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Integrated omics analysis reveals a correlation between gut microbiota and egg production in captive African penguins (*Spheniscus demersus*)

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Highlights

- Firmicutes and Proteobacteria are predominant bacteria in breeding African penguins.
- Microbiota and metabolites differ between penguins with different egg production.
- Egg production has correlations with differential microbial bacteria and metabolites.

Abstract

The egg production of captive African penguins differs considerably between individuals. An understanding of the physiological differences in African penguins with relatively greater and lesser egg production is meaningful for the captive breeding program of this endangered species. The objective of this study was to investigate differential microbial composition and metabolites in captive African penguins with different egg production. Fecal samples were collected from captive female African penguins during the breeding season. The results of 16 S rRNA gene sequencing showed that African penguins with different egg production had similar microbial diversities, whereas a significant difference was observed between their microbial community structure. African penguins with relatively greater egg production exhibited a higher relative abundance of Alphaproteobacteria, Rhizobiales, Bradyrhizobiaceae, Bradyrhizobium and *Bosea*. Meanwhile, penguins with relatively lesser egg production had an increased proportion of Klebsiella and Plesiomonas. We further identified a total of 1858 metabolites in female African penguins by liquid chromatography-mass spectrometry analysis. Among these metabolites, 13 kinds of metabolites were found to be significantly differential between African penguins with different egg production. In addition, the correlation analysis revealed that the egg production had significant correlations with most of the differential microbial bacteria and metabolites. Our findings might aid in understanding the potential mechanism underlying the phenomenon of abnormal egg production in captive African penguins, and provide novel insights into the relationship between gut microbiota and reproduction in penguins.

Introduction

African penguin (*Spheniscus demersus*) is a species of penguin that lives in the south and southwest coast of South Africa. Currently, the wild population of African penguins is sharply declining due to human intervention, infectious disease and rapid climate change (Parsons et al., 2016, Santiago-Moreno et al., 2019, Abolnik et al., 2023). The International Union for Conservation of Nature has classified African penguin as an endangered species. To prevent this species from extinction, it is urgent to ensure the reproductive success of captive population. High reproductive efficiency is critical for the conservation of endangered animals (Herrick, 2019). Egg production is a key reproductive trait for birds. Currently in the captivity of endangered birds, one of the main reproductive concerns is abnormal egg production (Le Loc'h et al., 2017). The egg-laying number per year varies between healthy individuals, which causes limitations on the captive breeding program of penguins (Emmerson et al., 2019). Therefore, it is of great significance to explore the physiological differences in African penguins with different egg production.

The formation of the egg is a non-sterile process. It can be influenced by bacteria. In birds, the anatomically terminal parts of reproductive, urinary and digestive tracts connect to the cloaca (Lee et al., 2020). It has been demonstrated that the intestinal bacteria can directly transfer from the cloaca into the oviduct and even the body-cavity (Ding et al., 2017, Lee et al., 2019). The colonization of these bacteria is able to affect oviductal microbiota which may have an impact on the formation of eggs (Niu et al., 2023). Additionally, glucocorticoids can be metabolized and converted into testosterone by bacteria (Ridlon et al., 2013). Evidence for a correlation between testosterone levels and microbial diversity of cloaca suggests that gut microbiota may affect the reproductive behaviors in birds (Comizzoli et al., 2021). Numerous studies have also indicated a close relationship between gut microbiota and reproductive output in avian species, including domestic birds (chicken, duck and goose) and crested ibis (*Nipponia nippon*) (Wang et al., 2020a, Ran et al., 2021, Gong et al., 2023, Li et al., 2023). Furthermore, the disturbance of gut microbiota induced by various factors may lead to reduced reproductive performance (Ricke et al., 2022). Relatively higher Firmicutes abundance has been recognized as an intestinal biomarker for chickens with decreased egg production (Wang et al., 2021). The modulation of gut microbiota has been demonstrated to improve the egg production in low-yield birds (Wang et al., 2020b). Nevertheless, the correlation between gut microbiota and reproductive outcome of African penguins has not been elucidated yet. Understanding this correlation may provide us a new perspective in solving the reproductive problem of African penguins.

Reproductive outcome of wild animals may be influenced by living environment, diet, diseases and even population fluctuations (Comizzoli et al., 2021, Barbanti et al., 2022, Wang et al., 2022). Therefore, it is difficult to ascert reproduction-related microbial alterations owing to many unpredictable interference factors in the wild. However, the habitat environment and diet of captive animals are both controlled in the zoo, which provides proper conditions for studying the potential correlation between microbiota and reproductive trait in endangered species.

The aim of this study was to explore differential microbial composition and metabolites in captive African penguins with different egg production through integrated omics analysis combined with 16 S rRNA gene sequencing and liquid chromatography-mass spectrometry (LC-MS) analysis. The possible correlation between gut microbiota and egg production in captive African penguins was also determined.

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Section snippets

Animals and sample collection

All captive African penguins involved in this study were healthy determined by timely physical examination, and they lived in the same controlled environment in Shanghai Zoo. A total of eight female individuals from a group of 37 African penguins were chosen for this study based on their egg-laying data in the past four years (2020–2023). The age and egg-laying information of each African penguin was shown in Table 1. These penguins were divided into two groups: (1) the group with relatively…

Egg production data of African penguins in this study

The egg production of African penguins was significantly different between two groups in this study ($P < 0.05$, Fig. 1). The GEP group exhibited significantly increased average egg number per year (Fig. 1A) and average clutch number per year (Fig. 1B) compared with the LEP group ($P < 0.05$).…

Microbial composition in African penguins with different egg production

Fig. 2 represents the top 10 microbial compositions (data of mean taxa) at phylum and class levels in African penguins with different egg production, respectively. In the GEP group, at the phylum level,…

Discussion

The fecal microbial composition of captive African penguins in the breeding season has been evaluated in this study. A previous study has indicated that fecal samples are sufficient for characterizing the gut microbial communities across different groups in birds (Berlow et al., 2020). Thus, the fecal microbiota could reflect the gut microbiota in birds. There is an obvious difference in the microbial composition of African penguins between non-breeding and breeding season. Our previous study…

Conclusions

The present study provides implications for the husbandry and conservation of captive African penguins. African penguins with relatively greater egg production exhibited a higher relative abundance of Alphaproteobacteria, Rhizobiales, Bradyrhizobiaceae, *Bradyrhizobium* and *Bosea*. Meanwhile, penguins with relatively lesser egg production had an increased proportion of *Klebsiella* and *Plesiomonas*. A total of 13 kinds of metabolites were found to be significantly differential between the groups. In…

CRediT authorship contribution statement

Jingle Jiang: Conceptualization, Formal analysis, Investigation, Resources, Project administration, Writing-original draft, Visualization. **Di Hu:** Investigation, Resources, Visualization. **Enle Pei:** Conceptualization; Supervision, Writing-review & editing, Funding acquisition.…

Declaration of Competing Interest

The authors declare that they have no competing interests.…

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