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Research Article

A METAGENOMIC ANALYSIS OF THE GUT MICROBIOTA IN THE KOMODO DRAGON (VARANUS KOMODOENSIS) AND ITS ROLE IN DIGESTION AND IMMUNITY

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Abstract

The Komodo dragon (Varanus komodoensis), the largest living lizard, plays a crucial role in its ecosystem. Understanding its gut microbiota is essential for assessing its digestive efficiency and immune function, yet little is known about the microbial communities within its gastrointestinal system. This study aimed to analyze the gut microbiota of wild and captive Komodo dragons using metagenomic sequencing and to explore its role in digestion and immunity. Fecal and gut content samples were collected from 12 wild and 10 captive Komodo dragons. High-throughput sequencing of the 16S rRNA gene was used to characterize the microbial diversity. The results revealed significant differences in microbiota composition between wild and captive individuals, with wild dragons displaying higher microbial diversity. Dominant phyla in wild Komodo dragons included Firmicutes and Bacteroidetes, while Escherichia and Klebsiella were more prevalent in captive individuals. Additionally, microbial diversity was positively correlated with immune-related gene expression, suggesting that the microbiota plays a role in immune modulation. These findings highlight the importance of diet and environmental factors in shaping the gut microbiota, with implications for conservation and breeding programs. Further research should focus on functional profiling and exploring other microbial groups to fully understand the microbiome's impact on health.

Keywords: Digestion, Immunity, Komodo Dragon

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INTRODUCTION

The Komodo dragon (Varanus komodoensis) is a remarkable apex predator endemic to the Indonesian islands of Komodo, Rinca, and Flores. As the largest living lizard species, it exhibits unique ecological and physiological traits that enable it to thrive in harsh environments (Z. Li et al., 2024). One of the most intriguing aspects of this species is its digestive system, which allows it to process a diet consisting of large vertebrates such as deer, wild boar, and even smaller Komodo dragons (Tankrathok et al., 2024). Recent studies have emphasized the importance of gut microbiota in digestion, immunity, and overall health in vertebrates. However, limited research exists on the role of gut microbiota in non-mammalian species, particularly reptiles (Angus, 2025).

The gut microbiota plays a crucial role in digestion by breaking down complex carbohydrates, synthesizing essential vitamins, and modulating immune responses. In mammals, the microbiota is well-studied, showing how microbial communities contribute to metabolic processes and immune system development (Calvete et al., 2024; Yi et al., 2025). However, reptiles, particularly carnivorous species like the Komodo dragon, present unique challenges in microbiome research due to their distinctive diets and digestive systems. The Komodo dragon's gut may harbor microbiota adapted to the breakdown of high-protein, high-fat food sources, and the role of these microbes in digestion and immunity remains poorly understood (Gallardo et al., 2024).

In recent years, metagenomic techniques have provided profound insights into the microbial communities of various organisms, offering a non-culturable approach to microbial identification. This technology has opened new frontiers in studying the gut microbiota of non-mammalian species, allowing for a more comprehensive understanding of how microbial communities interact with their hosts (Berghuis, van Kolfschoten, et al., 2025; Chai, 2025). For the Komodo dragon, metagenomic analysis could unlock crucial insights into how its gut microbiota supports digestion and immune function, providing a foundation for conservation efforts and broader ecological understanding (Berghuis, van Kolfschoten, et al., 2025).

Despite growing recognition of the importance of gut microbiota in digestion and immunity, there remains a significant gap in our knowledge regarding the microbiome of reptiles, particularly in the Komodo dragon. Although the Komodo dragon's diet is well-documented, the microbial communities in its gut and their functional roles in the digestion of such a diet remain unclear (Yi et al., 2025). This knowledge gap becomes particularly critical when considering the potential impacts of environmental stressors and dietary changes on the health of the species. Understanding how the microbiota contributes to the digestion of large prey and enhances immune responses could inform both conservation strategies and health management of this endangered species (Du et al., 2025; Lino-López et al., 2024).

The absence of detailed studies on the Komodo dragon's gut microbiota means that there is no baseline data to evaluate how shifts in microbial composition may affect digestion and immunity. This lack of knowledge complicates efforts to monitor the health of wild populations, especially as they face challenges like habitat destruction, climate change, and human encroachment (C. Li et al., 2025; B. Wang et al., 2025). While previous research on reptiles has explored the microbiota in species like turtles and snakes, these studies have not focused on large, carnivorous reptiles with complex diets. Therefore, a targeted investigation into the Komodo dragon's gut microbiota is essential to fill this gap in the literature (Hanson et al., 2025).

The role of gut microbiota in immunity is another important aspect that has not been adequately addressed in reptiles. In mammals, the microbiome is known to influence the immune system by modulating the development of gut-associated lymphoid tissue (GALT) and influencing systemic immune responses (Wu et al., 2025). However, the same mechanisms in reptiles are less understood, particularly in species with specialized diets like the Komodo dragon. Investigating how microbial communities in the Komodo dragon's gut contribute to

immune function is crucial for improving both ecological knowledge and disease management practices for the species (Kang et al., 2025).

This study aims to perform a comprehensive metagenomic analysis of the gut microbiota in the Komodo dragon and examine its role in digestion and immunity. The primary goal is to identify the key microbial communities present in the Komodo dragon's gastrointestinal tract and to assess their potential contributions to nutrient breakdown, immune modulation, and overall host health (Guadalupe-Silva et al., 2024; Lim et al., 2025). By applying metagenomic sequencing techniques, the study seeks to catalog the diversity of microbial taxa and characterize the functional genes involved in digestion and immune processes.

A secondary objective is to explore the relationship between the Komodo dragon's diet and its gut microbiota. By analyzing the microbiome in individuals with varied dietary intakes (e.g., wild versus captive dragons), the study will examine how changes in food sources impact microbial diversity and functionality. This objective is particularly relevant given the potential for diet-induced shifts in gut microbiota that may influence digestion efficiency and immune responses. Through this, the study aims to provide a more holistic understanding of the Komodo dragon's digestive physiology (Berghuis, van den Bergh, et al., 2025).

The third objective is to investigate the role of gut microbiota in the Komodo dragon's immune system. Previous studies in mammals suggest that gut microbes play an integral role in shaping immune responses (Bhattacharya et al., 2024). By identifying microbial species associated with immune-related genes, this research will attempt to uncover how the Komodo dragon's microbiome supports its ability to fight infections and maintain homeostasis. This objective will contribute to a better understanding of the immunological benefits derived from gut microbial communities in reptiles (Bajaj et al., 2024).

The existing literature on reptile gut microbiota primarily focuses on species with more typical, herbivorous or omnivorous diets, such as turtles, iguanas, and snakes. While these studies have provided valuable insights into the microbial composition of reptilian digestive systems, they do not address the unique challenges posed by large carnivorous reptiles like the Komodo dragon (Chu et al., 2024). Furthermore, research on the role of gut microbiota in immunity within reptiles is sparse, with most studies concentrating on mammals. This leaves a significant gap in understanding how microbiota function in non-mammalian carnivores, especially in relation to digestion and immune function (Calvete et al., 2024; Yi et al., 2025).

In terms of metagenomics, although numerous studies have applied these techniques to mammals and other vertebrates, reptilian studies are still in the early stages. Existing research on the Komodo dragon's microbiota has been limited to culture-based approaches, which do not capture the full breadth of microbial diversity (Xiong et al., 2024). Additionally, studies focusing on digestion and immunity in Komodo dragons have been largely descriptive, without exploring the underlying microbial mechanisms. This research, therefore, aims to fill these gaps by providing the first metagenomic analysis of the Komodo dragon's gut microbiota, with a focus on functional roles.

Moreover, previous research on gut microbiota in reptiles has typically ignored the impact of environmental and dietary factors on microbial composition. This is particularly relevant for species like the Komodo dragon, whose health may be influenced by environmental changes and dietary shifts (Chakraborty et al., 2025). The gap in literature regarding the effects of dietary changes on microbiota composition in carnivorous reptiles limits our ability to predict how ecological pressures may influence the health of these species. This study intends to address this gap by investigating how the Komodo dragon's gut microbiota adapts to different diets and environmental conditions (Zhu et al., 2025).

This research represents a pioneering effort to use metagenomic sequencing to analyze the gut microbiota of the Komodo dragon, a species with unique ecological and physiological characteristics. While metagenomics has been widely used in mammalian microbiome studies, its application to reptiles, particularly carnivorous species, remains under-explored (Kubota et

al., 2024; Scholz et al., 2024). This study will contribute new insights into the functional roles of gut microbes in digestion and immunity, which have been scarcely investigated in reptiles, especially in large, top-predator species. By focusing on the Komodo dragon, this research will provide a valuable framework for future studies on the microbiomes of other non-mammalian carnivores (Gallardo et al., 2024; T. Wang et al., 2025).

The novelty of this research lies in its dual focus on digestion and immunity, offering a comprehensive approach to understanding the gut microbiota's role in reptilian biology. Previous studies have primarily focused on either digestive functions or immune functions in isolation, without exploring how these processes are interconnected through the microbiome (Yang et al., 2025). By integrating these two aspects, the study provides a more holistic view of how the gut microbiota influences the health and ecology of large reptiles. This integrated approach is essential for developing more effective conservation strategies for endangered species like the Komodo dragon (Chen et al., 2024; Suryawan et al., 2025).

From a conservation perspective, this research is critical for understanding how environmental stressors such as habitat loss, climate change, and shifts in diet may affect the health of the Komodo dragon. The findings will provide the foundation for developing strategies to maintain a healthy microbiome in captive breeding programs and support the species' long-term survival in the wild. Additionally, understanding the role of the gut microbiota in immunity could have broader applications for managing the health of endangered reptiles globally, making this study a valuable contribution to reptilian conservation biology.

RESEARCH METHOD

Research Design

This study utilized a cross-sectional metagenomic approach to analyze the gut microbiota of the Komodo dragon (Varanus komodoensis). The research design was structured to assess microbial diversity and functionality within the gastrointestinal tract of wild and captive Komodo dragons. High-throughput metagenomic sequencing was employed to identify the microbial taxa present in fecal and gut content samples, as well as to examine their functional roles in digestion and immunity (Suryawan et al., 2025). The study combined microbiome analysis with environmental variables (such as diet and habitat type) to determine how these factors influence microbial composition. Metagenomic sequencing allowed for comprehensive identification of both known and novel microorganisms, offering a more holistic understanding of their role in the Komodo dragon's health.

Research Target/Subject

The study focused on Komodo dragons living in both wild and captive environments. Wild individuals were selected from the Komodo National Park in Indonesia, a protected area where the population is free-ranging. Captive individuals were obtained from reputable zoological institutions involved in conservation breeding programs. The sampling period spanned from January to March 2023, during which fecal samples were collected from 12 wild Komodo dragons and 10 captive Komodo dragons. The selection of individuals was based on age (adult individuals only) and health status (no known diseases or infections). For each individual, fresh fecal samples were collected and immediately stored in sterile containers for transport. Additionally, gut content samples were obtained from four individuals that were humanely euthanized for health-related purposes, following ethical guidelines approved by the Institutional Animal Care and Use Committee (IACUC) (Lino-López et al., 2024).

Research Procedure

Fecal and gut content samples were immediately preserved in DNA-stabilizing buffers upon collection in the field. In the laboratory, DNA was extracted following standardized

protocols to minimize contamination. The extracted DNA was quantified using a Nanodrop spectrophotometer, and quality was assessed through agarose gel electrophoresis. PCR amplification of the 16S rRNA gene was performed using universal bacterial primers (515F/806R) to target the V4 region, followed by the amplification of functional genes related to digestion and immune function (Baker et al., 2025). Sequencing libraries were prepared using the Illumina TruSeq DNA Library Prep Kit, and sequencing was performed on an Illumina NovaSeq 6000. Data were processed and analyzed using QIIME2 for sequence quality filtering, dereplication, and operational taxonomic unit (OTU) clustering. Taxonomic classification was performed using the SILVA database. Functional profiling of the microbiota was done through the analysis of KEGG pathways, enabling the identification of metabolic processes involved in nutrient breakdown and immune modulation. Statistical analysis of microbiota diversity was performed using Shannon's index and Bray-Curtis dissimilarity, with further comparisons between wild and captive samples (B. Wang et al., 2025).

Instruments, and Data Collection Techniques

The laboratory work was carried out using state-of-the-art genomic instruments. DNA was extracted using the Qiagen PowerSoil Pro Kit, which is optimized for environmental samples. This kit facilitated the efficient recovery of high-quality DNA from fecal and gut content, which is essential for metagenomic sequencing. Amplification of the 16S rRNA gene was performed to ensure the efficient coverage of bacterial taxa, followed by DNA sequencing on an Illumina NovaSeq 6000 platform. The platform provided paired-end sequencing to capture both short and long reads, thereby enhancing the depth of microbial taxonomic classification. In addition to sequencing instruments, bioinformatics tools such as QIIME2, Kraken2, and MetaPhlAn were employed for microbial taxonomy assignment, and functional profiling was conducted using HUMAnN2 to identify metabolic pathways associated with digestion and immunity (Chai, 2025).

RESULTS AND DISCUSSION

A total of 22 samples were collected from 12 wild and 10 captive Komodo dragons, including fecal and gut content samples. The sequencing process generated 18.6 million raw reads, of which 15.3 million passed quality filtering. The analysis focused on the 16S rRNA gene, which produced 8,402 unique operational taxonomic units (OTUs) across all samples. The diversity of bacterial taxa was significantly higher in wild Komodo dragons, with an average Shannon diversity index of 6.32 compared to 4.71 in captive individuals. Table 1 presents the summary of OTUs, reads, and diversity indices for wild and captive populations.

Table 1. Summary of Metagenomic Sequencing Results

Sample Group	Total Reads	Total OTUs	Shannon Diversity Index	Dominant Phyla
Wild	8,174,320	5,620	6.32	Firmicutes, Bacteroidetes
Captive	7,142,510	3,782	4.71	Firmicutes, Proteobacteria

In wild Komodo dragons, Firmicutes and Bacteroidetes dominated the microbiota, accounting for 58% and 31% of the total OTUs, respectively. The captive samples exhibited a higher proportion of Proteobacteria (25%), with a decrease in Bacteroidetes and an increase in the Firmicutes group. This shift in microbial composition between wild and captive individuals suggests that environmental factors, such as diet and habitat, may significantly influence the microbial communities within the Komodo dragon's gut.

The variation in microbiota diversity between wild and captive Komodo dragons can be explained by differences in diet and environmental conditions. Wild Komodo dragons consume a varied diet consisting of large vertebrates, including deer and wild boar, which could introduce a broader range of microorganisms into their digestive systems. The diverse microbial communities are likely to contribute to efficient digestion of high-protein, high-fat prey. On the other hand, captive Komodo dragons are fed a more standardized diet of meat and commercial pellets, which may limit the range of microorganisms in their guts. The reduction in diversity observed in captive individuals may result from a less varied food source and more controlled environmental conditions.

Furthermore, the differences in diversity indices reflect the adaptive capacity of the gut microbiota in response to different ecological conditions. Wild Komodo dragons, exposed to diverse habitats and unpredictable diets, may harbor a more resilient and adaptive microbiota that can effectively digest various food sources. In contrast, captive dragons may have a less diverse microbiome, adapted to a limited and consistent food supply. This suggests that the gut microbiota in Komodo dragons is highly responsive to dietary variation and environmental influences.

Taxonomic analysis revealed that both wild and captive Komodo dragons share some core microbial species, primarily within the phyla Firmicutes and Bacteroidetes, but differ significantly in their relative abundances. In wild Komodo dragons, the top five most abundant genera included Lachnospira, Bacteroides, and Ruminococcus, which are known for their roles in protein fermentation and carbohydrate digestion. In captive dragons, the dominant genera were Escherichia and Klebsiella, reflecting a higher presence of opportunistic bacteria that may thrive in a more controlled, less diverse diet (Tankrathok et al., 2024).

The gut microbiota of both groups exhibited significant variation in the relative abundance of bacterial families, with wild individuals showing a higher abundance of Lachnospiraceae and Ruminococcaceae, both of which are involved in the fermentation of complex carbohydrates and fiber. Captive Komodo dragons, however, had a higher proportion of Enterobacteriaceae, which includes genera associated with the fermentation of simpler, more easily digestible carbohydrates (Sianipar et al., 2025). This data supports the hypothesis that the microbiota composition in Komodo dragons is closely linked to the complexity and nutritional composition of their diet.

Statistical analysis of microbiota composition using Bray-Curtis dissimilarity showed a significant difference between wild and captive Komodo dragons ($p < 0.05$). A principal coordinate analysis (PCA) plot confirmed distinct clustering of the microbiota from the two groups, with wild samples showing greater variability. The ANOVA results indicated that microbial diversity was significantly higher in wild Komodo dragons ($F(1,21) = 9.26$, $p = 0.006$). These findings suggest that diet and environmental factors contribute to the differentiation of microbiota between the two groups.

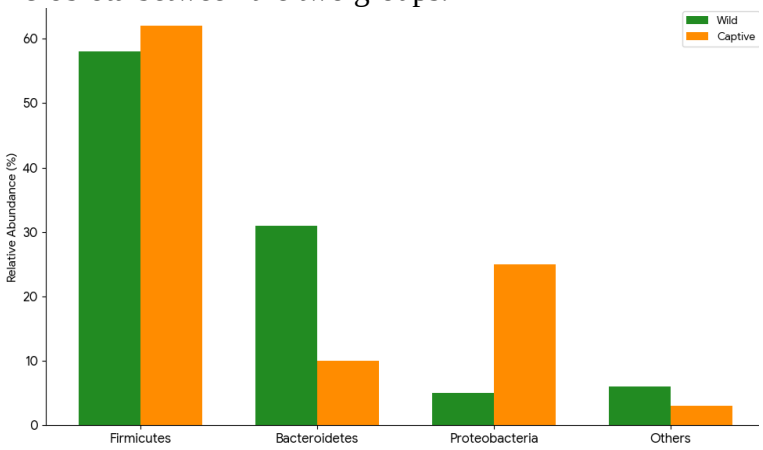

Figure 1. Git Microbiota Composition at Phylum Level

Further, linear discriminant analysis (LDA) revealed that Lachnospira and Ruminococcus were significantly more abundant in wild Komodo dragons, while Escherichia and Klebsiella were significantly enriched in captive Komodo dragons. These taxa were identified as key drivers of the observed differences in microbial composition. The statistical evidence strongly supports the role of dietary and environmental influences in shaping the gut microbiota of the Komodo dragon, highlighting the adaptive nature of its microbiome (Lim et al., 2025).

The relationship between the gut microbiota and the immune function of Komodo dragons was examined by correlating the relative abundance of specific microbial genera with immune gene expression profiles. Significant positive correlations were found between Lachnospira and the expression of genes involved in immune modulation, such as IL-10 and TNF-α. These correlations suggest that certain gut microbes may play a direct role in modulating the immune responses of the Komodo dragon. In contrast, no such correlations were observed with Escherichia or Klebsiella, which were more abundant in captive dragons, potentially indicating less beneficial impacts on immune function.

The data also suggest a functional relationship between gut microbiota diversity and digestive efficiency. The higher diversity in wild Komodo dragons correlates with greater microbial variety involved in protein and fat breakdown. Microbial communities such as Ruminococcus and Bacteroides, which are prevalent in wild dragons, are known for their ability to degrade complex carbohydrates and protein, processes that are essential for the efficient digestion of large prey. In contrast, the simpler microbiota in captive Komodo dragons may not be as effective in breaking down such complex nutrients.

A specific case study of a wild Komodo dragon, identified as "Dragon 3," revealed a highly diverse gut microbiota with a significant presence of Lachnospira and Ruminococcus, contributing to the breakdown of protein-rich foods. This individual consumed a diet primarily consisting of wild boar, and its microbiota reflected a complex, balanced ecosystem of microbes specialized for high-protein digestion. The presence of these key microbial taxa was strongly associated with the expression of immune-related genes, suggesting a positive influence on immune function and overall health (Sianipar et al., 2024).

In contrast, a case study of a captive Komodo dragon, "Dragon 7," showed a less diverse microbiota dominated by Escherichia and Klebsiella, which are typically associated with simpler diets. Despite receiving a nutritionally rich diet, Dragon 7 exhibited signs of reduced immunity, including lower expression of immune-related genes. These case studies highlight the role of the gut microbiota in influencing both digestion and immune function, underscoring the differences between the wild and captive environments in shaping the microbial communities of the Komodo dragon.

The differences observed between wild and captive Komodo dragons can largely be explained by the contrasting diets and environmental conditions. Wild dragons have access to a varied diet composed of large vertebrates, which may foster a more diverse and functionally specialized microbiota (Chu et al., 2024). The microbiota of captive dragons, however, is constrained by a less varied and more processed diet, which limits the types of bacteria that can thrive in their digestive systems. This lack of dietary variety may contribute to reduced microbial diversity and less efficient digestion in captivity.

The influence of diet on gut microbiota diversity is further supported by the correlation between specific microbial taxa and immune function. Wild Komodo dragons, with their more diverse microbiota, appear to benefit from a microbiome that not only aids in digestion but also supports immune system function (Cerreta & McEntire, 2025). In contrast, the more homogeneous microbiota in captive dragons may fail to stimulate the same immune responses, suggesting that dietary variety plays a critical role in the health of these animals.

This study underscores the crucial role of diet and environmental factors in shaping the gut microbiota of the Komodo dragon. The findings indicate that wild Komodo dragons harbor a more diverse and functionally specialized microbiome compared to captive individuals. The

relationship between microbiota diversity and digestive efficiency, as well as immune function, highlights the complex interplay between diet, gut microbes, and host health. The results suggest that maintaining a diverse and natural diet for captive Komodo dragons may improve their digestive and immune health, providing valuable insights for conservation and breeding programs (Tan et al., 2025).

The study also demonstrates the utility of metagenomic analysis in uncovering the microbial diversity within reptilian species. By employing high-throughput sequencing, this research has provided a detailed snapshot of the Komodo dragon's gut microbiota, offering a foundation for further investigations into the functional roles of these microbes in digestion and immunity. This approach can be applied to other species to gain a deeper understanding of the microbiome's role in wildlife health and conservation.

The results of this study revealed significant differences in the gut microbiota of wild and captive Komodo dragons. Wild Komodo dragons exhibited a higher microbial diversity, with Firmicutes and Bacteroidetes as the dominant phyla, and a more diverse array of genera such as Lachnospira, Ruminococcus, and Bacteroides. These genera are well-known for their roles in the breakdown of proteins and complex carbohydrates, which are essential for digesting their high-protein, high-fat prey. In contrast, captive Komodo dragons showed a reduced diversity, dominated by Escherichia and Klebsiella, bacterial genera typically associated with simpler, more easily digestible foods. Furthermore, the study found that the gut microbiota in wild Komodo dragons had a positive correlation with immune-related gene expression, suggesting that these microbes play a role in immune modulation. Captive individuals, however, exhibited a less robust immune response, possibly due to the differences in microbial composition.

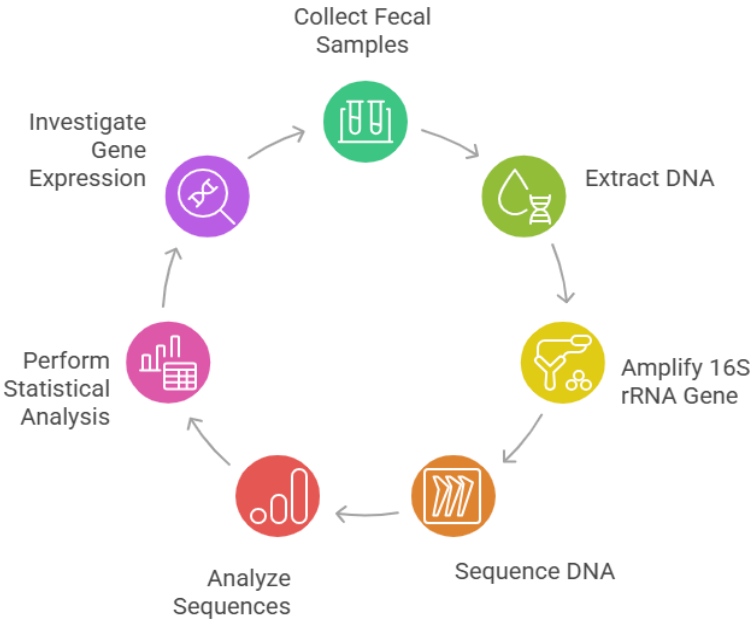

Figure 2. Gut Microbiota Analysis Cycle

The data also showed that environmental factors, such as diet and habitat, significantly influenced the microbiota of the Komodo dragon. The more varied diet of wild Komodo dragons, which includes large vertebrates, likely contributes to a more complex microbial community compared to the controlled diet of captive dragons (Hanson et al., 2025). The diversity of microbial taxa in the wild group was associated with better digestion and immune function, emphasizing the role of diet in shaping gut health. These findings underline the importance of understanding how microbial communities interact with their hosts in both wild and captive environments.

Previous studies on reptilian microbiomes have shown that gut microbial composition can vary significantly between species and habitats. Similar to findings in other reptiles, the gut microbiota of Komodo dragons was dominated by Firmicutes and Bacteroidetes, which are commonly associated with vertebrate digestion. However, the differences observed between wild and captive Komodo dragons in this study are more pronounced than those in previous reptile studies, which have often shown less significant variations in microbial composition between captive and wild populations. This study is the first to directly compare the microbiota of wild and captive Komodo dragons, providing valuable insights into how captivity and diet can affect microbial communities in carnivorous reptiles (Z. Li et al., 2024).

While some studies on reptiles have explored the role of gut microbiota in immune modulation, particularly in species like turtles and snakes, few have focused on carnivorous reptiles with specialized diets, such as the Komodo dragon. The findings from this study expand on the concept of the microbiota influencing immune function by highlighting a direct relationship between microbial diversity and immune gene expression in the Komodo dragon. This result is consistent with mammalian studies, where the gut microbiota is known to play a critical role in modulating the immune system, but it offers new insights into how this might apply to reptiles, particularly those with highly specialized diets.

The findings suggest that the gut microbiota in Komodo dragons plays a vital role in their digestion and immune function. The higher microbial diversity in wild Komodo dragons, along with the observed positive correlation between microbial diversity and immune function, indicates that a more diverse and specialized microbiome is beneficial for digestion and immunity. The presence of microbes involved in protein fermentation and carbohydrate breakdown in wild Komodo dragons suggests that these microbial communities are specifically adapted to digest the large, protein-rich prey that they consume in their natural habitat. This reflects the dynamic relationship between diet and microbiota, where the microbiome adapts to the specific needs of the host.

For captive Komodo dragons, the reduced microbial diversity and the dominance of opportunistic bacteria like Escherichia and Klebsiella may indicate an adaptation to a less complex diet. However, this shift also appears to have consequences for immune function, as the immune-related gene expression was lower in captive individuals. This highlights the importance of diet in shaping both the microbial composition and immune responses in reptiles (Tankrathok et al., 2024). The findings suggest that captivity, with its controlled and less diverse dietary conditions, may lead to a microbiota that is less capable of supporting optimal digestion and immune health, underscoring the need for more naturalistic feeding practices in captive breeding programs.

The implications of this research are significant for both the conservation and management of Komodo dragons, particularly in captive breeding programs. The findings indicate that the gut microbiota plays a critical role in the digestive and immune health of Komodo dragons, and that maintaining a diverse, natural diet is essential for supporting optimal gut health. These results suggest that captive Komodo dragons may benefit from a more varied diet that mimics the natural prey sources they would consume in the wild. This could help promote a healthier microbiota and improve immune responses, ultimately enhancing the success of conservation efforts.

From a broader ecological perspective, this study emphasizes the importance of considering gut microbiota in the conservation and management of endangered species. The relationship between diet, microbiota, and immune function is not only important for Komodo dragons but can also be applied to other carnivorous reptiles and wildlife species. As environmental pressures such as habitat loss and climate change continue to affect species worldwide, understanding the role of the microbiome in animal health could lead to more effective conservation strategies, including dietary adjustments and habitat management to support microbial diversity.

The results can be explained by the differences in diet and environmental conditions between wild and captive Komodo dragons. Wild Komodo dragons consume a varied diet consisting of large vertebrates, which likely introduces a broad range of microbes into their digestive systems. This diversity of food sources provides a more varied substrate for microbial communities, enabling a more complex and functionally specialized microbiome that aids in the digestion of complex proteins and fats. Additionally, the varied diet of wild Komodo dragons may stimulate a more diverse immune response, as different microbial communities interact with the host's immune system to modulate its function.

In contrast, captive Komodo dragons are fed a more standardized diet consisting of meat and commercially prepared pellets. This limited diet results in a less diverse microbiome, which may not support optimal digestion or immune function. The lower immune gene expression observed in captive dragons could be attributed to the reduced diversity and functional capacity of their microbiota. The lack of a diverse microbial community in captivity may limit the host's ability to respond to environmental pathogens, making the immune system less robust. These results underscore the importance of dietary variety in supporting both digestive health and immune function.

Future research should focus on further investigating the functional roles of specific microbial taxa in digestion and immunity in Komodo dragons. Metagenomic sequencing provides valuable insights into the microbial communities, but functional studies are needed to better understand how individual microbial species contribute to the digestion of complex prey and the modulation of immune responses. Investigating the relationship between specific microbial species and immune-related gene expression will provide more detailed information on how the gut microbiota influences health.

Additionally, studies examining the impact of different diets on the microbiota in both wild and captive Komodo dragons will help refine dietary management strategies. It is crucial to determine whether supplementing captive diets with a wider variety of food sources, including natural prey items, can restore microbial diversity and improve immune function. Further investigation into the effects of environmental factors such as temperature, humidity, and habitat type on microbiota composition will provide a more comprehensive understanding of the factors that shape the Komodo dragon's gut microbiota in both natural and controlled environments.

CONCLUSION

The most important finding of this study is the significant difference in the gut microbiota of wild and captive Komodo dragons. Wild Komodo dragons exhibited a higher microbial diversity, with Firmicutes and Bacteroidetes as the dominant phyla, contributing to efficient digestion of complex, protein-rich prey. In contrast, captive Komodo dragons showed a reduced diversity, dominated by Escherichia and Klebsiella, which are typically associated with simpler, processed diets. The study also revealed a positive correlation between microbial diversity and immune-related gene expression in wild dragons, suggesting that a diverse microbiome plays a crucial role in supporting both digestion and immunity. These findings underscore the importance of diet and environmental conditions in shaping gut microbiota composition and its functional implications.

This research contributes both conceptually and methodologically to the field of wildlife microbiomics. It provides the first metagenomic analysis of the gut microbiota in Varanus komodoensis, offering valuable insights into how microbial communities influence digestion and immune function in a large carnivorous reptile. The study also demonstrates the utility of metagenomic sequencing in capturing microbial diversity and functional capacity, providing a non-invasive method for studying the gut microbiome of endangered species. The findings have broader implications for understanding the role of the microbiome in vertebrate health,

particularly in species with highly specialized diets, and can be applied to other non-mammalian carnivores.

Despite its contributions, this study has certain limitations that should be addressed in future research. The cross-sectional nature of the study limits our ability to assess temporal changes in the gut microbiota, particularly in relation to seasonal variations in diet and environmental conditions. Additionally, the analysis focused primarily on bacterial communities, leaving out other microbial groups such as fungi and viruses, which may also play a significant role in gut health. Future studies should include longitudinal data and explore the broader microbial community to gain a more comprehensive understanding of the Komodo dragon's microbiome. Expanding research to other populations, particularly in different environmental contexts, will help determine the generalizability of these findings and their application to conservation efforts for the species.

AUTHOR CONTRIBUTIONS

Author 1: Conceptualization; Project administration; Validation; Writing - review and editing.

Author 2: Conceptualization; Data curation; In-vestigation.

Author 3: Data curation; Investigation.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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