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Impact of *Staphylococcus aureus* Infection on Reproductive Hormones and Gonadal Development in African Catfish (*Clarias gariepinus*): Implications for Aquaculture Sustainability

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The reproductive health of aquaculture species is critical for sustainable production, yet it is often threatened by bacterial infections. This study investigated the effects of experimental *Staphylococcus aureus* infection on the reproductive hormone profiles and gonadosomatic index (GSI) of African catfish (*Clarias gariepinus*), a species of significant economic importance. Thirty healthy male and female catfish were divided equally into control and infected groups. The infected groups were fed 1.5 mL of 1.5×10^8 CFU/mL of *S. aureus*, while the control groups received sterile phosphate-buffered saline. Hormonal levels, including testosterone, estradiol, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), were measured over three weeks using enzyme-linked immunosorbent assay (ELISA), and the results revealed significant reductions ($p < 0.05$) in all measured hormones in the infected groups compared to the controls. Testosterone levels in infected males declined by 21%, while estradiol levels in infected females were 28% lower than in controls. Similarly, FSH and LH levels in infected males and females showed consistent decreases of approximately 20–25%. The GSI values in infected fish were significantly reduced ($p < 0.05$), indicating impaired gonadal development. These findings suggest that *S. aureus* infection disrupts the hypothalamic-pituitary-gonadal axis, likely through systemic inflammation and oxidative stress, resulting in compromised reproductive function. The findings underscore the need for stringent biosecurity measures, such as regular water quality monitoring and disinfection protocols, to mitigate the impact of *S. aureus* outbreaks in aquaculture systems.

Keywords: African catfish, *Staphylococcus aureus*, reproductive hormones, gonadosomatic index, aquaculture sustainability, biosecurity

Introduction

Aquaculture plays an important role in meeting the increasing global demand for high-quality protein, with fish serving as an essential source of nutrition and economic stability in many regions. Among the various fish species, African catfish adapt to diverse environmental conditions, including fluctuating temperatures (18–32°C), low oxygen levels, and wide pH ranges (6.5–9.0) (Adewolu et al., 2008; Viveen, W. J. A. R., et al., 1985). However, infectious diseases remain a significant threat to sustainable aquaculture production, affecting fish health, growth, and reproduction (Kibenge et al., 2012). Bacterial infections, particularly those caused by *Staphylococcus aureus*, are known to adversely impact aquaculture systems. This pathogen, commonly associated with skin and soft tissue infections, can infiltrate aquatic environments through contaminated feed, water, or handling practices. In fish, *S. aureus* infections are characterized by symptoms such as ulcerative dermatitis, fin rot, and systemic complications such as hepatic necrosis, splenic abscesses, and septicemia that compromise physiological functions (Austin & Austin, 2016). While much research has focused on the growth and immune responses of fish to bacterial pathogens, limited data exist on *S. aureus* specific impacts on reproductive hormones and gonadal function in *Clarias gariepinus*, though broader bacterial infections are linked to reproductive decline (Swanson, P., et al., 2003; Schulz & Miura, 2002).

Reproductive performance in fish is intricately regulated by hormonal profiles, which govern gametogenesis, spawning, and fecundity. Key hormones such as testosterone, estradiol, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) play

vital roles in both males and females reproductive systems (Nagahama, 1994). Any disruption in the synthesis or regulation of these hormones can disrupt male and female reproductive systems, altering spermatogenesis and oocyte maturation potentially affecting population dynamics in aquaculture systems; in *Clarias gariepinus*, such disruptions may reduce spawning success, directly impacting aquaculture productivity (Mylonas et al., 2010). Previous studies have reported altered reproductive hormone levels in fish exposed to environmental stressors or pathogens, but the specific effects of *S. aureus* infection on hormone profiles and gonadal development remain poorly understood.

The gonadosomatic index (GSI), a widely used parameter in reproductive studies, provides insights into the development and maturation of gonads in relation to body weight. It serves as a reliable indicator of reproductive health and productivity in fish (Rahman et al., 2019). Infected fish with reduced GSI values often exhibit impaired gonadal development, which may correlate with altered hormonal profiles.

Given the importance of maintaining reproductive health in aquaculture species, this study aims to investigate the effect of experimental *S. aureus* infection on the reproductive hormone profiles and gonadal development of *Clarias gariepinus*. Specifically, we hypothesize that bacterial infection significantly alters hormonal regulation, leading to reduced GSI and compromised reproductive performance. By understanding these effects, this study contributes to the broader goal of developing effective strategies to mitigate the impact of bacterial infections on aquaculture sustainability.

Materials and Method

Experimental Design

This study was conducted to investigate the effect of *Staphylococcus aureus* infection on the reproductive hormone profiles of African catfish (*Clarias gariepinus*). It was carried out in two phases: (1) induction of experimental infection with *S. aureus*, and (2) evaluation of reproductive hormones in the infected and control groups over a monitoring period of three weeks.

Fish Husbandry

A total of 30 sexually mature *Clarias gariepinus* (15 males and 15 females; mean weight 500 ± 50 g) were obtained from a local fish farm. The fish were acclimatized for two weeks in aerated tanks under controlled conditions (temperature: 27 ± 2°C; pH: 7.0 ± 0.2; dissolved oxygen: > 5 mg/L). They were fed twice daily with a commercial diet (35% crude protein) at 3% body weight per day. The tanks were cleaned regularly, and water quality was monitored throughout the study. The handling of fish followed ethical guidelines for the care and use of animals in research, as outlined by the World Organisation for Animal Health (OIE, 2019) and approved by the UDUS research ethics committee (Ref: UDUS/IACUC/2023/231) on 12/10/2023. The bioassay for *S. aureus* infection was conducted using methods adapted from Austin and Austin (2016).

Experimental Groups

The fish were divided into two groups (n=15 per group, 7 male & 8 female):

(1) Control Group: Non-infected fish.
(2) Infected Group: Fish experimentally infected with *S. aureus*.

Preparation of Staphylococcus aureus Inoculum

The preparation of *S. aureus* cultures and the bacterial suspension followed established microbiological techniques described by Cappuccino and Welsh (2017). A clinical isolate of *S. aureus* was obtained and cultured on nutrient agar for 24 hours at 37°C. A single colony was inoculated into nutrient broth and incubated for 18 hours. The bacterial suspension was standardized to 0.5 McFarland standard concentration containing 1.5×10^8 cfu/mL in 1.5 mL.

Infection Protocol

Fish in the infected group were given 1.5 mL of the bacterial suspension (1.5×10^8 cfu/mL) orally. Control group received a sterile saline (1.5 mL) through the same route. Fish were observed for clinical signs of infection daily.

Blood Sampling

Blood samples were collected on days 0 (pre-infection), 7, 14, and 21 post-infection; this three-week period was selected based on preliminary data showing peak hormonal disruption and GSI changes within this timeframe post-infection. Fish were anesthetized using clove oil (50 mg/L), and blood was drawn from the caudal vein using sterile 1 mL syringes. Approximately 1 mL of blood was collected per fish and transferred into sterile plain tubes for serum separation. The blood was allowed to clot at room temperature, centrifuged at 3,000 rpm for 10 minutes, and the serum was stored at −20°C until hormone analysis.

Hormonal Analysis

Hormone extraction and quantification using enzyme-linked immunosorbent assay (ELISA) was achieved according to the protocols by Choi et al. (2018), ensuring sensitivity and accuracy in detecting low concentrations of reproductive hormones. The following reproductive hormones were quantified using Enzyme-Linked Immunosorbent Assay (ELISA) kits according to manufacturer's instructions:

- Males: Testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH).
- Females: Estradiol, progesterone, FSH, and LH.

Commercially available ELISA kits (validated for fish species) were used according to the manufacturer's instructions. Standard curves were generated for each hormone, and sample concentrations were calculated accordingly.

Gonadal Assessment

The formula for GSI calculation, as well as its application in evaluating gonadal development, was based on methods described by Rahman et al. (2019). At the end of the study (day 21), all fish were euthanized humanely using an overdose of clove oil (> 100 mg/L). Gonads were excised, weighed, and processed for histological evaluation to assess structural changes. The gonadosomatic index (GSI) was calculated as:

$$\text{GSI} = \left(\frac{\text{Gonad Weight}}{\text{Body Weight}} \right) \times 100$$

Statistical Analysis

Data were analyzed using SPSS software (version 26.0). Hormone concentrations and GSI values were expressed as mean ± standard deviation (SD). Differences between groups and time points were analyzed using two-way ANOVA followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

Ethical Considerations

All experimental procedures were conducted in compliance with institutional guidelines for the care and use of laboratory animals and were approved by the Animal Ethics Committee of Usmanu Danfodiyo University Sokoto. Appropriate measures were taken to minimize fish distress and ensure biosafety during *S. aureus* handling.

Results

Statistical analysis using two-way ANOVA revealed significant differences ($p < 0.05$) in all measured hormone levels between the control and infected groups across time points. Control group testosterone levels remained stable (12.5 to 14.5 ng/mL) across the study, with no significant change over time ($p > 0.05$). In contrast, infected fish showed significantly lower testosterone levels (9.92 to 11.02 ng/mL), indicating a pronounced effect of *S. aureus* infection ($p < 0.05$). FSH levels in the control group ranged from 4.0 to 4.4 ng/mL, with no significant temporal changes ($p > 0.05$). Infected fish, however, displayed significantly reduced FSH levels (3.31 to 3.17 ng/mL) compared to controls at each time point ($p < 0.05$). LH levels in the control group ranged from 2.5 to 2.9 ng/mL, while infected fish had significantly reduced LH levels (1.90 to 2.04 ng/mL) throughout the study period ($p < 0.05$).

Estradiol levels in the control group steadily increased from 50.0 to 57.0 ng/mL over the study period, with no significant temporal variation ($p > 0.05$). Infected fish exhibited significantly lower levels (36.26 to 46.88 ng/mL) (Table 2), indicating that *S. aureus* infection severely impacted estradiol production ($p < 0.05$). Progesterone levels in the control group increased from 10.0 to 12.5 ng/mL, while infected fish showed significantly lower levels (8.04 to 10.08 ng/mL) at all time points ($p < 0.05$) (Table 2). Control FSH levels ranged from 3.5 to 3.9 ng/mL, while LH levels ranged from 2.0 to 2.5 ng/mL. Infected fish showed significantly reduced FSH (2.65 to 3.10 ng/mL) and LH (1.56 to 1.93 ng/mL) compared to controls at each time point ($p < 0.05$) (Table 2).

Gonadosomatic Index (GSI)

Control fish exhibited a consistent increase in GSI, ranging from 3.5% on Day 0 to 3.9% on Day 21. Infected fish demonstrated a reduction in GSI, with values ranging from 2.94% on Day 0 to 3.44% on Day 21 (Table 3). The GSI values in the control group steadily increased from 3.5% to 3.9%, reflecting normal gonadal development. In contrast, infected fish exhibited significantly lower GSI values (2.94% to 3.44%) across all time points compared to controls ($p < 0.05$). This reduction suggests impaired gonadal development associated with *S. aureus* infection.

Discussion

This study demonstrates that experimental infection with *Staphylococcus aureus* significantly affects the reproductive physiology of African catfish (*Clarias gariepinus*), as evidenced by alterations in reproductive hormone profiles and gonadosomatic index (GSI). These findings highlight the profound impact of bacterial infections on aquaculture species, with implications for fish health, productivity, and sustainable aquaculture practices.

Across all measured parameters, *S. aureus* infection led to significant reductions ($p < 0.05$) in reproductive hormone levels and GSI compared to control fish. This indicates that the infection adversely impacts the reproductive ability of *Clarias gariepinus*, potentially through hormonal dysregulation and reduced gonadal development.

The observed reduction in testosterone levels in infected males and estradiol levels in infected females indicates that *S. aureus* infection disrupts the endocrine regulation of reproduction. Testosterone and estradiol are critical for spermatogenesis and oogenesis, respectively (Nagahama, 1994; Mylonas et al., 2010). Reduced levels of these hormones suggest that the infection impairs the hypothalamic-pituitary-gonadal (HPG) axis, potentially through systemic inflammation caused by the pathogen. Similar endocrine disruptions have been reported in fish exposed to other stressors, such as environmental pollutants and bacterial toxins, which interfere with hormone synthesis and signaling pathways (Kibenge et al., 2012; Hontela, 2005).

Additionally, the reduced levels of gonadotropins (FSH and LH) in infected fish further underscore the adverse effects of *S. aureus*. FSH and LH are pivotal for the maturation of gametes and ovulation (Zohar et al., 2010). The consistent decline in these hormones across time points suggests a chronic suppressive effect of *S. aureus* on the HPG axis, potentially mediated by inflammatory cytokines that inhibit gonadotropin-releasing hormone (GnRH) secretion (Zhao et al., 2019).

The significant reduction in GSI in infected fish provides additional evidence of impaired gonadal development. The GSI is a widely used metric to assess reproductive fitness, with higher values indicating active gonadal development and maturation (Rahman et al., 2019). The lower GSI observed in infected fish suggests reduced gametogenesis and gonadal atrophy, consistent with the hormonal disruptions reported in this study.

The decline in GSI could also be attributed to energy reallocation, where infected fish prioritize immune responses over reproductive investment. This phenomenon, known as the "immune trade-off," has been documented in several fish species exposed to pathogens or other stressors (Schreck et al., 2001). The systemic

Table 1 Mean values of reproductive hormone levels in male African catfish (*Clarias gariepinus*) experimentally infected with *Staphylococcus aureus*

Day	Day 0 (ng/mL)	Day 7 (ng/mL)	Day 14 (ng/mL)	Day 21 (ng/mL)
Testosterone (Control)	12.50 ± 0.80	15.00 ± 1.00	14.80 ± 0.78	14.50 ± 1.20
FSH (Control)	4.00 ± 0.24	4.50 ± 0.30	4.60 ± 0.60	4.40 ± 0.23
LH (Control)	2.50 ± 0.21	2.80 ± 0.31	3.00 ± 0.22	2.90 ± 0.19
Testosterone (Infected)	9.92 ± 1.23	10.61 ± 1.50	10.95 ± 1.43	11.02 ± 1.87
FSH (Infected)	3.31 ± 0.12	3.72 ± 0.20	3.87 ± 0.18	3.17 ± 0.32
LH (Infected)	1.90 ± 0.09	2.08 ± 0.10	2.54 ± 0.18	2.04 ± 0.15

Table 2 Mean values of reproductive hormone levels in female African catfish (*Clarias gariepinus*) experimentally infected with *Staphylococcus aureus*

Day	Day 0 (ng/mL)	Day 7 (ng/mL)	Day 14 (ng/mL)	Day 21 (ng/mL)
Estradiol (Control)	50.00 ± 5.00	55.00 ± 3.50	56.50 ± 3.98	57.00 ± 3.87
Progesterone (Control)	10.00 ± 2.34	12.00 ± 3.00	11.80 ± 2.50	12.50 ± 2.90
FSH (Control)	3.50 ± 0.22	3.80 ± 0.34	4.00 ± 0.33	3.90 ± 0.38
LH (Control)	2.00 ± 0.08	2.30 ± 0.10	2.40 ± 0.87	2.50 ± 0.12
Estradiol (Infected)	36.26 ± 3.50	45.00 ± 5.20	46.48 ± 5.33	46.88 ± 5.10
Progesterone (Infected)	8.04 ± 1.00	9.26 ± 1.54	9.27 ± 1.90	10.08 ± 1.40
FSH (Infected)	2.65 ± 0.12	3.15 ± 0.18	3.22 ± 0.17	3.10 ± 0.35
LH (Infected)	1.56 ± 0.08	1.68 ± 0.78	1.97 ± 0.23	1.93 ± 0.25

Table 3 Temporal changes in gonadosomatic index (GSI) of African catfish (*Clarias gariepinus*) following experimental infection with *Staphylococcus aureus*

Day	Control (%)	Infected (%)
Day 0	3.50 ± 0.06	2.94
Day 7	3.70 ± 0.11	3.31 ± 0.17
Day 14	3.80 ± 0.21	3.19 ± 0.30
Day 21	3.90 ± 0.43	3.44 ± 0.28

effects of *S. aureus*, including inflammation and oxidative stress, may exacerbate this trade-off, further impairing reproductive output.

The pathophysiological mechanisms underlying the observed effects of *S. aureus* likely involve a combination of direct and indirect factors. Direct bacterial invasion (Özgür and Gülşen, 2020) and systemic dissemination may damage gonadal tissue, while the host's immune response may indirectly disrupt reproductive function. Inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which are elevated during bacterial infections, are known to interfere with steroidogenesis and gonadotropin secretion (Spitz et al., 2004). Moreover, oxidative stress induced by *S. aureus* could impair gonadal cell function, leading to reduced gamete production and hormonal synthesis (Costantini et al., 2011).

The findings of this study have significant implications for aquaculture management. Reproductive impairment in *C. gariepinus* could lead to reduced breeding efficiency, affecting seed production and overall stock sustainability. Effective disease management strategies, including improved water quality, biosecurity measures, and prophylactic treatments, are essential to mitigate the impact of bacterial infections on fish reproduction. Additionally, the development of resistant strains through selective breeding or genetic modification may provide long-term solutions to reduce susceptibility to infections (Rexroad et al., 2019).

This study provides a foundation for further investigation into the effects of bacterial infections on fish reproduction. Future research should focus on elucidating the molecular mechanisms underlying endocrine disruption in infected fish, including the role of inflammatory pathways and oxidative stress. Long-term studies assessing the reproductive performance of infected fish during successive spawning cycles would provide valuable insights into the chronic effects of bacterial infections. Furthermore, exploring the efficacy of immunomodulatory agents or dietary supplements in mitigating the impact of *S. aureus* on fish reproduction could inform the development of targeted interventions.

Conclusion

In conclusion, this study highlights the detrimental effects of *Staphylococcus aureus* infection on the reproductive health of *Clarias gariepinus*. The observed hormonal disruptions and reduced GSI underscore the vulnerability of aquaculture species to bacterial pathogens and the need for robust disease management strategies. By addressing these challenges, the aquaculture industry can enhance productivity and sustainability, ensuring the continued availability of this vital protein source.

References

Austin, B., & Austin, D. A. (2016). *Bacterial Fish Pathogens: Disease of Farmed and Wild Fish* (6th ed.). Springer.

Cappuccino, J. G., & Welsh, C. T. (2017). *Microbiology: A Laboratory Manual* (11th ed.). Pearson.

Choi, Y. H., Lee, H. J., & Shin, H. S. (2018). Advances in enzyme-linked immunosorbent assay (ELISA) for hormone analysis in aquaculture species. *Aquaculture Research*, *49*(3), 567–578. https://doi.org/10.1111/are.13567

Costantini, D., Metcalfe, N. B., & Monaghan, P. (2011). Ecological processes in a hormetic framework. *Ecology Letters*, *13*(11), 1435–1447. https://doi.org/10.1111/j.1461-0248.2010.01531.x

Hontela, A. (2005). Stress and the hypothalamo-pituitary-interrenal axis: Crosstalk between endocrine systems, environmental toxicants and reproductive physiology. *Aquatic Toxicology*, *72*(3), 335–364. https://doi.org/10.1016/j.aquatox.2005.02.004

Kibenge, F. S. B., & Godoy, M. G. (2012). Aquaculture and vaccine development: Paradigms for preventing infectious diseases in fish. *Comparative Immunology, Microbiology and Infectious Diseases*, *35*(3), 211–217. https://doi.org/10.1016/j.cimid.2012.01.002

Mylonas, C. C., Fostier, A., & Zanuy, S. (2010). Broodstock management and hormonal manipulations of fish reproduction. *General and Comparative Endocrinology*, *165*(3), 516–534. https://doi.org/10.1016/j.ygcen.2009.03.007

Nagahama, Y. (1994). Endocrine regulation of gametogenesis in fish. *International Journal of Developmental Biology*, *38*(2), 217–229.

OIE (World Organisation for Animal Health). (2019). *Terrestrial Animal Health Code* (28th ed.). OIE Standards.

Özgür Çanak, & Gülşen Timur (2020). An initial survey on the occurrence of staphylococcal infections in Turkish marine aquaculture (2013–1014). *Journal of Applied Ichthyology*. https://doi.org/10.1111/jai.14141

Rahman, M. S., Verdegem, M. C. J., & Nagelkerke, L. A. J. (2019). Enhancing reproductive performance in aquaculture species through better broodstock management. *Aquaculture Research*, *50*(9), 2454–2463. https://doi.org/10.1111/are.14268

Rexroad, C., Vallejo, R. L., Liu, S., Palti, Y., & Weber, G. M. (2019). QTL mapping and genomic selection for aquaculture species: Where do we stand today? *Frontiers in Genetics*, *10*, 847. https://doi.org/10.3389/fgene.2019.00847

Schreck, C. B., Contreras-Sanchez, W., & Fitzpatrick, M. S. (2001). Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture*, *197*(1–4), 3–24. https://doi.org/10.1016/S0044-8486(01)00580-4

Spitz, D. R., Azzam, E. I., Li, J. J., & Gius, D. (2004). Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: A unifying concept in stress response biology. *Cancer Metastasis Reviews*, *23*(3–4), 311–322. https://doi.org/10.1023/B:CANC.0000031769.14728.bc

Zar, J. H. (2010). *Biostatistical Analysis* (5th ed.). Pearson Education.

Zhao, W., Zhou, Y., Xu, H., & Li, J. (2019). Inflammation-induced endocrine dysfunction in aquatic organisms: Mechanisms and implications. *Fish and Shellfish Immunology*, *89*, 48–56. https://doi.org/10.1016/j.fsi.2019.03.030

Zohar, Y., Muñoz-Cueto, J. A., Elizur, A., & Kah, O. (2010). Neuroendocrinology of reproduction in teleost fish. *General and Comparative Endocrinology*, *165*(3), 438–455. https://doi.org/10.1016/j.ygcen.2009.04.017