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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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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.

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.

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