Agr. Nat. Resour. 59 (2025) xxx–xxx

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Impacts of α-tocopherol (vitamin E) and L-ascorbic acid (vitamin C) on reproductive performance in female yellow mystus (*Hemibagrus spilopterus*)

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Article Info

Article history:
Received 16 July 2024
Revised 11 December 2024
Accepted 17 December 2024
Available online 28 February 2025

Keywords:
Estrogen,
Fecundity,
Growth,
Progesterone,
Synergistic effect

Abstract

Importance of the work: The success of breeding in fish is influenced by egg quality, which can be enhanced through vitamin supplementation in the diet.
Objectives: To evaluate the effects of vitamins C and E, both individually and in combination, on the growth, reproductive performance and sex hormone levels in female yellow mystus.
Materials and Methods: Female yellow mystus aged 1 yr were fed a commercial diet with 32% protein, supplemented with: 1) no vitamins (the control); 2) vitamin C at 1,000 mg/kg; 3) vitamin E at 500 mg/kg; or 4) a combination of 1,000 mg/kg vitamin C + 500 mg/kg vitamin E. Data were collected on growth, ovary weight, gonadosomatic index (GSI), fecundity and hormone levels after 30 d and 60 d of treatment, while the fertilization rate and the hatching rate were evaluated only at 60 d.
Results: There were synergistic effects from adding vitamin C and E. Fish fed the combined vitamin supplementation showed improved GSI, ovary weight, estrogen and progesterone levels. Vitamin C supplementation alone (at 30 d) also increased fecundity. By day 60, all vitamin-supplemented groups showed improved fecundity, fertilization and hatching rates.
Main finding: The combination of vitamin C and vitamin E significantly improved reproductive parameters in yellow mystus, consisting of fecundity, fertilization rates, hatching rates and sex hormones, offering potential for enhanced fry production.

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https://doi.org/10.34044/j.anres.2025.59.1.14

Introduction

Improving nutritional quality, including the use of feed supplements, has been shown to enhance both growth and reproductive performance in fish (Gioacchini et al., 2023). The yellow mystus (*Hemibagrus spilopterus* Ng & Rainboth, 1999), is a freshwater fish species with high nutritional and economic value in Southeast Asia, where it is known for its high-quality meat that is rich in protein and omega-3 fatty acids and is highly prized, particularly among health-conscious consumers (Aryani et al., 2002; Mesomya et al., 2002; Adebiyi et al., 2011). Despite its popularity, raising this species using aquaculture remains limited due to an insufficient supply of fingerlings (Durand et al., 2022).

Vitamin supplements, particularly vitamins C and E, are essential for enhancing aquaculture productivity by supporting the growth, maintenance and reproductive processes in fish (Gasco et al., 2018; Hernandez de-Dios et al., 2022). Vitamin C, crucial for fish reproductive organ function, has been shown to enhance gonadal development and egg production in species, including rainbow trout (*Oncorhynchus mykiss*; Hwang et al., 2002) and goldfish (*Carassius auratus*; James and Vasudhevan, 2011). Vitamin E, a lipid-soluble antioxidant, improves ovary health and egg quality in various fish species, influencing reproductive hormone synthesis and gonadal development (Fernández-Palacios et al., 1998; James et al., 2008; Hamre, 2011; Nagarajan et al., 2011; Miller et al., 2012; Huang and Liu, 2021; Shaha et al., 2022). The combined supplementation of vitamins C and E synergistically enhances reproductive performance in fish, increasing fecundity by improving overall health and gonadal function (Izquierdo et al., 2001; Nguyen et al., 2012; Lebold et al., 2013; Gao et al., 2014; Hernández et al., 2019). Therefore, supplementation with vitamins C and E is also expected to enhance reproductive efficiency in yellow mystus.

Other related research (Somnuek et al., 2023) revealed that male yellow mystus fed diets supplemented with vitamins C and E showed improved semen quality and sex hormone levels, suggesting that such supplementation may also improve reproductive outcomes in female yellow mystus, despite the current lack of scientific evidence in this area.

Therefore, the current study aimed to explore the effects of vitamin C and vitamin E supplementation on the growth, fecundity and sex hormones processes in female yellow mystus. The results could potentially enhance hatchery production of yellow mystus fingerlings and, in turn, contribute to the growth of aquaculture production for this species.

Materials and Methods

Preparation of animals

In total, 360 female yellow mystus, all aged 1 yr were sampled, with an initial average weight (± SD) of 214.41 ± 7.74 g. The fish were acclimated for 2 wk in cages positioned within an earthen pond, with weekly water changes. They were fed floating commercial pellet feed containing 32% protein, administered twice daily at a rate of 3% of their body weight during the acclimation period. Following this, the fish were equally distributed across 12 netted cages, each measuring 2 m × 2 m × 1.5 m (30 fish per cage).

Feed preparation

Commercial floating pellets containing 32% protein were used as the basal feed. The feed treatments were prepared following the methodology of Somnuek et al. (2023). For the vitamin C-supplemented diet, 1,000 mg/kg of vitamin C (L-ascorbic acid) was dissolved in 5% weight per volume (w/v) water. The resulting vitamin C solution was sprayed onto the experimental diet and then coated with an oil layer. Similarly, the vitamin E-supplemented diet was prepared by dissolving 500 mg/kg of vitamin E (α-tocopherol acetate) sourced from Sigma (St. Louis, MO, USA) in vegetable oil at a concentration of 5% w/v. Then, this vitamin E solution was sprayed onto the experimental diet. For the control diet, equivalent amounts of water and vegetable oil to those used in the experimental diets were added, ensuring consistent oil and moisture levels across all diets at a rate of 5% v/w. All diets were stored at 4°C in a refrigerator. The nutritional composition of the experimental diets was analyzed using the methodology outlined by the Association of Official Analytical Chemists (2016).

Experimental design

The experiment was conducted using a completely randomized design, with four treatments. Each treatment was replicated three times, with each replication consisting of 30 female yellow mystus, totaling 90 fish per treatment group. The experimental fish were fed one of the following diets: 1) feed without vitamin supplementation (the control); 2) feed supplemented with vitamin C at 1,000 mg/kg (Vit C); 3) feed supplemented with vitamin E at 500 mg/kg (Vit E); or 4) feed supplemented with both vitamin C at 1,000 mg/kg and vitamin E at 500 mg/kg (Vit C + Vit E). During the study, the fish were fed twice daily (0900 hours and 1600 hours) with commercial diets containing 32% crude protein, at a rate of 5% of their biomass weight per day. Fish growth was measured every 30 days throughout the 60 d of the experiment.

Data collection

Growth performance

Within each experimental group, 5 fish out of 30 per replication were randomly selected for measurement, focusing on minimizing stress on the fish during the procedure while adhering to logistic constraints, as well as methodologies from other studies (Ashley, 2007; Sneddon et al., 2016). The selected fish underwent regular measurements of weight and length every 30 d. The daily weight gain (DWG) and specific growth rate (SGR) parameters were calculated using Equations 1 and 2, as outlined by Rahman and Arifuzzaman (2021):

$$\text{DWG} = \frac{\text{Final weight - Initial weight}}{\text{Number of rearing days}} \quad (1)$$

$$\text{SGR} = \frac{[\text{Ln (final weight) - Ln (Initial weight)} \times 100}{\text{Number of rearing days}} \quad (2)$$

where all weights are in grams.

On days 30 and 60, five fish per replication were randomly selected for analysis of growth, fecundity and sex hormones.

Analysis of reproductive performance

The ovaries were accessed by opening the stomach, as the ovaries are located beneath the gastrointestinal tract. Prior to data recording, any residual blood was carefully removed. The ovary weight and body weight of each individual were measured to the nearest milligram. The gonadosomatic index (GSI) was calculated using Equation 3:

$$\text{GSI} = \frac{\text{Gonad weight (g)} \times 100}{\text{Body weight (g)}} \quad (3)$$

where all weights are in grams.

Fecundity was assessed by collecting subsamples of eggs (each 1 g) from each ovary and placing them in a jar with a 1% formalin solution, followed by counting the number of eggs. Fecundity was calculated using Equation 4:

$$\text{Fecundity} = \frac{\text{Mean number of eggs in subsample} \times \text{Weight of ovary}}{\text{Weight of subsample}} \quad (4)$$

where all weights are in grams.

The ovaries were excised, the ovulated eggs removed and then thoroughly mixed with fresh sperm. During agitation for 2 min, three 5 mL samples of fertilized eggs were drawn into a standard micropipette and transferred into three clean Petri dishes. Each Petri dish was placed on a black background and the eggs were counted under a stereoscope. Transparent eggs were considered fertilized eggs while opaque eggs were counted as unfertilized eggs based on a mean count from the three samples, which were subsequently used as the number of fertilized and unfertilized eggs, based on calculations using Equations 5 and 6:

$$\text{Egg quality} = \frac{\text{Number of normal eggs} \times 100}{\text{Total number of eggs}} \quad (5)$$

$$\text{Fertilization rate} = \frac{\text{Number of fertilized eggs} \times 100}{\text{Total number of eggs (fertilized + unfertilized)}} \quad (6)$$

After 48 hr, the number of hatched eggs (fish larvae) was counted and hatching rates were calculated using Equation 7:

$$\text{Hatching rate} = \frac{\text{Number of eggs hatched x 100}}{\text{Total number of fertilized eggs}} \quad (7)$$

The water conditions were: water temperature range = 25−30°C; pH range = 6.5−7.5; dissolved oxygen = >4–5 mg/L; light intensity range = 200−500 lux; and water circulation speed = 0.2−0.4 L/s. The procedures followed the methodologies described by Bobe and Labbé (2010), Rahman et al. (2023) and Migaud et al. (2013) and included both visual assessment and microscopic examination.

Analysis of sex hormones

Blood samples were collected on days 30 and 60 from five females per replication. The samples were stored in an ice flask and transported to the laboratory within 6 hr. Next, they were each centrifuged at 3,000×*g* for 15 min and the serum fraction was collected in a 1 mL plastic tube and stored at -20°C. The estrogen and progesterone levels were detected using competitive enzyme-linked immunosorbent assay (ELISA) with a microplate reader at an absorbance of 405 nm. The ELISA kit used had the lowest reading of 0.0023 ng/mL.

Water quality

Water samples were collected weekly at 0600 hours and 1500 hours. The levels of DO, pH and temperature were measured using a DO meter (YSI model 52), a pH meter (YSI model pH100) and a thermometer (HI93150), respectively, all sourced from Hanna Instruments (Woonsocket, RI, USA). Total ammonia was determined using the phenate method, as described by Boyd (1990).

Statistical analysis

Data on growth, fecundity, eggs quality, fertilization rate, hatchability rate and sex hormones levels were tested for normal distributions using the Shapiro-Wilk test. Data with non-normal distribution were transformed as necessary. One-way analysis of variance was used, with means compared using Duncan's new multiple-range test at the 95% confidence level ($p < 0.05$). Data were presented as mean ± SD values.

Ethics statement

The research procedures and methods were carried out under the supervision of the Sub-committee on Animal Husbandry and Use at Rajamangala University of Technology Isan, Thailand. A committee overseeing the ethical aspects of laboratory animal use granted permission (No. U1-04362-2559).

Results

Growth rate

The growth parameters of the female yellow mystus fed with vitamin C, vitamin E or a combination of vitamin C + vitamin E supplements did not differ significantly on day 30. However, on day 60 of feeding, the group receiving the combined vitamin C + vitamin E diet had a significantly higher final weight than the groups fed only vitamin C or only vitamin E and the control group (Table 1). Other growth parameters (DWG and SGR) were not significantly different among treatments.

Reproductive performance

Gonadosomatic index and ovary weight

The GSI and ovary weight of the female yellow mystus fed diets supplemented with vitamin C + vitamin E for 30 d and 60 d were significantly higher than for the control group. On day 30, the combination of vitamin C and vitamin E significantly enhanced the GSI (5.00 ± 0.72%) and ovary weights (10.98 ± 1.67 g), compared to the control without vitamins (GSI = 3.06 ± 0.50% and ovary weight = 6.68 ± 1.15 g). After 60 d of supplementation, the GSI and ovary weight (5.00 ± 0.72% and 10.98 ± 1.67 g, respectively) of the group receiving both vitamins remained significantly

Table 1 Growth performance (mean ± SD) of female yellow mystus fish fed diets supplemented with vitamin C, vitamin E or vitamin C + vitamin E

Parameter	Vitamin supplementation				*p* value
	No vitamins	Vitamin C	Vitamin E	Vitamins C + E	
Day 30					
Initial length (cm)	26.66 ± 4.61	26.33 ± 1.52	25.66 ± 2.22	26.48 ± 0.57	0.969
Final length (cm)	29.98 ± 1.43	30.78 ± 1.09	29.61 ± 2.21	30.71 ± 2.56	0.852
Initial weight (g)	212.33 ± 10.69	213.66 ± 10.21	214.33 ± 6.65	217.33 ± 6.80	0.911
Final weight (g)	224.33 ± 4.04	229.66 ± 4.51	226.33 ± 4.72	230.33 ± 4.93	0.386
Daily weight gain (g/d)	0.40 ± 0.32	0.53 ± 0.40	0.40 ± 0.12	0.43 ± 0.07	0.914
Specific growth rate (%/d)	0.18 ± 0.14	0.24 ± 0.18	0.18 ± 0.05	0.19 ± 0.03	0.922
Day 60					
Final length (cm)	32.00 ± 1.00	33.33 ± 0.57	32.00 ± 2.00	32.33 ± 2.08	0.696
Final weight (g)	238.58 ± 2.72^{a}	241.31 ± 1.99^{a}	241.23 ± 0.34^{a}	247.29 ± 3.66^{b}	0.015
Daily weight gain (g/d)	0.44 ± 0.15	0.46 ± 0.14	0.45 ± 0.11	0.50 ± 0.09	0.936
Specific growth rate (%/d)	0.19 ± 0.07	0.20 ± 0.07	0.20 ± 0.05	0.21 ± 0.04	0.975

Row values (mean ± SD) with different lowercase superscripts are significantly ($p < 0.05$) different.

higher than those of the control group (GSI = 3.06 ± 0.50% and ovary weight = 6.68 ± 1.15 g). However, although no significant differences were observed between the GSI and ovary weight of the groups supplemented with vitamin C alone, vitamin E alone and the control group (Table 2), there was a clearly increasing trend for the groups receiving either vitamin C or vitamin E.

Fecundity

On day 30, the group supplemented with both vitamin C and vitamin E had significantly higher fecundity (30,650 ± 6,464 eggs) compared to the groups receiving only vitamin C (22,880 ± 1,091 eggs), only vitamin E (19,051 ± 3,154 eggs) and the control group (13,660 ± 3,010 eggs). By day 60, the groups receiving vitamin E alone (31,138 ± 4,038 eggs) and the combination of vitamin C + vitamin E (35,029 ± 2,715 eggs) continued to show significantly higher fecundity compared to the control group (20,240 ± 5,986 eggs).

Fertilization and hatching rates

On both day 30 and day 60, the fertilization and hatching rates of the groups supplemented with vitamin C, vitamin E, or with the combination of both vitamins were significantly higher than those observed in the control group (Table 2).

Estrogen and progesterone levels

On day 30, the fish fed diets supplemented with either vitamin C or vitamin E alone tended to have higher estrogen and progesterone levels than the control group, although these differences were not statistically significant. However, the group receiving both vitamin C and vitamin E in the diets had a significantly higher serum estrogen level than the other groups. However, on day 60, there were no significant differences in the estrogen levels across the treatments, although an increasing trend was noticeable. The results for the progesterone levels on day 60 were similar to those on day 30, with the group receiving a combination of vitamin C and vitamin E having higher progesterone levels than the other groups, including the control group (Table 3).

Water quality

The water quality in the experimental ponds met the standards set by American Public Health Association (2017). The DO levels were in the range 4.15–4.80 mg/L, while temperatures were in the range 28.20–32.20°C, while the pH levels were consistently in the range 6.50–7.30 and ammonia (NH_3) concentrations were 0.001 mg/L. The values for these parameters indicated that the water conditions were suitable for fish rearing throughout the experimental period.

Table 2 Gonadosomatic index, ovary weight, fecundity, fertilization rate and hatching rate of yellow mystus fed diets supplemented with vitamin C, vitamin E and vitamin C + vitamin E

Reproductive performance	Vitamin supplementation				*p* value
	No vitamins	Vitamin C	Vitamin E	Vitamins C + E	
Day 30					
Gonadosomatic index	3.06 ± 0.50[a]	4.11 ± 0.62[ab]	4.03 ± 0.54[ab]	5.00 ± 0.72[b]	0.029
Ovary weight (g)	6.68 ± 1.15[a]	9.06 ± 1.16[ab]	8.76 ± 0.95[ab]	10.98 ± 1.67[b]	0.021
Fecundity (eggs)	13,660 ± 3,010[a]	22,880 ± 1,091[b]	19,051 ± 3,154[ab]	30,650 ± 6,464[c]	0.005
Day 60					
Gonadosomatic index)	3.24 ± 0.74[a]	4.17 ± 0.65[ab]	4.08 ± 0.44[ab]	4.72 ± 0.31[b]	0.048
Ovary weight (g)	7.47 ± 1.62[a]	9.66 ± 1.51[ab]	9.46 ± 0.99[ab]	11.16 ± 0.83[b]	0.047
Fecundity (eggs)	20,240 ± 5,986[a]	28,066 ± 7,009[ab]	31,138 ± 4,038[b]	35,029 ± 2,715[b]	0.043
Fertilization rate (%)	68.36 ± 4.05[a]	78.86 ± 1.97[b]	76.90 ± 3.63[b]	77.80 ± 2.02[b]	0.011
Hatching rate (%)	57.26 ± 5.08[a]	68.53 ± 3.65[b]	70.23 ± 2.18[b]	69.63 ± 1.68[b]	0.005

Row values (mean ± SD) with different lowercase superscripts are significantly ($p < 0.05$) different.

Table 3 Estrogen and progesterone levels in female yellow mystus fed diets supplemented with vitamin C, vitamin E, or vitamin C + vitamin E

Vitamin supplementation	Estrogen hormone level (ng/mL)		Progesterone hormone level (ng/mL)	
	Day 30	Day 60	Day 30	Day 60
No vitamins	0.105 ± 0.057[a]	0.271 ± 0.047[a]	0.133 ± 0.054[a]	0.244 ± 0.189[a]
Vitamin C	0.165 ± 0.097[a]	0.449 ± 0.114[a]	0.226 ± 0.166[a]	0.239 ± 0.070[a]
Vitamin E	0.166 ± 0.044[a]	0.414 ± 0.116[a]	0.225 ± 0.092[a]	0.197 ± 0.114[a]
Vitamins C + E	0.443 ± 0.077[b]	0.467 ± 0.182[a]	0.756 ± 0.228[b]	0.619 ± 0.258[b]
p value	0.002	0.283	0.004	0.046

Column values (mean ± SD) with different lowercase superscripts are significantly ($p < 0.05$) different.

Discussion

Effects of vitamin supplementation on growth performance

Although growth was not a primary objective in the current study, growth enhancement was observed in the group supplemented with a combination of vitamin C and E for 60 d, clearly demonstrating their synergistic effects on growth. Similar growth enhancement, along with improved reproductive performances, has been reported due to the synergy of these vitamins in species such as Nile tilapia (*Oreochromis niloticus*; Nascimento et al., 2014) and blue gourami (*Trichopodus trichopterus*; Vasudhevan et al., 2017). However, the growth enhancement observed in the current study could have been confounded by gonad weight, as the treatment also significantly increased ovary weight, which comprised 3–5% of the body weight. This was supported by other research findings in male yellow mystus that had received similar treatments but did not show growth enhancement, despite an increase in the GSI (Somnuek et al., 2023). In males, gonad weight is relatively small (0.29–0.46% of body weight) and therefore does not greatly confound overall body weight.

Effects of vitamin supplementations on reproductive performance

Five parameters were assessed to evaluate the reproductive performance of yellow mystus in response to the vitamin supplementation in the diets.

GSI is a parameter used to assess the reproductive performance of living organisms, particularly concerning the development and maturation of gonads. In fish, it has been found to highly correlate with the expression of the vitellogenin genes in *Notopterus notopterus* and *Anematichthys armatus* (Panprommin et al., 2015). In the current study, there were synergistic effects from the combination of vitamin C and E on GSI enhancement, which implied that these vitamins, by certain mechanisms, facilitated better vitellogenesis. These findings aligned with the significant increase in estrogen in the group receiving the same supplements (vitamins C + E) for 30 d. Estrogen is primarily produced by the granulosa cells of developing follicles and directly controls vitellogenesis (Devlin and Nagahama, 2002; Guiguen et al., 2010; Pang and Thomas, 2010; Aizen and Thomas, 2015). In addition, the synergistic effects of these vitamins was clearly demonstrated by the significant increase in the progesterone level in the group that received a combination of vitamin C and E. Progesterone plays crucial roles during the later maturation stages of oocytes (Nagahama and Yamashita, 2008; Thomas, 2012; Aizen et al., 2018). In addition, there were positive effects of these vitamins on the reproductive performance of yellow mystus on fecundity, which significantly increased in fish that had received either vitamin C or vitamin E or their combinations in both the 30 d and 60 d treatments.

Although the precise mechanisms remain unclear, these vitamins are known to enhance antioxidant protection and foster a cellular environment conducive to the development of reproductive cells (Andrade et al., 2007; Khara et al., 2016; Keogh et al., 2017). The interaction between these vitamins is driven by two key mechanisms: simultaneous protection against oxidation in both aqueous and lipid environments; and the regeneration of vitamin E from its radicals by vitamin C (Ortuño et al., 2001). The results of other studies support the potential of vitamins C and E to improve reproductive cell quality and reproductive health across various species (Lee and Dabrowski, 2003; Ortuño et al., 2003; Chen et al., 2004; Gao et al., 2014). For example, in Nile tilapia, a dietary inclusion of 400 mg/kg of these vitamins was identified as optimal for growth and reproductive success (Nascimento et al., 2014). Recent research indicated that a nanocomposite of vitamins C and E, delivered with chitosan, significantly improved the growth performance and immune response of this species (Sherif et al., 2024). Similarly, in blue gourami, a diet containing 300 mg/kg of vitamin E and 200 mg/kg of vitamin C was recommended to achieve optimal growth and reproduction (Vasudhevan et al., 2017). A related study (Somnuek et al., 2023) to the current one showed that vitamins C + E supplementation improved the reproductive performance of male yellow mystus. Furthermore, various studies that have targeted different reproductive performance traits also reported similar synergistic effects of vitamins C and E: improved fertilization rates that contributed to successful egg hatching in koi carp (*Cyprinus carpio*; Betsy et al., 2021); improved egg fertility in Japanese flounder (*Paralichthys olivaceus*; Gao et al., 2014); increased fertilization rates and hatching success in African catfish (*Clarias gariepinus*; Ovissipour et al., 2014); and improved hatching rates and larval survival of gilthead sea bream (*Sparus aurata*; Izquierdo et al., 2019).

Notably, the effects of vitamins C or E alone cannot be overlooked, since there was a positive trend toward increment of the GSI despite not being significantly different from the control. In addition, fertilization rates and hatching rates increased in the groups that had received vitamin C, vitamin E, or their combination, without the synergistic effects.

It is well known that these individual vitamins are beneficial for fish reproduction due to their antioxidant properties, which can improve overall fish health and may positively affect egg fertility and quality. For example, studies have indicated that supplementing vitamin C can enhance reproductive efficiency in fish, including increasing egg fertility, with one study in Nile tilapia showing that vitamin C supplementation in feed improved reproductive performance and egg fertility (Fathi et al., 2013). Supplementing vitamin E in feed has also been reported to positively impact egg fertility and egg quality in rainbow trout (Bilguven, 2014), to improve egg fertility in gilthead sea bream (Tocher et al., 2002) and improve the GSI, fertilization rate, fecundity and hatching rate of butter catfish (*Ompok bimaculatus*; Mandal et al., 2024).

Notably, the specific effects of vitamin C and vitamin E can vary depending on fish species, dosage, duration of supplementation and environmental factors (Rahimnejad et al., 2021). Thus, further research is necessary to gain a comprehensive understanding of their effects on different fish species and reproductive systems (Gao et al., 2014; Khara et al., 2016).

Effects of vitamin supplementation on estrogen and progesterone

Based on the current results, supplementation for 30 d with vitamin C combined with vitamin E increased the estrogen levels in female yellow mystus, while progesterone production was elevated with the same diet after both 30 d and 60 d. These results aligned with a related study on male yellow mystus, where a combination of vitamin C and E resulted in significantly elevated levels of the sex hormone testosterone (Somnuek et al., 2023). Although the exact mechanisms through which vitamins C and E influence steroidogenesis are still being explored, both vitamins are known to support steroid hormone production through antioxidant properties and direct involvement in the synthesis of key hormones, with evidence suggesting that vitamin C may help to maintain normal functioning of the endocrine system, including the regulation of estrogen hormones (Hayati et al., 2022). In Nile tilapia, vitamin C supplementation has been shown to affect the expression of genes involved in the synthesis of steroid hormones, including estrogen (Abdel-Tawwab et al., 2008). Additionally, a study on hybrid red tilapia indicated that vitamin E supplementation may influence the expression of genes involved in androgen hormone synthesis, potentially impacting progesterone levels (Griesh et al., 2024).

Conclusion

This study was the first to examine the effects of vitamin C, vitamin E and their combination on fecundity and sex hormones in female yellow mystus. Supplementation for 30 d with vitamin C, vitamin E, or both, significantly impacted female hormone levels. Vitamin C enhanced fecundity. The combined supplementation of both vitamins positively impacted the GSI, fecundity and the levels of estrogen and progesterone. Extending the supplementation period to 60 d further enhanced fish weight and sex hormones in yellow mystus breeders. Vitamin C and vitamin E increased fish fecundity, with the highest impact resulting from a combination of the two vitamins, which influenced fish weight, GSI, ovary weight, fecundity and progesterone levels.

Based on these findings, dietary supplementation with 1,000 mg/kg of vitamin C and 500 mg/kg of vitamin E for 30–60 d could be recommended to enhance the GSI, ovary weight, fecundity, fertilizing rates and hatching rates in female yellow mystus. This intervention should improve the fry produced by increasing fecundity and improving fertilization rates and hatching success. However, future research should investigate varying dosages of the combined vitamins to optimize both efficacy and cost. Additionally, experiments should extend beyond the breeding season.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The Faculty of Agriculture and Technology at Rajamangala University of Technology Isan, Surin campus provided funding. Additional assistance and support were provided by the Fisheries Laboratory at Rajamangala University of Technology Isan, Surin campus, as well as the Animal Science Laboratory at the Faculty of Agriculture and Agricultural-Industry, Surindra Rajabhat University.

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