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Evaluation of the Histology of Gonads in Ramirezi (*Mikrogeophagus ramirezi*) as a Response to Variations in Feeding Ratio

(EVALUASI HISTOLOGI GONAD IKAN RAMIREZI (MIKROGEOPHAGUS RAMIREZI) SEBAGAI RESPONS TERHADAP VARIASI RASIO PAKAN)

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ABSTRACT

Nutritional management is a crucial factor in optimizing the reproductive performance of fish. This study was aimed to evaluate the histological characteristics of the gonads of ramirezi (*Mikrogeophagus ramirezi*) in response to variations in feed ratios. The feeding trial was conducted over a period of 45 days with four different treatments. The experimental design involved the administration of artificial feed and Tubifex worms in varying ratios: (A) 3:0, (B) 0:3, (C) 2:1, and (D) 1:2. Gonads (testes and ovaries) were collected at the beginning and end of the treatment. Histological analysis using hematoxylin-eosin staining, to assess the developmental stages based on tissue structure and germ cell stages. Observations revealed significant differences in gonadal maturity levels among the treatments. In the testes, treatment A exhibited a predominance of spermatogonia, while the treatment with a 1:2 ratio (treatment D) displayed seminiferous tubule lumens filled with spermatozoa. The ovaries showed development from Primary Growth Oocyte (PG) to the vitellogenic stage, particularly in treatment D. The combination of artificial feed and Tubifex worms at a 1:2 ratio (treatment D) significantly accelerated gonadal maturation in both male and female *M. ramirezi*. These findings underscore the importance of integrating high-quality natural feed in the management of broodstock and reproduction within small-scale aquaculture systems.

Keywords: *Mikrogeophagus ramirezi*; gonadal histology; Tubifex worms; reproductive maturity; nutritional strategy

ABSTRAK

Pengelolaan nutrisi merupakan faktor penting dalam mengoptimalkan performa reproduksi ikan. Penelitian ini bertujuan mengevaluasi histologis gonad ikan ramirezi (*Mikrogeophagus ramirezi*) sebagai respons terhadap variasi rasio pakan. Uji coba pemberian pakan dilakukan selama 45 hari dengan empat perlakuan. Rancangan percobaan yang digunakan yaitu pemberian pakan buatan dan cacing sutera (*Tubifex worm*) dengan perbandingan rasio yang berbeda.: (A) 3:0, (B) 0:3, (C) 2:1, and (D) 1:2. Gonad (testis dan ovarium) diambil pada awal dan akhir perlakuan, untuk analisis histologi menggunakan pewarnaan hematoksilin-eosin, guna menilai tahap perkembangan berdasarkan struktur jaringan dan stadium sel germinal. Hasil pengamatan menunjukkan adanya perbedaan nyata dalam tingkat kematangan gonad antar perlakuan. Pada testis, perlakuan A menunjukkan dominasi spermatogonium, sementara perlakuan dengan rasio 1:2 (perlakuan D) menunjukkan lumen tubulus seminiferus yang penuh dengan spermatozoa. Ovarium menunjukkan perkembangan dari oosit Primary Growth Oocyte (PG) menuju tahap vitellogenik, terutama pada perlakuan D. Kombinasi pakan buatan dan cacing sutera 1:2 (perlakuan D) secara signifikan mempercepat pematangan gonad pada ikan jantan dan betina *M. ramirezi*. Hasil ini menunjukkan pentingnya integrasi pakan alami berkualitas tinggi dalam manajemen indukan dan reproduksi pada sistem budidaya skala kecil.

Kata-kata kunci: *Mikrogeophagus ramirezi*; histologi gonad; cacing sutera (*Tubifex* \
worm); kematangan reproduksi; strategi nutrisi

INTRODUCTION

The reproductive performance of ornamental fish is a key parameter in determining the success of captive breeding programs, particularly for species such as Ramirezi (*Mikrogeophagus ramirezi*), family of Cichlid that is highly valued in the global aquarium trade (Azizah *et al*., 2024a). As demand increases, there is a pressing need to optimize breeding protocols that enhance gonadal development, spawning frequency and overall fecundity (Azizah *et al*., 2024b; Torsabo *et al*., 2024). One critical factor influencing reproductive success in fish is nutrition, especially during gonad maturation. Nutritional inputs not only support somatic growth but also playing a crucial role in the regulation of endocrine pathways and gametogenesis (Singh *et al*., 2021).

Among various dietary sources, live feeds such as *Tubifex* worms (*Tubifex* sp.) are widely known for their high protein content and digestibility, which make them beneficial for broodstock conditioning (Ghafoor *et al*., 2020; Gisbert *et al*., 2022). However, the use of live feed alone may not provide a balanced nutrient profile and poses risks related to pathogen transmission. Conversely, artificial diets are formulated to meet specific nutritional requirements and offer consistent quality, but they may lack certain natural stimulants found in live feed that promote reproductive readiness (Glencross, 2020; Watts and D'Abramo, 2021). Therefore, combining artificial feed with live feed sources in varying ratios could potentially yield synergistic benefits by improving both the nutritional balance and palatability, thus enhancing gonadal development (Gule and Geremew, 2022).

Histological analysis of gonads provides direct evidence of reproductive stage and gamete quality, allowing for the assessment of the impact of different fee-ding regimes on reproductive physiology (Abdollahpour, 2020; Samal *et al*., 2025). Previous studies have demonstrated that dietary composition affects not only gonadosomatic indices and spawning performance but also gonadal histoarchitecture, including oocyte maturation in females and spermatogenesis in males (Sharma *et al*., 2024; Al-Khalaifah *et al*., 2025). Despite

this, limited research has been conducted on the histological outcomes of varying feed ratios in *M. ramirezi*. This study was aimed to investigate the effect of different ratios of artificial feed and *Tubifex* worms on the gonadal histology of Ramirezi, providing insight into optimal broodstock nutrition strategies for ornamental fish aquaculture.

RESEARCH METHODS

Experimental Fish and Feeding Trial

A total of 120 healthy *M. ramirezi* (blue electric variety), aged approximately three months with an average weight of 0.503 ± 0.140 g, were obtained from ornamental fish breeders in the Bogor region. Following an acclimation period, ten fish were randomly distributed into each of ten aquariums (40 x 30 x 30 cm^3) filled with clean borehole water sourced from the University of Lampung. Each treatment was conducted in triplicate. The experimental design involved feeding the fish with different ratios of artificial feed and bloodworms: (A) 3:0, (B) 0:3, (C) 2:1, and (D) 1:2. In treatment A, the fish were given 100% artificial feed three times daily. In treatment B, the fish received 100% bloodworms three times daily. Treatment C involved feeding artificial feed at 08:00 and 12:00, followed by bloodworms at 17:00. Conversely, in treatment D, bloodworms were administered at 08:00 and 12:00, followed by artificial feed at 17:00.

Histology Analysis

Gonadal histological analysis was conducted by randomly collecting samples from fish at the start of the experiment (n = 6) from the stock and at the end (n= 9 per treatment group). Fish were anesthetized using clove oil at a concentration of 1 mL/L, with the anesthetic process lasting approximately 20–30 minutes. After anesthetization, the fish trunk was dissected and fixed in 10% neutral buffered formalin (NBF) for 24 hours, then rinsed with 70% ethanol and stored at room temperature until further processing. Histological preparations were carried out at the Lampung Veterinary Center using standard procedures: tissues were dehydrated through a graded ethanol series 70%, 80%, 85%, 90%, 95% I, 95% II, 100% I dan 100% II 70%, 80%, 85%, 90%, 95% I, 95% II, 100% I dan 100% II, and then embedded in paraplast, next sectioned transversely at 5 µm thickness. The sections included the full abdominal cavity and were stained with hematoxylin and eosin. Gonadal development stages were then identified microscopically based on the classification system by Tang *et al*. (2015).

Data Analysis

Data analysis of gonad development of ramirezi broodstocks were analyzed qualitatively by observing gonad morphology based on the criteria found and analyzed descriptively.

RESULTS AND DISCUSSION

Microscopic observations of the histological preparations of the testis of *M. ramirezi* reveal that on day 0 (Figure 1a), the testicular tissue structure is predominantly composed of spermatogonia (SG), with minimal or no presence of advanced germ cells such as spermatocytes (SC), spermatids (ST), and spermatozoa (SZ). This indicates that spermatogenesis is not yet actively occurring, and the testis is in the early developmental phase of the reproductive cycle. On day 45 of treatment A (Figure 1b), a more active spermatogenesis process begins to emerge, evidenced by the increased presence of spermatocytes and spermatids, as well as the initial appearance of spermatozoa within the tubular lumen. The presence of various stages of germ cell differentiation suggests a progressive activation of testicular function. Further development is observed in Figures 1c to 1e, each representing the testis on day 45 under treatments B, C and D, respectively.

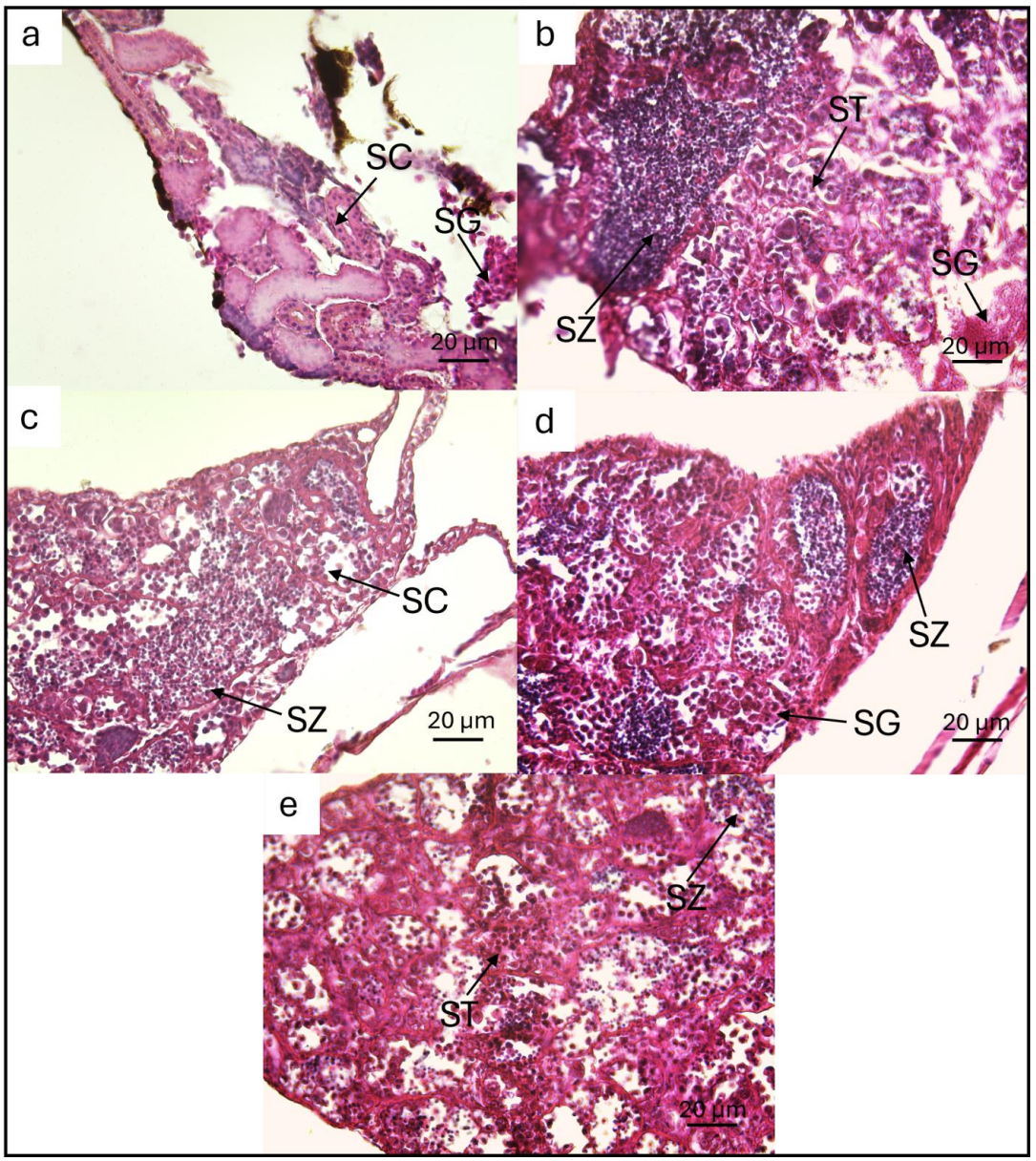

Figure 1. Testicles histology of ramirezi (*Mikrogeophagus ramirezi*). (a) Testicular day-0; (b) Testicular day-45 at A Treatment; (c) Testicular day-45 at B Treatment; (d) Testicular day-45 at C Treatment; (e) Testicular day-45 at D Treatment. SG, spermatogonia; SC, spermatocytes; ST, spermatids; SZ, spermatozoa; The scale bars in the testicular sections represent 20 µm.

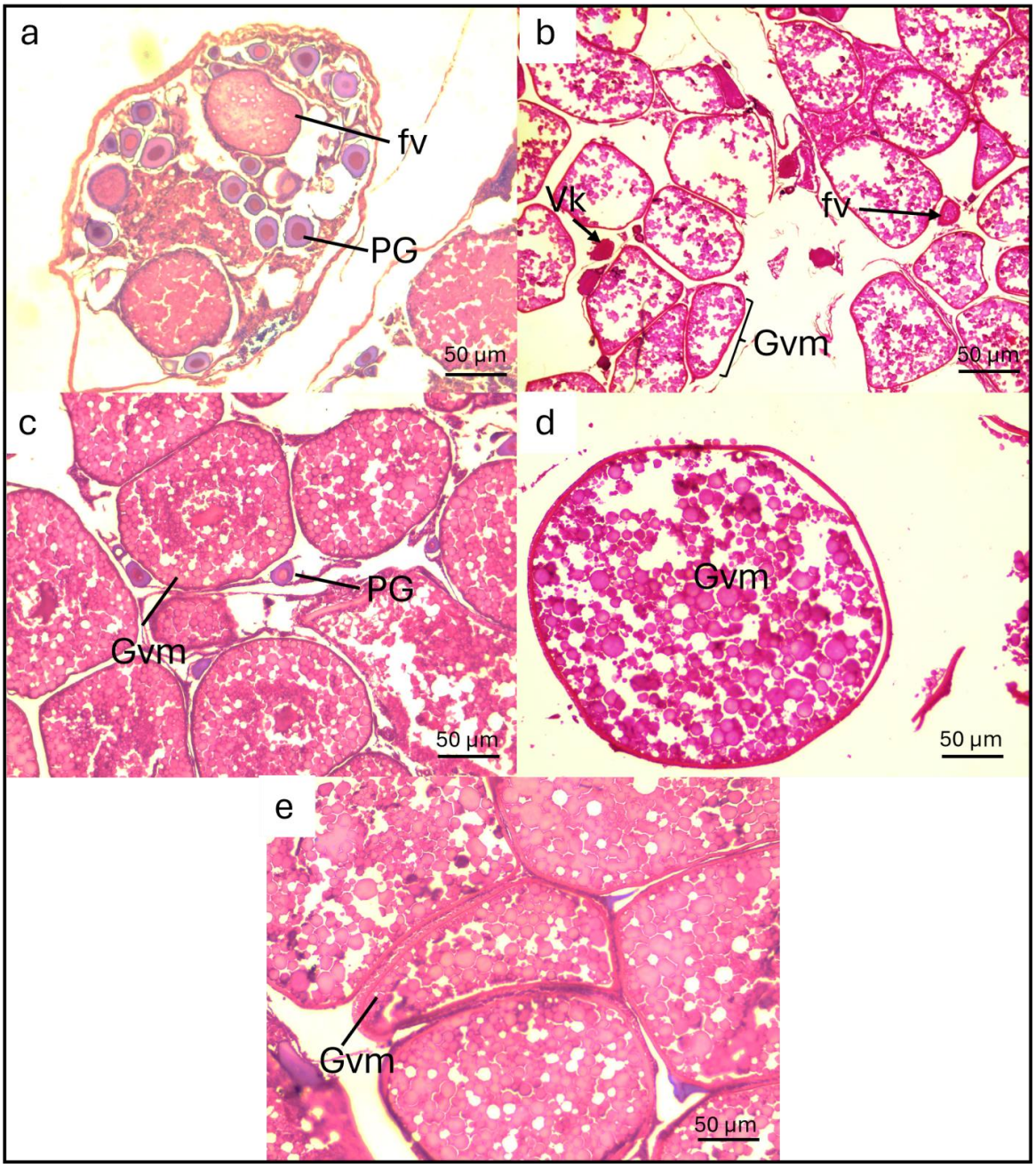

Figure 2. Ovarian histology of ramirezi (*Mikrogeophagus ramirezi*). (a) Ovarian day-0; (b) Ovarian day-45 at A Treatment; (c) Ovarian day-45 at B Treatment; (d) Ovarian day-45 at C Treatment; (e) Ovarian day-45 at D Treatment. PG, primary growth oocyte; GVM, germinal vesicle migration; fv, follicle vitellogenin; VK, Vesicle; The scale bars in the ovarian sections represent 50 µm.

Table 1. Microscopic characteristics of the testis in *Mikrogeophagus ramirezi* with different ratios of artificial feed and bloodworms (A) 3:0, (B) 0:3, (C) 2:1, and (D) 1:2

Treatments	Dominant Germinal Cell Stage	Microscopic Characteristics
Initial control, Day-0	Spermatogonium (SG)	Small round cells are distributed along the walls of the seminiferous tubules; differentiation is not yet evident; the tubules appear empty, lacking spermatocytes or spermatozoa.
A, Day-45	Spermatocytes (SC), Spermatids (ST), Initial SZ	Spermatocytes begin to develop; several spermatids are visible; spermatozoa start to appear slightly in the lumen of the tubules; the process of spermatogenesis becomes active.
B, Day-45	Spermatocytes (SC), Spermatids (ST), Spermatozoa (SZ)	The various stages of germinal cells are clearly observed; the number of spermatozoa begins to increase; the lumen of the tubules becomes densely filled with spermatozoa; spermatogenesis is actively occurring.
C, Day-45	Spermatid (ST), Spermazoa (SZ)	Spermatozoa predominantly occupy the lumen of the tubules; a small amount of SG and SC remains at the periphery; indicating that spermiogenesis is nearly complete; the seminiferous tubules are densely filled with SZ.
D, Day-45	Spermatozoa (SZ)	The lumen of the tubule is filled with spermatozoa; the presence of SG and SC is minimal; indicating that the process of spermiogenesis has reached its peak and spermiation has occurred.

Table 2. Microscopic characteristics of the ovarium in *Mikrogeophagus ramirezi* with different ratios of artificial feed and bloodworms (A) 3:0, (B) 0:3, (C) 2:1, and (D) 1:2.

Treatments	Dominant Germinal Cell Stage	Microscopic Characteristics
Initial control, Day-0	Primary Growth Oocyte (PG)	The oocyte is small in size, with a dense and homogeneous cytoplasm; the nucleus is centrally located; vitellogenesis has not yet occurred; the vitellogenic follicle (fv) has not developed.
A, Day-45	Germinal Vesicle Migration (Gvm)	The oocyte enlarges, initiating the migration of the nucleus towards the periphery; the cytoplasm begins to contain vitellogenic granules; mild vacuolization is observed.
B, Day-45	Germinal Vesicle Migration (Gvm)	Oocyte is characterized by Gvm dominance; the nucleus is eccentric; vitellogenesis activity is heightened; numerous vacuoles appear in the cytoplasm.
C, Day-45	Vitellogenic Oocyte (V)	The oocyte is significantly large, with the cytoplasm filled with vitelline granules; vitellogenic vesicles are distinctly visible; the ovary is in the late maturation phase, approaching ovulation.
D, Day-45	Germinal Vesicle Migration (Gvm)	Large oocyte with the nucleus positioned at the periphery of the cell; the cytoplasm begins to fill with vitellogenic vesicles; the mid-stage of vitellogenesis is predominant.

Further development is observed in Figures 1c to 1e, each representing the testis on day 45 under treatments B, C and D, respecttively. Figure 1c illustrates a more uniform distribution of SG, SC, ST, and SZ, reflecting that the formation of male gamete has occurred optimally. Figure 1d shows a significant increase in the number of spermatozoa, with the dominance of SZ in the lumen indicating that spermiogenesis has reached its final stage, and many germ cells have undergone complete differentiation. Treatment D (Figure 1e) displays a clear dominance of spermatozoa, with only a few SC and ST still visible, indicating that the spermatogenesis process has peaked and most tubules have undergone spermiasis. This pattern reflects the differences in effecttiveness among treatments in stimulating testicular development, with treatment D appearing to be the most successful in inducing sexual maturation and optimal spermatozoa production. The histological development characteristics of the testes, as illustrated in Table 1, reflect the dynamics of the spermatogenesis cycle in response to the administered treatments. The presence of germ cells from the early stage (SG) to the final stage (SZ) indicates that spermatogenesis occurs normally and reaches maturity within 45 days post-treatment. According to Chen and Ge (2013), puberty in female ornamental fish is typically initiated around 45 days post-fertilization (dpf), but this timing is strongly correlated with achieving a certain body size. In zebrafish, gonadal differrentiation is completed around 35 dpf in females and 45 dpf in males. Puberty in females begins at approximately 45 dpf, but this can vary depending on growth rate and environmental conditions. The spermatogenesis process in teleosts involves the mitotic division of spermatogonia, meiosis of spermatocytes, and the differentiation of spermatids into spermatozoa within the seminiferous tubules (Ding *et al*., 2023). The observed differences in the quantity of SZ across treatments suggest that the effecttiveness of the treatments in stimulating testicular activity varies.

An increase in the number of SZ in treatments C and D indicates optimal hormonal or nutritional stimulation supporting spermatogenesis. This finding aligns with the report of Sepehrfar *et al*. (2023), which state that environmental factors and the administration of bioactive substances (such as hormones, phytohormones, or immunestimulant compounds) can expedite gonadal maturation. The maturation process of sperm occurs in a gradual and organized manner along the seminiferous tubules, allowing different sections of the tubule to be at various stages of spermatogenesis. According to Batista-Silva *et al.* (2022), spermatogenic cells within the testicular tubules do not develop synchronously. Each segment of the tubule can exhibit different stages of spermatogenesis, resulting in a wave of sperm maturation throughout the tubules. Environmental changes can rapidly trigger morphological alterations in the testes and either accelerate or stimulate the wave of spermatogenesis, leading to a more varied distribution of sperm cell stages within the tubules.

The dominance of SZ in treatment D further suggests that the process of spermiogenesis has been completed and spermiation has occurred, which is crucial for the success of fertilization in artificial reproduction programs (Ramos-Júdez *et al*., 2022). This is also suspected to be due to the timing of feeding and the high nutritional content of the feed provided in sufficient quantity and nutrients for the maturation of the gonads in ramirezi. According to Pantoni *et al*. (2022), the elevated nutritional content in live feed, such as Tubifex worms, acelerates gonadal maturation in fish. The nutritional composition of tubifex worms is notably high, comprising 57% protein and 13.3% fatty acids (Syahputra and Isma, 2020). The high levels of protein and fatty acids in Tubifex worms, along with the nutritional content of the artificial feed used, which contains 47.5% protein and 6.5% fat (as per the feed packaging composition), can

enhance the overall nutritional profile of the feed provided.

Histological observations of the ovaries in *M. ramirezi* reveal varying levels of oocyte development across different treatments. In Figure 2a, which represents day 0 prior to treatment, the ovarian structure is predominantly characterized by primary growth oocytes (PG) that possess dense cytoplasm and have not yet undergone yolk accumulation (vitellogenesis). Vitellogenic follicles (fv) are not yet visibly developed, indicating that the ovaries are in the early stages of development, prior to the initiation of the vitellogenic cycle (Table 2).

Structural changes in the ovaries begin to manifest on day 45 post-treatment. Figures 2b, 2c and 2e illustrate significant development, marked by the emergence of oocytes exhibiting germinal vesicle migration (Gvm), which signifies the commencement of vitellogenesis and oocyte enlargement. Oocytes at this stage display morphological changes, including nuclei that are beginning to migrate towards the cell periphery and cytoplasm that appears to be undergoing vacuolization (Table 2). In Figure 2d, a more mature oocyte in the Gvm stage is observed, characterized by abundant yolk accumulation and clearly visible vesicles (V), indicating that a majority of the oocytes have entered or are nearing the final vitellogenic phase, suggesting readiness for ovulation.

The morphological variation of oocytes under each treatment reflects differing levels of ovarian development influenced by the applied treatments. The dominance of PG on day 0 indicates that the ovaries are in the previtellogenic phase, as described by Corriero *et al*. (2021), during which oocytes have not yet actively synthesized yolk proteins. The appearance of Gvm on day 45 in several treatments indicates a transition to the vitallogenic stage, characterized by increased ovarian endocrine activity and marked by the accumulation of vitellogenin from plasma into the oocytes.

The increase in the number of oocytes at the Gvm and V stages, as illustrated in Figure 2d, suggests that the treatments effectively accelerated the gonadal maturation process. The germinal vesicle migration process itself serves as a crucial indicator that oocytes are preparing to enter the final phase of development, namely ovulation (Mahalingam and Santhanam, 2023). The effectiveness of the treatments in promoting oocytes towards the final stage of vitellogenesis demonstrates the success of hormonal stimulation or the influence of bioactive compounds administered, whether through feed or injection.

CONCLUSION

The variation in the feed ratio between artificial feed and natural feed (Tubifex worms) greatly influence the histological development of the gonads in *M. ramirezi*. Microscopic observations reveal that in male gonads, the treatment with the highest ratio of feeding with a ratio of one part artificial feed to two parts natural feed in the form of Tubifex worms results in the most optimal maturation of the testes. In female gonads, the same treatment also accelerates the development of oocytes towards the vital-logenic stage, characterized by the formation of vitellin granules and the migration of the germinal vesicle to a peripheral position, indicating readiness for the ovulation process.

SUGGESTION

The present study provides evidence that the combination of artificial feed and Tubifex worms influences gonadal histology and reproductive maturation in *M.geophagus ramirezi*. The feeding approach can be tested on other ornamental or economically important species to determine its broader applicability in aquaculture.

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