–124.

CHUNG, H. Y. et al. Molecular inflammation hypothesis of aging based on the anti-aging mechanism of calorie restriction. Microscopy Research and Technique, v. 59, n. 4, p. 264–272, 15 nov. 2002.

CHUNG, H. Y. et al. The Molecular Inflammatory Process in Aging. Antioxidants & Redox Signaling, v. 8, n. 3–4, p. 572–581, mar. 2006.

COSTANTINI, D. Oxidative stress in ecology and evolution: lessons from avian studies. Ecology Letters, v. 11, n. 11, p. 1238–1251, 2 nov. 2008.

COSTANTINI, D. et al. From molecules to living systems: historical and contemporary issues in oxidative stress and antioxidant ecology. Functional Ecology, v. 24, n. 5, p. 950–959, 16 out. 2010.

COSTANTINI, D. et al. Induced bacterial sickness causes inflammation but not blood oxidative stress in Egyptian fruit bats (Rousettus aegyptiacus). Conservation Physiology, v. 10, n. 1, 2022.

COSTANTINI, D. et al. Oxidative damage varies in response to bacterial, fungal and viral antigen challenges in bats. Journal of Experimental Biology, v. 226, n. 21, 1 nov. 2023.

COSTANTINI, D.; MØLLER, A. P. Carotenoids are minor antioxidants for birds. Functional Ecology, v. 22, n. 2, p. 367–370, 28 abr. 2008.

COSTANTINI, D.; MØLLER, A. P. Does immune response cause oxidative stress in birds? A meta-analysis. Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology, v. 153, n. 3, p. 339–344, 2009.

CRAY, C. Acute Phase Proteins in Animals. Em: Progress in Molecular Biology and Translational Science. [s.l.] Elsevier B.V., 2012. v. 105p. 113–150.

CRAY, C.; ZAIAS, J.; ALTMAN, N. H. Acute phase response in animals: a review. Comparative medicine, v. 59, n. 6, p. 517–26, dez. 2009.

DIETERICH, S. et al. Gene Expression of Antioxidative Enzymes in the Human Heart Increased Expression of Catalase in the End-Stage Failing HeartCirculation. [s.l: s.n.]. Disponível em: <http://www.circulationaha.org>.

DRÖGE, W.; SCHIPPER, H. M. Oxidative stress and aberrant signaling in aging and cognitive decline. Aging Cell, v. 6, n. 3, p. 361–370, 27 jun. 2007.

FINKEL, T.; HOLBROOK, N. J. Oxidants, oxidative stress and the biology of ageing. Nature, v. 408, n. 6809, p. 239–247, nov. 2000.

GABAY, C.; KUSHNER, I. Acute-Phase Proteins and Other Systemic Responses to Inflammation. New England Journal of Medicine, v. 340, n. 6, p. 448–454, 11 fev. 1999.

GARRATT, M.; BROOKS, R. C. Oxidative stress and condition-dependent sexual signals: More than just seeing red. Proceedings of the Royal Society B: Biological SciencesRoyal Society, , 2012.

GRUYS, E. et al. Acute phase reaction and acute phase proteins. Journal of Zhejiang University-SCIENCE B, v. 6, n. 11, p. 1045–1056, 1 nov. 2005.

GUERRERO-CHACÓN, A. L. et al. Metabolic cost of acute phase response in the frugivorous bat, Artibeus lituratus. Mammal Research, v. 63, n. 4, p. 397–404, 30 out. 2018.

HABIG, W. H.; PABST, M. J.; JAKOBY, W. B. Glutathione S-Transferases. Journal of Biological Chemistry, v. 249, n. 22, p. 7130–7139, nov. 1974.

HADWAN, M. H.; ABED, H. N. Data supporting the spectrophotometric method for the estimation of catalase activity. Data in Brief, v. 6, p. 194–199, 1 mar. 2016.

HANADHITA, D.; SATYANINGTIJAS, A. S.; AGUNGPRIYONO, S. Bats Oxidative Stress Defense. Jurnal Riset Veteriner Indonesia (Journal of The Indonesian Veterinary Research), v. 3, n. 1, 30 jan. 2019.

JAESCHKE, H. Mechanisms of Liver Injury. II. Mechanisms of neutrophil-induced liver cell injury during hepatic ischemia-reperfusion and other acute inflammatory conditions. American Journal of Physiology-Gastrointestinal and Liver Physiology, v. 290, n. 6, p. G1083–G1088, jun. 2006.

KNOLLE, P. A.; GERKEN, G. Local control of the immune response in the liver. Immunological Reviews, v. 174, n. 1, p. 21–34, 19 abr. 2000.

LANGLOIS, M. R.; DELANGHE, J. R. Biological and clinical significance of haptoglobin polymorphism in humans. Clinical Chemistry, v. 42, n. 10, p. 1589–1600, 1 out. 1996.

LEMASTERS, J. J.; JAESCHKE, H. Oxidative Stress and Inflammation in the Liver. Em: The Liver. [s.l.] Wiley, 2020. p. 714–727.

LEVINE, R. L. et al. Carbonyl assays for determination of oxidatively modified proteins. Em: [s.l: s.n.]. p. 346–357.

MACHADO, A. M. et al. Effects of yacon flour associated with an energy restricted diet on intestinal permeability, fecal short chain fatty acids, oxidative stress and inflammation markers levels in adults with obesity or overweight: a randomized, double blind, placebo controlled clinical trial. Archives of Endocrinology and Metabolism, v. 64, n. 5, p. 597–607, 30 mar. 2020.

MARRI, V.; RICHNER, H. Immune response, oxidative stress and dietary antioxidants in great tit nestlings. Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology, v. 179, p. 192–196, 1 jan. 2015.

MORENO, K. R. et al. Sick bats stay home alone: fruit bats practice social distancing when faced with an immunological challenge. Annals of the New York Academy of Sciences, v. 1505, n. 1, p. 178–190, 1 dez. 2021.

NATHAN, C.; CUNNINGHAM-BUSSEL, A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. Nature Reviews Immunology, v. 13, n. 5, p. 349–361, 25 maio 2013.

OLIVEIRA, J. M. et al. Exposure to the insecticide endosulfan induces liver morphology alterations and oxidative stress in fruit-eating bats (*Artibeus lituratus*). International Journal of Experimental Pathology, v. 98, n. 1, p. 17–25, 27 fev. 2017.

OLIVEIRA, J. M. et al. Exposure to deltamethrin induces oxidative stress and decreases of energy reserve in tissues of the Neotropical fruit-eating bat Artibeus lituratus. Ecotoxicology and Environmental Safety, v. 148, p. 684–692, 1 fev. 2018.

OTÁLORA-ARDILA, A. et al. Metabolic Cost of the Activation of Immune Response in the Fish-Eating Myotis (Myotis vivesi): The Effects of Inflammation and the Acute Phase Response. PLOS ONE, v. 11, n. 10, p. e0164938, 28 out. 2016.

OTÁLORA-ARDILA, A. et al. The effect of short-term food restriction on the metabolic cost of the acute phase response in the fish-eating Myotis (Myotis vivesi). Mammalian Biology, v. 82, p. 41–47, 1 jan. 2017.

PEREIRA, F. D. et al. Oxidative Stress and Antioxidant Defense in the Heart, Liver, and Kidney of Bat Species with Different Feeding Habits. International Journal of Molecular Sciences, v. 24, n. 22, p. 16369, 15 nov. 2023.

PIKULA, J. et al. Phagocyte activity reflects mammalian homeo- and hetero-thermic physiological states. BMC Veterinary Research, v. 16, n. 1, p. 232, 6 dez. 2020.

RAMPELOTTO, P. H. et al. Oxidative Stress and Antioxidant Defense in the Brain of Bat Species with Different Feeding Habits. International Journal of Molecular Sciences, v. 24, n. 15, p. 12162, 29 jul. 2023.

SCHNEEBERGER, K.; CZIRJÁK, G. Á.; VOIGT, C. C. Inflammatory challenge increases measures of oxidative stress in a free-ranging, long-lived mammal. Journal of Experimental Biology, v. 216, n. 24, p. 4514–4519, 1 jan. 2013.

SCHNEEBERGER, K.; CZIRJÁK, G. Á.; VOIGT, C. C. Frugivory is associated with low measures of plasma oxidative stress and high antioxidant concentration in free-ranging bats. Naturwissenschaften, v. 101, n. 4, p. 285–290, 11 abr. 2014.

SELTMANN, A. et al. Differences in acute phase response to bacterial, fungal and viral antigens in greater mouse-eared bats (Myotis myotis). Scientific Reports, v. 12, n. 1, p. 15259, 10 set. 2022.

SOHAL, R. S.; MOCKETT, R. J.; ORR, W. C. Mechanisms of aging: an appraisal of the oxidative stress hypothesis. Free Radical Biology and Medicine, v. 33, n. 5, p. 575–586, set. 2002.

SOHAL, R. S.; ORR, W. C. Relationship between Antioxidants, Prooxidants, and the Aging Process. Annals of the New York Academy of Sciences, v. 663, n. 1, p. 74–84, 17 nov. 1992.

VIOLA, M. F.; HERRERA, L. G.; DA CRUZ-NETO, A. P. The acute phase response in bats (*Carollia perspicillata*) varies with time and dose of the immune challenge. Journal of Experimental Biology, v. 225, n. 24, 15 dez. 2022.

VOIGT, C. C. et al. The immune response of bats differs between pre-migration and migration seasons. Scientific Reports, v. 10, n. 1, p. 17384, 15 out. 2020.

WILHELM FILHO, D. et al. Antioxidant defenses, longevity and ecophysiology of South American bats. Comparative Biochemistry and Physiology - C Toxicology and Pharmacology, v. 146, n. 1- 2 SPEC. ISS., p. 214–220, 2007.

WILKINSON, G. S.; SOUTH, J. M. Life history, ecology and longevity in bats. Aging cell, v. 1, n. 2, p. 124–131, 2002.

GENERAL CONCLUSION

In this study, we investigated the impact of the APR on the energy balance of bats, focusing on differences between two species, one insectivorous and the other frugivorous. Initially, we hypothesized that immunological challenges would lead to the mobilization of glycogen, protein, or fatty acid reserves in bats. However, our results demonstrated that the immune challenge did not compromise the energy balance in *Molossus molossus* and *Artibeus lituratus*. While a combination of immune stimulation and prolonged food deprivation might reveal glycemic homeostasis mechanisms not detected in our experimental design, it is important to note that previous studies suggest an adaptation of the bat immune system to bacterial pathogens. Moreover, our findings are consistent with earlier research indicating that activation of the APR in response to bacterial antigens does not pose a significant threat to oxidative balance in both frugivorous and insectivorous species. Despite the observed differences in antioxidant levels and oxidative markers between the species, we could not conclude that diet determines bat susceptibility to oxidative stress, as neither species exhibited significant alterations during the simulated immune challenge.

Although studies on oxidative responses in bats have increased across various contexts, important gaps remain. Bats are known to exhibit remarkable longevity relative to other animals of similar size and metabolic rate, and they display characteristics that confer increased tolerance to oxidative damage. Nevertheless, the physiological mechanisms underlying this capacity, as well as how bats manage these challenges at the molecular level, remain poorly understood. Therefore, the results of this study offer new insights into the immunological and metabolic processes in bats, suggesting that, despite the complex interactions among diet, immune response, and oxidative stress, bats are highly adapted to physiological challenges. However, further research is needed, particularly into the molecular specificities underpinning their resistance to oxidative stress and their immune strategies, thus opening new avenues for future investigation.

GENERAL REFERENCES

ADAMO, S. A. et al. Illness-induced anorexia may reduce trade-offs between digestion and immune function. Animal Behaviour, v. 79, n. 1, p. 3–10, jan. 2010.

AMARAL, T. S. et al. Low energy reserves are associated with fasting susceptibility in Neotropical nectar bats Glossophaga soricina. Brazilian Journal of Biology, v. 79, n. 2, p. 165–168, 1 abr. 2019.

ANINDITA, P. D. et al. Detection of coronavirus genomes in Moluccan naked-backed fruit bats in Indonesia. Archives of Virology, v. 160, n. 4, p. 1113–1118, 4 abr. 2015.

AUDET, D.; FENTON, M. B. Heterothermy and the Use of Torpor by the Bat Eptesicus fuscus (Chiroptera: Vespertilionidae): A Field Study. Physiological Zoology, v. 61, n. 3, p. 197–204, maio 1988.

AUSTAD, S. N.; FISCHER, K. E. Mammalian Aging, Metabolism, and Ecology: Evidence From the Bats and MarsupialsJournal of Gerontology: BIOLOGICAL SCIENCES. [s.l: s.n.]. Disponível em: <http://geronj.oxfordjournals.org/>.

AVIELLO, G. et al. Brain control of appetite during sickness. British Journal of Pharmacology, v. 178, n. 10, p. 2096–2110, 2 maio 2021.

BARTELS, W.; LAW, B. S.; GEISER, F. Daily torpor and energetics in a tropical mammal, the northern blossom-bat Macroglossus minimus (Megachiroptera). Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology, v. 168, n. 3, p. 233–239, 7 abr. 1998.

BILBO, S. D.; NELSON, R. J. Melatonin Regulates Energy Balance and Attenuates Fever in Siberian Hamsters. [s.l: s.n.].

BOLLEN, M.; KEPPENS, S.; STALMANS, W. Specific features of glycogen metabolism in the liverBiochem. J. [s.l: s.n.].

BROESSNER, G. et al. Update on therapeutic temperature management. Critical Care, v. 16, n. S2, p. A1, 7 jun. 2012.

BROOK, C. E.; DOBSON, A. P. Bats as ‘special’ reservoirs for emerging zoonotic pathogens. Trends in Microbiology, v. 23, n. 3, p. 172–180, mar. 2015.

BROSNAN, J. Comments on metabolic needs for glucose and the role of gluconeogenesis. European Journal of Clinical Nutrition, v. 53, n. S1, p. s107–s111, 1 abr. 1999.

BRUNET-ROSSINNI, A. K. Reduced free-radical production and extreme longevity in the little brown bat (Myotis lucifugus) versus two non-flying mammals. Mechanisms of Ageing and Development, v. 125, n. 1, p. 11–20, jan. 2004.

BRUNET-ROSSINNI, A. K.; AUSTAD, S. N. Ageing Studies on Bats: A Review. Biogerontology, v. 5, n. 4, p. 211–222, ago. 2004.

CABRERA-MARTINEZ, L. V.; HERRERA, M. L. G.; CRUZ-NETO, A. P. The energetic cost of mounting an immune response for Pallas’s longtongued bat (Glossophaga soricina). PeerJ, v. 2018, n. 6, 2018.

CABRERA-MARTINEZ, L. V.; HERRERA M., L. G.; CRUZ-NETO, A. P. Food restriction, but not seasonality, modulates the acute phase response of a Neotropical bat. Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology, v. 229, p. 93–100, 1 mar. 2019.

CARLTON, E. D.; DEMAS, G. E.; FRENCH, S. S. Leptin, a neuroendocrine mediator of immune responses, inflammation, and sickness behaviors. Hormones and Behavior, ago. 2012.

CHELOMBITKO, M. A. Role of Reactive Oxygen Species in Inflammation: A Minireview. Moscow University Biological Sciences Bulletin, v. 73, n. 4, p. 199–202, 12 out. 2018.

CHESLEY, L.; IV, S. Neutrophil:Lymphocyte Ratio as a Possible Indicator of Chronic Anthropogenic Stress in Bats (Mammalia: Chiroptera). [s.l: s.n.]. Disponível em: <http://hdl.handle.net/10415/2683>. Acesso em: 23 set. 2024.

CRAY, C. Acute Phase Proteins in Animals. Em: Progress in Molecular Biology and Translational Science. [s.l.] Elsevier B.V., 2012. v. 105p. 113–150.

CRAY, C.; ZAIAS, J.; ALTMAN, N. H. Acute phase response in animals: a review. Comparative medicine, v. 59, n. 6, p. 517–26, dez. 2009.

DHABHAR, F. S. Stress-induced augmentation of immune function - The role of stress hormones, leukocyte trafficking, and cytokines. Brain, Behavior, and Immunity, v. 16, n. 6, p. 785–798, 2002.

EL-BELTAGI, H. S.; MOHAMED, H. I. Reactive Oxygen Species, Lipid Peroxidation and Antioxidative Defense Mechanism. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, v. 41, n. 1, p. 44, 28 maio 2013.

EVANS, S. S.; REPASKY, E. A.; FISHER, D. T. Fever and the thermal regulation of immunity: the immune system feels the heat. Nature Reviews Immunology, v. 15, n. 6, p. 335–349, 15 jun. 2015.

EXTON, M. S. Infection-Induced Anorexia: Active Host Defence Strategy. Appetite, v. 29, n. 3, p. 369–383, dez. 1997.

FIELD, H. E. Bats and Emerging Zoonoses: Henipaviruses and SARS. Zoonoses and Public Health, v. 56, n. 6–7, p. 278–284, 9 ago. 2009.

FREITAS, M. B. et al. Metabolic responses induced by fasting in the common vampire bat Desmodus rotundus. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology, v. 173, n. 8, p. 703–707, 1 nov. 2003.

FREITAS, M. B. et al. Effects of short-term fasting on energy reserves of vampire bats (Desmodus rotundus). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, v. 140, n. 1, p. 59–62, jan. 2005.

FREITAS, M. B. et al. Reduced insulin secretion and glucose intolerance are involved in the fasting susceptibility of common vampire bats. General and Comparative Endocrinology, v. 183, p. 1–6, 1 mar. 2013.

FREITAS, M. B.; WELKER, A. F.; PINHEIRO, E. C. Seasonal variation and food deprivation in common vampire bats (Chiroptera: Phyllostomidae). Brazilian Journal of Biology, v. 66, n. 4, p. 1051–1055, nov. 2006.

FREITAS, MB. et al. Energy metabolism and fasting in male and female insectivorous bats Molossus molossus (Chiroptera: Molossidae). Brazilian Journal of Biology, v. 70, n. 3, p. 617–621, ago. 2010.

GABAY, C.; KUSHNER, I. Acute-Phase Proteins and Other Systemic Responses to Inflammation. New England Journal of Medicine, v. 340, n. 6, p. 448–454, 11 fev. 1999.

GOMES, B. R. B. et al. Increase of reactive oxygen species in different tissues during lipopolysaccharide-induced fever and antipyresis: an electron paramagnetic resonance study. Free Radical Research, v. 52, n. 3, p. 351–361, 4 mar. 2018.

GONG, S. et al. Dynamics and Correlation of Serum Cortisol and Corticosterone under Different Physiological or Stressful Conditions in Mice. PLOS ONE, v. 10, n. 2, p. e0117503, 20 fev. 2015.

GONZALEZ, V.; BANERJEE, A. Molecular, ecological, and behavioral drivers of the bat-virus relationship. iScience, v. 25, n. 8, p. 104779, ago. 2022.

GRUYS, E. et al. Acute phase reaction and acute phase proteins. Journal of Zhejiang University-SCIENCE B, v. 6, n. 11, p. 1045–1056, 1 nov. 2005.

GUERRERO-CHACÓN, A. L. et al. Metabolic cost of acute phase response in the frugivorous bat, Artibeus lituratus. Mammal Research, v. 63, n. 4, p. 397–404, 30 out. 2018.

HOU, C.-C. et al. Oxidative stress and pyrogenic fever pathogenesis. European Journal of Pharmacology, v. 667, n. 1–3, p. 6–12, 30 set. 2011.

HOUTEN, S. M.; WANDERS, R. J. A. A general introduction to the biochemistry of mitochondrial fatty acid β-oxidation. Journal of Inherited Metabolic Disease, v. 33, n. 5, p. 469–477, 2 out. 2010.

INGVARTSEN, K. L.; BOISCLAIR, Y. R. Leptin and the regulation of food intake, energy homeostasis and immunity with special focus on periparturient ruminantsDomestic Animal Endocrinology. [s.l: s.n.].

JANEWAY, C. A.; MEDZHITOV, R. Innate Immune Recognition. Annual Review of Immunology, v. 20, n. 1, p. 197–216, abr. 2002.

JOHNSON, R. W. Immune and endocrine regulation of food intake in sick animals. Domestic Animal Endocrinology, v. 15, n. 5, p. 309–319, set. 1998.

KNOLLE, P. A.; GERKEN, G. Local control of the immune response in the liver. Immunological Reviews, v. 174, n. 1, p. 21–34, 19 abr. 2000.

KRAUS-FRIEDMANN, N. Hormonal regulation of hepatic gluconeogenesis. Physiological Reviews, v. 64, n. 1, p. 170–259, jan. 1984.

LAURIDSEN, C. From oxidative stress to inflammation: redox balance and immune system. Poultry Science, v. 98, n. 10, p. 4240–4246, 1 out. 2019.

LEMASTERS, J. J.; JAESCHKE, H. Oxidative Stress and Inflammation in the Liver. Em: The Liver. [s.l.] Wiley, 2020. p. 714–727.

LOCHMILLER, R. L.; DEERENBERG, C. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos, v. 88, n. 1, p. 87–98, 24 jan. 2000.

LUIS, A. D. et al. A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? Proceedings of the Royal Society B: Biological Sciences, v. 280, n. 1756, p. 20122753, 7 abr. 2013.

LUO, J. et al. Flight rapidly modulates body temperature in freely behaving bats. Animal Biotelemetry, v. 9, n. 1, p. 45, 3 dez. 2021.

MARAIS, M.; MALONEY, S. K.; GRAY, D. A. The metabolic cost of fever in Pekin ducks. Journal of Thermal Biology, v. 36, n. 2, p. 116–120, mar. 2011.

MARKOWSKA, M.; MAJEWSKI, P. M.; SKWARŁO-SOŃTA, K. Avian biological clock – Immune system relationship. Developmental & Comparative Immunology, v. 66, p. 130–138, 1 jan. 2017.

MARTIN, L. B.; WEIL, Z. M.; NELSON, R. J. Fever and sickness behaviour vary among congeneric rodents. Functional Ecology, v. 22, n. 1, p. 68–77, 29 fev. 2008.

MELHADO, G.; HERRERA M., L. G.; DA CRUZ-NETO, A. P. Bats respond to simulated bacterial infection during the active phase by reducing food intake. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, v. 333, n. 8, p. 536–542, 1 out. 2020.

MORENO, K. R. et al. Sick bats stay home alone: fruit bats practice social distancing when faced with an immunological challenge. Annals of the New York Academy of Sciences, v. 1505, n. 1, p. 178–190, 1 dez. 2021.

MURPHY, K. et al. Janeway´s Immunobiology. 8th. ed. New York: Garland Science, Taylor & Francis Group, LLC, 2012.

NEWSHOLME, P.; NEWSHOLME, E. A. Rates of utilization of glucose, glutamine and oleate and formation of end-products by mouse peritoneal macrophages in culture. Biochemical Journal, v. 261, n. 1, p. 211–218, 1 jul. 1989.

OTÁLORA-ARDILA, A. et al. Metabolic Cost of the Activation of Immune Response in the Fish-Eating Myotis (Myotis vivesi): The Effects of Inflammation and the Acute Phase Response. PLOS ONE, v. 11, n. 10, p. e0164938, 28 out. 2016.

OTÁLORA-ARDILA, A. et al. The effect of short-term food restriction on the metabolic cost of the acute phase response in the fish-eating Myotis (Myotis vivesi). Mammalian Biology, v. 82, p. 41–47, 1 jan. 2017.

PIKULA, J. et al. Phagocyte activity reflects mammalian homeo- and hetero-thermic physiological states. BMC Veterinary Research, v. 16, n. 1, p. 232, 6 dez. 2020.

POND, C. M. Adipose tissue and the immune system. Prostaglandins, Leukotrienes and Essential Fatty Acids, v. 73, n. 1, p. 17–30, jul. 2005.

SCHEIERMANN, C.; KUNISAKI, Y.; FRENETTE, P. S. Circadian control of the immune system. Nature Reviews Immunology, v. 13, n. 3, p. 190–198, 8 mar. 2013.

SCHREIBER, R.; ZECHNER, R. Lipolysis meets inflammation: arachidonic acid mobilization from fat. Journal of Lipid Research, v. 55, n. 12, p. 2447–2449, 1 dez. 2014.

SELTMANN, A. et al. Differences in acute phase response to bacterial, fungal and viral antigens in greater mouse-eared bats (Myotis myotis). Scientific Reports, v. 12, n. 1, p. 15259, 10 set. 2022.

SIMMONS, P. S. et al. Increased proteolysis. An effect of increases in plasma cortisol within the physiologic range. Journal of Clinical Investigation, v. 73, n. 2, p. 412–420, 1 fev. 1984.

SOCORRO FARIA, S. et al. The neutrophil-to-lymphocyte ratio: a narrative review. ecancermedicalscience, v. 10, 12 dez. 2016.

SOON, G. S. T.; TORBENSON, M. The Liver and Glycogen: In Sickness and in Health. International Journal of Molecular Sciences, v. 24, n. 7, p. 6133, 24 mar. 2023.

STAWSKI, C.; GEISER, F. Fat and fed: frequent use of summer torpor in a subtropical bat. Naturwissenschaften, v. 97, n. 1, p. 29–35, 16 jan. 2010.

STOCKMAIER, S. et al. No fever and leucocytosis in response to a lipopolysaccharide challenge in an insectivorous bat. Biology Letters, v. 11, n. 9, p. 20150576, set. 2015.

SU, L.-J. et al. Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. Oxidative Medicine and Cellular Longevity, v. 2019, p. 1–13, 13 out. 2019.

SUN, L. et al. Metabolic Reprogramming in Immune Response and Tissue Inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology, v. 40, n. 9, p. 1990–2001, 1 set. 2020.

TEAGUE O'MARA, M. et al. Heart rate reveals torpor at high body temperatures in lowland tropical free-tailed bats. Royal Society Open Science, v. 4, n. 12, 20 dez. 2017.

TRIANA-LLANOS, C. et al. The acute phase response elicited by a viral-like molecular pattern increases energy expenditure in Artibeus lituratus. Biologia, v. 74, n. 6, p. 667–673, 15 jun. 2019.

VAN NIEKERK, G. et al. Sickness-Associated Anorexia: Mother Nature's Idea of Immunonutrition? Mediators of Inflammation, v. 2016, p. 1–12, 2016.

VIOLA, M. F.; HERRERA, L. G.; DA CRUZ-NETO, A. P. The acute phase response in bats (*Carollia perspicillata*) varies with time and dose of the immune challenge. Journal of Experimental Biology, v. 225, n. 24, 15 dez. 2022.

VIOLA, M. F.; HERRERA M., L. G.; CRUZ-NETO, A. P. Combined effects of ambient temperature and food availability on induced innate immune response of a fruit-eating bat (Carollia perspicillata). PLOS ONE, v. 19, n. 5, p. e0301083, 24 maio 2024.

VOIGT, C. C. et al. The immune response of bats differs between pre-migration and migration seasons. Scientific Reports, v. 10, n. 1, p. 17384, 15 out. 2020.

WIBBELT, G. et al. Emerging diseases in Chiroptera: why bats? Biology Letters, v. 6, n. 4, p. 438–440, 23 ago. 2010.

WILHELM FILHO, D. et al. Antioxidant defenses, longevity and ecophysiology of South American bats. Comparative Biochemistry and Physiology - C Toxicology and Pharmacology, v. 146, n. 1- 2 SPEC. ISS., p. 214–220, 2007.

WILKINSON, G. S.; SOUTH, J. M. Life history, ecology and longevity in bats. Aging cell, v. 1, n. 2, p. 124–131, 2002.

YU, W. et al. Reactive Oxygen Species Bridge the Gap between Chronic Inflammation and Tumor Development. Oxidative Medicine and Cellular Longevity, v. 2022, p. 1–22, 28 jun. 2022.

ZHANG, X. et al. Metabolic substrate utilization in stress-induced immune cells. Intensive Care Medicine Experimental, v. 8, n. S1, p. 28, 18 dez. 2020.