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Significance of Micronutrients in Broodstock Nutrition and their Role in Enhancing Gonadal Development in Fishes

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Introduction

Broodstock nutrition plays a decisive role in the reproductive success of fish. In aquaculture, the quality and quantity of seed production largely depend on the nutritional status of the broodfish**.** While macronutrients, such as proteins, lipids, and carbohydrates, supply energy and structural components, micronutrients regulate physiological, biochemical, and hormonal processes that directly influence reproduction. Nutrition is a vital factor affecting fish during the reproductive stage and represents a significant component that can limit maturation, fecundity, and larval survival(Izquierdo et al., 2001). In captive fish, the quality of the administered diet can affect gonadal development and fertility rate if there is a deficiency of essential nutrients(Sharma et al., 2001). Micronutrients such as zinc, selenium and manganese (Gargotra et al., 2024),and vitamin A, vitamin C, vitamin E (Alava et al., 1993) Can significantly enhance growth, gonadal maturation, ovarian development, and reproductive performances under captive condition. Deficiency results in impaired gonadal development, poor egg and sperm quality, reduced fertilization and hatchability, and an overall decline in reproductive performance (Pandey., 2024). For example, when the iron concentration of rainbow trout eggs was low, the hatching rate was poor (Hirao et al., 1955) and Vitamin B_6 is pivotal in steroidogenesis and folate metabolism; deficiency impairs nucleic acid synthesis, suppresses cell division, and reduces egg hatchability (Halver, 1989). Although research on the nutrition of fish broodstock has accelerated in recent years, there remains a lack of comprehensive understanding of their nutritional requirements and dietary micronutrient needs (Watanabe et al., 1997; Izquierdo et al., 2001). Compared to macronutrients, micronutrients have been the subject of less research, and only a limited number of studies have explored their needs in fish (Watanabe, 1988; Hilton, 1989).

Micronutrients in Broodstock Nutrition

Micronutrients are essential nutrients required in minute quantities but play a decisive role in regulating metabolism, reproduction, immunity, and cellular integrity in aquatic organisms. In broodstock nutrition, micronutrients—primarily vitamins (Fat and water soluble) like A, D, E, K, C and B-complex and minerals and trace elements (magnesium, selenium, zinc, iodine, etc.) are fundamental for gonadal development, gametogenesis, egg and sperm quality, fertilization success and larval viability (Kumar et al., 2018; Sarmento et al., 2017)

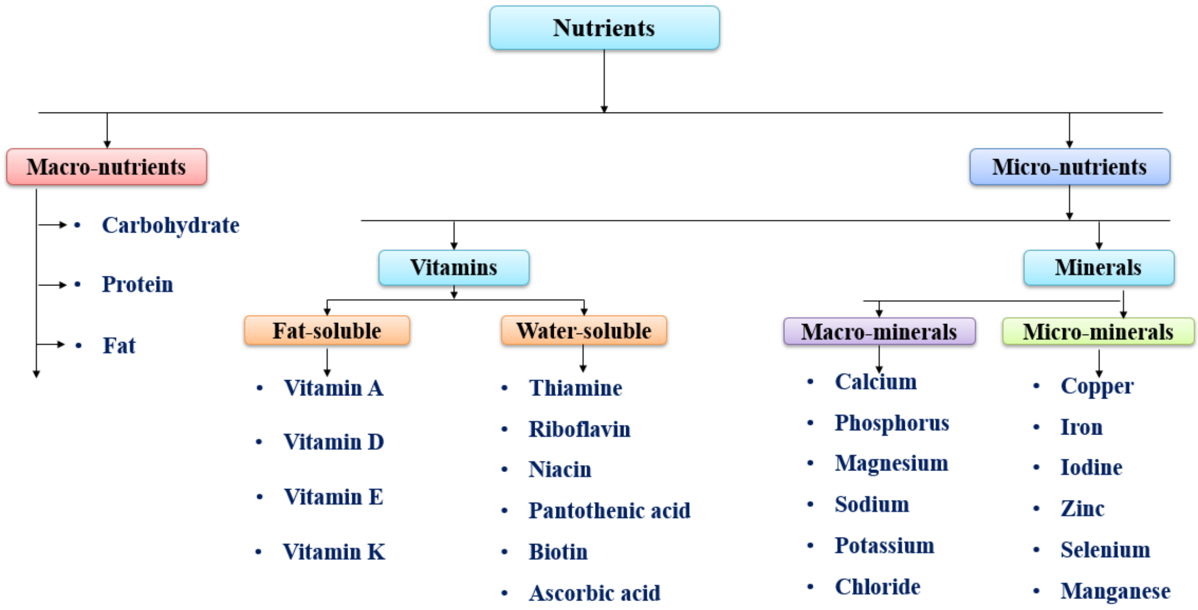

Fig: 1 Classification of nutrients

Physiological Role of Micronutrients in Broodstock Nutrition

I. Regulation of Enzyme Systems

Most of the reproductive processes are enzyme driven and required micronutrients, acts as a cofactor, stabilizer and structural component of the enzymes. Micronutrients regulate enzymes involved in steroid hormone biosynthesis. Such as zinc supplementation can influence enzymes such as 3β-hydroxysteroid dehydrogenase, crucial for estrogen and testosterone synthesis and Iron participates in cytochrome P450 enzyme systems that catalyze steroidogenesis.

II. Regulation of Hormone Synthesis

Reproductive hormones like estrogen, testosterone and progesterone are derived from cholesterol via enzyme driven pathway. Micronutrients ensure efficient cholesterol transport in to mitochondria and involved in activation of steroidogenic enzymes like aromatase, and cytochrome P450.

III. **Antioxidant Defence mechanism**

Reproductive tissues are highly susceptible to reproductive stress as the gonads contain high level of Polyunsaturated fatty acids and during rapid cell division generate reactive oxygen species (ROS). Micronutrients protect cell membrane integrity and sperm viability from oxidative stress and scavenge ROS species.

IV. **Facilitate Steroidogenesis, Vitellogenesis, Spermatogenesis**

Micronutrients promote major reproductive processes like steroidogenesis and vitellogenesis through formation of sex steroid hormones from cholesterol and protects fish oocytes from oxidative stress. Facilitate gonadal development and synthesis of reproductive hormones and improve sperm motility and viability.

V. **Interaction with Endocrine System (HPG)**

Micronutrients are integral to the regulation of the HPG axis in fish broodstock, influencing endocrine signaling at hypothalamic, pituitary, and gonadal levels. Through their roles in enzyme systems, hormone synthesis, antioxidant defense, and gene expression, they ensure proper reproductive function and offspring quality. Strategic micronutrient management is therefore essential for maximizing reproductive performance in aquaculture.

Role of Vitamins in Gonadal Development of fish

Vitamins are essential micronutrients that play a pivotal role in regulating reproductive physiology and gonadal development in fishes. Although required in small quantities, they are crucial for coordinating key biological processes such as cell differentiation, hormone synthesis, antioxidant protection, and gametogenesis. Vitamins influence both structural and functional aspects of the gonads.

Role of Fat-soluble Vitamins in Gonadal Development

I. **Vitamin A**

Vitamin A (VA) is stored as retinyl esters and converted to retinaldehyde and retinoic acid (RA) in tissues. RA is a key signalling molecule in fish gonads. In many vertebrates, RA triggers germ-cell meiosis. Mechanistically, RA regulates expression of genes like *cyp26a1* and *aldh1a2* (retinoid metabolism) and may modulate aromatase (*cyp19*) expression, linking to estrogen synthesis. Deficiency impairs germ-cell progression, while excess RA can disrupt normal differentiation. VA enhances steroidogenesis and vitellogenin production (Silva et al., 2022). Similarly, Okure et al., (2024) demonstrated that dietary vitamin A supplementation significantly enhances the reproductive performance of African catfish broodstock. This improvement was associated with elevated retinol deposition in eggs and increased ovarian estradiol concentrations, indicating stimulation of steroidogenesis and ovarian development.

II. Vitamin D

Dietary vitamin D_3 regulates gonadal function by enhancing aromatase-mediated estrogen synthesis, and its deficiency leads to reduced estradiol production, hormonal imbalance, and impaired reproductive development in both male and female gonads (Kinuta et al., 2000). In broodstock management, maintaining sufficient vitamin D supports follicle development and egg shell quality. Vitamin D_3 helps in oocyte maturation by inhibiting the TGF-β1 pathway, which normally keeps the egg inactive. It reduces natriuretic peptide precursor c (NPPC) and cyclic guanosine monophosphate (cGMP) levels involved in maintaining oocyte meiotic arrest, allowing the oocyte to resume meiosis and mature properly. It promotes granulosa cell growth by increasing cyclin-dependent kinase 4 (CDK4) and Cyclin D1 (cell division factors) and decreasing P21 (growth inhibitor) (Li et al., 2024).

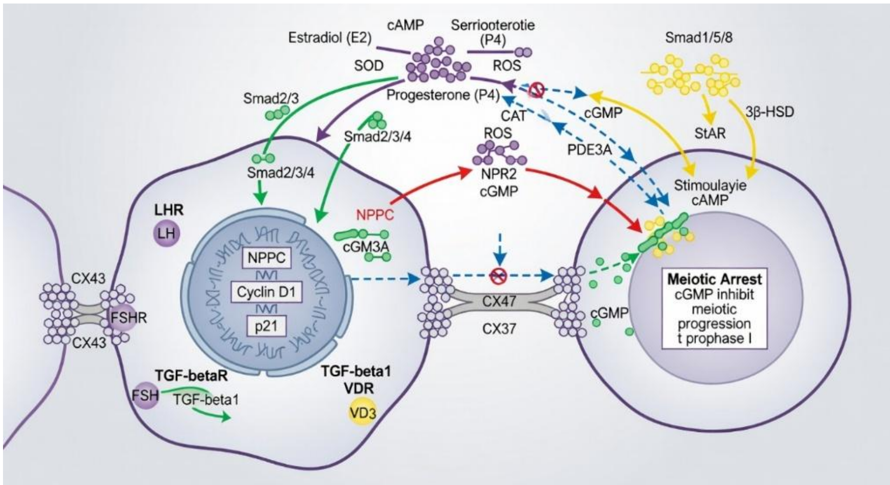

Fig 2: Molecular mechanism by which vitamin D3 promotes follicular development.

III. Vitamin E

Vitamin E (tocopherol) plays a crucial role in fish reproduction, particularly in gonadal development, oocyte maturation, and overall reproductive performance. Its primary function is as a lipid-soluble antioxidant (Roy et al., 2009). During oogenesis, vitamin E is actively mobilized from storage tissues such as the liver and muscle and transported to the ovary via lipoproteins. Vitamin E prevents lipid peroxidation in ovarian tissues and developing eggs, thereby preserving membrane integrity and ensuring proper functioning of reproductive cells. Deficiency in vitamin E leads to delayed ovarian development, reduced fertility, poor egg hatchability, and decreased larval survival due to oxidative damage (Palace et al 2006). Vitamin E is critical during early embryonic development, where antioxidant defense systems are not fully developed. The vitamin deposited in eggs acts as a protective agent against reactive oxygen species (ROS) (Bilguven et al., 2014).

IV. Vitamin K

Vitamin K (VK) plays a crucial role in gonadal development through two primary pathways: activation of the pregnane X receptor (PXR) and its function as a cofactor in γ-glutamyl carboxylation of vitamin K–dependent proteins (VKDPs). VK enhances testosterone production in fish, this effect is likely mediated via PXR activation, which controls genes involved in cholesterol metabolism and steroid hormone synthesis. Increased testosterone levels improve germ cell development, sperm quality, and reproductive performance. VK is also significant for gametogenesis through its role in VKDP activation. Proteins such as GAS6 and Protein S, which require VK-dependent carboxylation, are involved in cell survival, proliferation, and signaling within gonadal tissues (Beato et al., 2024).

Role of Water-soluble Vitamins in Gonadal Development

Vitamin	Role in Gonadal Development	Deficiency Effects	Reference
Vitamin C	Enhances gonadosomatic index (GSI) by promoting cellular proliferation and differentiation in gonadal tissues. Protects spermatozoa from oxidative damage by scavenging reactive oxygen species (ROS), thereby preserving membrane fluidity and mitochondrial function.	Increased oxidative damage, impaired steroidogenesis, and degeneration of germinal epithelium.	(Sarmento et al., 2017; Wang et al., 2023)
Vitamin B1 (Thiamine)	Supports embryogenesis, maternal nutrient transfer, and larval survival; essential for reproductive success.	Early Mortality Syndrome (EMS), poor egg quality, and increased larval mortality.	(Jaroszewska et al., 2009)
Vitamin B2 (Riboflavin)	Supports ovarian development, steroidogenesis, and energy supply required for gametogenesis.	Reduced growth and impaired reproductive performance.	(NRC, 2011)
Vitamin B3 (Niacin)	Plays a key role in energy metabolism necessary for gonadal maturation and reproductive processes.	Reduced fecundity and poor growth.	(Halver and Hardy, 2002)
Vitamin B6 (Pyridoxine)	Regulates hormone synthesis and is involved in proper gamete development.	Reduced fertility and abnormal embryogenesis.	(NRC, 2011)
Vitamin B9 (Folate)	Essential for oocyte development, DNA synthesis, embryonic cell division, and gonadal maturation.	Poor egg development, deformities, and reduced hatchability.	(Halver and Hardy, 2002)

Vitamin	Role in Gonadal Development	Deficiency Effects	Reference
Vitamin B12 (Cobalamin)	Crucial for cell proliferation in gonadal tissues and embryonic development.	Anemia, reduced growth, and reproductive failure.	(NRC, 2011)

Role of minerals in Gonadal Development of fish

Minerals play critical roles in fish reproduction, including oogenesis, spermatogenesis, vitellogenesis and steroidogenesis, largely via structural, enzymatic and endocrine functions. Macrominerals like Ca and P are central to eggshell formation (vitellogenin is Ca-bound) and energy metabolism, while microelements (Fe, Zn, Cu, Mn, Se, I, Co, etc.) support hemoglobin (O_2 transport), antioxidant enzymes (SOD, GPx), hormone synthesis and cell signalling(El-Fotoh et al., 2022). Deficiencies or excesses (e.g. low Mn in trout, or Cu exposure in marine fish) impair fecundity, egg size, fertility and embryo survival. Mechanistically, minerals serve as cofactors for steroidogenic enzymes (e.g. Fe-heme in cytochromes), regulate gene expression.

I. Zinc

Zinc is an essential trace mineral plays a crucial role in reproductive performance, gonadal development, and seed quality in fish broodstock. Acts as a cofactor in the synthesis and activity of reproductive hormones such as FSH, LH. These hormones control gamete formation and maturation (El-Fotoh et al., 2020). It is essential for cell division and genetic processes. It participates in DNA transcription, protein synthesis, and enzyme activation. Since gamete formation requires rapid cell division, zinc ensures the production of healthy and viable eggs and sperm (Untung et al., 2023). Supplementation has been shown to increase egg number, hatchability, sperm motility, and viability it enhances nutrient deposition in eggs and supports spermatogenesis, thereby improving fertilization success and larval survival (Kaliky et al., 2019). Zinc enhances spawning efficiency and seed production by improving metabolic activity and reproductive performance in broodstock (Gammanpila et al., 2007).

II. Selenium

Selenium (Se) is an essential trace element that plays a critical role in fish reproduction, particularly in gonadal development, through its involvement in antioxidant defense, endocrine regulation, gene expression, and maternal nutrient transfer. Structural component of selenoproteins such as glutathione peroxidase (GPx), which protects gonadal tissues from oxidative damage by reducing ROS and lipid peroxides. Developing gonads are metabolically active and therefore highly susceptible to oxidative stress, which can impair gametogenesis and cellular integrity (El-Dahhar et al., 2025). It plays a pivotal role in regulating steroidogenesis.

It enhances the expression of key steroidogenic genes such as *StAR*, *P450scc*, and *3β-HSD*, which are directly involved in the biosynthesis of sex steroids (Khorasaninasab et al., 2025). Selenium contributes to Vitellogenesis, the process of yolk protein synthesis by upregulating genes associated with yolk protein synthesis, including *vitellogenin (vtg)* and zona pellucida proteins (*zp2*) (Khorasaninasab et al., 2025)

Table 1: Dietary requirement of the trace minerals in fishes

Trace Mineral	Model Fish	Requirement Level (mg/kg diet)
Iron (Fe)	Atlantic salmon	33–100
	Channel catfish	30
	Red sea bream	150
	Eel	170
Copper (Cu)	Rainbow trout and carp	3
	Channel catfish	5
	Atlantic salmon	5
Manganese (Mn)	Channel catfish	2.4
	Rainbow trout and carp	12–13
	Atlantic salmon	7.5–10.5
	Juvenile stage fish	2–15
	Salmon and trout broodstock	>30
Zinc (Zn)	Rainbow trout and common carp	15–30
	Atlantic salmon	37–67
	Channel catfish	20
	Blue tilapia	20
	Red drum	20–25
Iodine (I)	Atlantic salmon	4.5
Selenium (Se)	Rainbow trout	0.15–0.38
	Channel catfish	0.25
	Gibel carp	1.18
Cobalt (Co)	General fish species	0.05–1.0

Note: Adapted from Chanda et al.,2015, reflecting Dietary requirement of the trace minerals in different fish species.

Interaction Between Micronutrients and Other Nutrients

Studies have shown that when micronutrients were applied in combination with other micronutrients have shown better outcomes compared to single micronutrients (Schoendorfer et al.,2012). Understanding how they are interacting with each other is very much essential while formulating the diet.

Calcium and Vitamin D

Vitamin D enhances intestinal calcium absorption by stimulating the synthesis of calcium-binding proteins calbindin, which actively transport calcium across intestinal cells. In the absence of adequate vitamin D, calcium absorption becomes inefficient, even if dietary calcium intake is sufficient.

Iron and Vitamin C

Vitamin C reduces ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), a form that is more soluble and readily absorbed in the intestine. This chemical transformation improves iron bioavailability, especially in diets containing inhibitors.

Iron Interaction

Copper is required for proper iron transport and hemoglobin formation; its deficiency can cause anemia despite adequate iron levels. High levels of zinc and iron compete for absorption, reducing their bioavailability. Vitamin A supports iron mobilization and red blood cell production, making it essential for effective iron utilization.

Zinc Interaction

Zinc is essential for vitamin A metabolism by regulating retinol-binding protein and enzymatic conversion. Its deficiency impairs vitamin A transport and utilization. Zinc absorption is enhanced by dietary protein and amino acids, while excessive zinc can inhibit copper absorption, potentially causing copper deficiency.

Folate and Vitamin B12

Folate and vitamin B12 function together in one-carbon metabolism and methylation reactions, which are essential for DNA synthesis, cell division, and neurological function. Vitamin B12 is required to activate folate; in its absence, folate becomes metabolically trapped in an inactive form, leading to a functional folate deficiency.

Recent advances and research trends in broodstock micronutrient nutrition

Recent Trends	Concept	Research Findings	References
Use of nano-minerals	• Transition from conventional mineral salts to nano-form minerals, particularly selenium nanoparticles (SeNPs). • Provide higher bioavailability, improved tissue	Nano-selenium significantly increased selenium deposition in broodstock tissues (liver and ovary) and progeny. This resulted in enhanced antioxidant defense through increased glutathione peroxidase (GPx) activity and reduced malondialdehyde (MDA) levels. Additionally, key genes involved in steroidogenesis (*star,*	(El-Dahhar et al., 2024; Khorasaninasab et al., 2025; Naiel et al., 2023)

Recent Trends	Concept	Research Findings	References
	retention, and enhanced biological activity at lower inclusion levels.	*P450scc, 3β-hsd*) and vitellogenesis (*vtg, zp2*) were upregulated. Improvements were observed in spawning frequency, fecundity, fertilization rate, and hatchability.	
Chelated minerals	• Use of chelated (organic) mineral forms instead of inorganic salts. • Exhibit superior bioavailability and enhance feed efficiency, immune response, and disease resistance.	The source of selenium influenced tissue distribution: sodium selenite was more effective in increasing blood selenium concentration and selenoenzyme activity, whereas selenized yeast showed greater efficiency in selenium deposition in roe and larvae.	(Jovanović et al., 2024)
Nutrigenomics and reproductive gene expression	• Focus on how nutrients regulate gene expression, epigenetic modifications, and reproductive pathways. • Key genes include *cyp19a1a* (aromatase), *StAR*, and *cyp11a1* (P450scc).	Selenium nanoparticles (SeNPs) upregulated key reproductive genes associated with steroidogenesis (*star, P450scc, 3β-hsd*) and vitellogenesis (*vtg, zp2*), indicating improved molecular regulation of gonadal development and reproductive performance.	(Khorasaninasab et al., 2025)

Conclusion

Micronutrients play a fundamental and multifaceted role in broodstock nutrition, directly influencing reproductive efficiency and gonadal development in fishes. They act as essential cofactors in enzymatic systems, regulators of endocrine function, and structural components in key physiological processes such as gametogenesis, vitellogenesis, and steroidogenesis. Minerals like zinc, selenium, iron, and manganese, along with vitamins such as A, D, E, and B-complex, contribute to hormonal balance within the hypothalamic–pituitary–gonadal (HPG) axis, ensuring proper maturation of gonads and high-quality gamete production. Adequate micronutrient supply enhances fecundity, fertilization rates, hatchability, and larval viability, while deficiencies can lead to impaired gonadal development, oxidative stress, endocrine disruption, and poor reproductive outcomes.

Furthermore, the interactions among micronutrients, including synergistic and antagonistic effects, highlight the complexity of their roles in fish physiology and the need for balanced dietary formulations. Despite their critical importance, species-specific requirements, bioavailability, and toxicity thresholds remain insufficiently understood, particularly during different reproductive stages. Therefore, optimizing micronutrient nutrition in broodstock diets is essential for sustainable aquaculture practices. Future research focusing on precise requirement levels, improved nutrient delivery systems, and non-invasive assessment methods will further enhance reproductive performance and support the production of high-quality offspring in fish culture systems.

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