Impacts of Feed Additives, Prebiotics and Vitamin E on Growth and Reproductive Performance of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758)

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ABSTRACT

In this study, the effects of prebiotics (mannan oligosaccharides, MOS) and vitamin E (VitE) on growth, reproductive capacity, and feed utilization of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) broodstock were investigated. Four experimental diets, namely a control (no supplementation), with 1% MOS, with 1% VitE, or with a combination of 1% MOS and 1% VitE, were each administered to three randomly selected cages of experimental fish over a 120-day period. The results revealed that, given alone, both 1% MOS and 1% VitE had a positive effect on the growth, feed conversion ratio (FCR), and survival rates of Nile tilapia, while only MOS increased villi length. A synergistic effect of MOS and VitE was evident for body weight, length, and average daily weight gain. Interestingly, for reproductive traits, both MOS and VitE enhanced sperm concentration and gonadosomatic indices (GSI) in both sexes, with VitE having a greater effect than MOS; a synergistic effect was also observed. However, no such effect was observed on sperm viability, even though MOS and VitE alone did increase sperm viability. This study also demonstrated the positive impact of MOS on enhancing progesterone and estradiol levels in female Nile tilapia broodstock, with these effects being more pronounced than the response to VitE supplementation and showing significant synergistic effects. For testosterone, the enhancing effect of either MOS or MOS+VitE supplementation was more efficient than supplementation with 1% VitE alone. This study suggests further exploration of 1% MOS+1% VitE as a feed supplement to enhance the reproductive performance of Nile tilapia broodstock.

Keywords: Growth performance, Mannan oligosaccharide, Nile tilapia, Prebiotic, Reproductive performance, Vitamin E

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is an economically important fish. It boasts significant annual production among freshwater species and is extensively cultivated across various countries, particularly in Africa, the Americas, and Asia (Arumugam *et al*., 2023). This fish species is favored for its rapid growth and ease of cultivation, making it highly desirable for farmers. Furthermore, tilapia enjoys widespread consumer acceptance. In 2017, Thailand's total tilapia production exceeded 185,000 t (Department of Fisheries, 2018), and was valued at over 6,000 million baht (1,721.42 million USD). In the commercial cultivation of Nile tilapia, achieving a high growth rate, disease resistance, and a high survival rate are essential (Tiengtam *et al*., 2015). Mannan oligosaccharides (MOS)

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are prebiotics derived from the macromolecule glucomannan, with a structure akin to cellulose in the animal intestinal mucosa. These structures, similar to the intestinal mucosa of tilapia, consist of columnar epithelial cells with a linear-like structure. Microvilli, responsible for nutrient absorption and increased food-contact surface area, further contribute to efficient nutrient absorption and aid in digestion (Connolly *et al*., 2010). The structure allows tilapia to efficiently absorb nutrients, enhancing its digestive process.

Dietary MOS supplementation strengthens animal immune systems, reduces intestinal pathogen growth, and enhances nutrient absorption efficiency in the intestines, while inhibiting the growth of unwanted bacteria (Dawood *et al*., 2020), This consequently promotes the health of the gastrointestinal tract, oral cavity, and skin in aquatic animals (Al-Ghazzewi and Tester, 2010), and also enhances intestinal tract growth and physical appearance (Gibson *et al*., 2005).

Vitamin E, known for its antioxidant properties, safeguards against oxidative stress and positively affects metabolic and physiological processes in animals. It enhances growth in aquatic animals and significantly benefits various fish species, including yellow catfish (*Pelteobagrus fulvidraco*) (Peng *et al*., 2009), hybrid snakehead (*Channa maculata*) (Lu *et al*., 2016), and black snapper (*Acanthopagrus schlegeli*) (Zhao *et al*., 2018). The absence of vitamin E inhibits seabass (*Sparus aurata*) growth (Ortuño *et al*., 2003), while supplementation reduces muscle lipid peroxidation and increases liver glutathione levels in tilapia hybrids (Huang and Huang, 2004). Additionally, it significantly boosts antioxidant capacity and affects tilapia lens membrane structure, guarding against oxidative stress (Huang and Lin, 2004; Prieto *et al*., 2008). Vitamin E also has been found to affect the reproductive system and reproductive performance in climbing perch; when juveniles were fed a diet containing vitamin E at 400 mg during the reproductive period, they displayed enhanced fertilization rate, spawning rate, and fecundity. Higher values of gonadosomatic index, ovarian development, and fecundity were observed in Nile tilapia compared to fish fed a control diet (Bera *et al*., 2022). The same study showed an increase in larval survival rate when fed a diet containing 100 mg·kg^{-1} vitamin E to the adult fish. Despite the apparent benefits that have been shown in several fish species, information about the effects of prebiotics and vitamin E in Nile tilapia is still scanty. Therefore, this experiment was conducted to evaluate the effect of MOS and vitamin E supplementation in enhancing sexual fertility, improving sperm quality, and elevating sex hormone levels of Nile tilapia. The knowledge gained from this study will contribute to the improvement of Nile tilapia breeding success.

MATERIALS AND METHODS

Preparation of laboratory animals

A total of 360 Nile tilapia broodstock were purchased from the Fisheries Cooperative, Tambon Nok Mueang, Mueang District, Surin Province, Thailand. These fish, aged seven months and with an average weight of 100 g, consisted of 120 males and 240 females. The experimental animals were stocked into cages measuring 2×2×1.5 m within an earthen pond of size 1,600 m^2 at a stocking density of 30 fish·cage^{-1}, comprising 10 males and 20 females. The fish were fed twice a day (at 8:00 a.m. and 4:00 p.m.) at a rate of 3% body weight for a period of two weeks. Daily water exchanges of 10% were performed. The experiment was conducted for 120 days.

Feed preparation

The experiment used floating pellet fish feed before commencing the experiment, and proximate analysis was conducted at the Analytical Laboratory Center, Faculty of Agriculture and Technology, Rajamangala University of Technology Isan, Surin Campus, Thailand. The analysis followed the AOAC (2016) method and revealed the basic nutritional compounds of the feed (without additives), which consisted of 29.26% protein, 9.32% fat, 7.42% ash, 7.42% fiber, and provided an energy content of 486 kcal·100 g^{-1} of feed.

The MOS feed was prepared by combining 10 g of mannan oligosaccharide dissolved in 10 mL of water with 1 kg of base feed (same feed as during acclimation). The ingredients were thoroughly mixed and then coated with 10 mL of squid oil. For the vitamin E-supplemented feed, 10 mL of vitamin E (alpha-tocopherol) was mixed with 10 mL of vegetable oil per 1 kg of feed. The mixture was combined and coated with 10 mL of squid oil. After all feedings, any remaining feed was collected, and growth performance data were calculated.

The experimental feed was separately packed in plastic bags and stored in a -20 °C freezer throughout the experimental period.

Experimental design

In the experiment, a completely randomized design (CRD) was implemented with four feed categories: without supplementation, with 1% MOS, with 1% VE, or with 1% MOS+1% VE. The experiment was conducted with three replications.

Data collection

Growth performance

Every 30 days, five fish were randomly taken from each cage, and total length and weight were measured to the nearest mm and g, respectively. Daily weight gain (DWG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR) were determined using the following formulas:

Daily weight gain = (final weight-initial weight) / number of rearing days

SGR = [(Ln (final weight)-Ln (initial weight)] / number of rearing days ×100

FCR = feed consumed / weight gained

SR = (number of fish / total number of fish)×100

The experiment was conducted for 120 days.

Assessment of sperm quality

A detailed microscopic assessment of sperm quality was made according to the method of Shamspour and Khara (2016).

Sperm motility (%): Briefly, the collected tests were handled with special care to prevent contamination with blood, mucus, and tissue fibers. Subsequently, they were placed on dry tissue, and minced into pieces to release sperm into a petri dish. Using 1 µL of sperm, it was added to 50 µL of 0.40% NaCl without a glass coverslip. Immediately, sperm were assessed for motility based on six levels: 0%, 20%, 40%, 60%, 80%, and 100% for evaluation.

Sperm viability (%): Live and dead sperm were differentiated using nigrosin-eosin. A single drop of fish semen was spread onto a slide and then stained with 1% eosin solution to achieve a contrasting background. The sample was counted under a 100x microscope. Live sperm were characterized by clear cells, while dead sperm exhibited purple-pink cells.

Sperm concentration (cells·mL^{-1}): Fish semen was diluted 100 times using 0.9% normal saline. A 20-µL drop of the sperm solution was placed on a hemacytometer and covered with a cover slide. The sample was then counted under a 40x microscope. The calculation was based on the method outlined by Hajirezaee *et al*. (2010).

Assessment of Gonadosomatic Index (GSI)

At harvest, a total of five fish per sex were randomly selected from each cage (with 3 replicates per treatment). These fish were individually weighed to the nearest gram, after which their gonads were extracted and weighed. The Gonadosomatic Index (GSI) was then calculated for each individual as a percentage of gonad weight relative to body weight using the method outlined by Shamspour and Khara (2016).

Serum hormone levels

On day 1 and day 120, blood samples were collected from caudal veins of one male and one female per replication. Subsequently, each sample was stored in a sterile test tube, placed in ice, and transported to the laboratory within 6 h. Following this, the blood samples were centrifuged at 3,000×g for 15 min. Only the serum fraction from the sterile test tubes was collected, and it was stored in 1-mL plastic tubes at -20 °C. Serum hormone levels, including female hormones progesterone and estradiol, and male hormone testosterone, were measured using specific and homologous competitive enzyme-linked immunosorbent assay (ELISA) methods. The hormone levels in the samples were calculated based on a standard curve, following the manufacturer's instructions. The competitive ELISA was performed using a microplate reader at an absorbance of 405 nm. The ELISA kit used was obtained from the University of California, Davis (USA), and had a minimum reading of 0.0023 ng·mL^{-1}.

Measurement of villi length

A sample of small intestine (jejunum) was collected from five fish of mixed gender randomly selected from each treatment group; these individuals were part of the collection taken for GSI estimates. Individual tissue samples were placed into an automated biopsy machine to produce paraffin blocks. Subsequently, the tissue was cut to a thickness of 5–6 µm, mounted onto a microscopic slide, and stained with hematoxylin and eosin. The stained slides were investigated and photographed under a microscope. The length of the villi was recorded using Aperio Image Scope version 12.3.2. 5030 (Bancroft and Gamble, 2013).

Statistical analysis

Data were analyzed using analysis of variance (ANOVA), and mean differences between groups were determined using Duncan's new multiple range test with a 95% confidence level.

Ethical statement

The experimental protocols used in this study were approved by a committee overseeing the moral and ethical aspects of using laboratory animals for scientific work (permission ID: U1-04327-2559).

RESULTS AND DISCUSSION

Growth performance and villi length

Nile tilapia receiving different dietary regimens for a period of 120 days exhibited significant ($p<0.05$) differences in average weight and length; the group receiving feed supplemented with 1% MOS+1% VitE showed the greatest final size (16.10±0.11 cm and 286.30±1.39 g). MOS supplementation alone significantly enhanced both weight and length of Nile tilapia compared to the control and the group fed vitamin E supplement. Vitamin E alone resulted in increased weight but not length relative to the control fish. The specific growth rate (SGR) was highest in the group receiving 1% MOS, with a rate of 0.83± 0.015% per day, which was significantly ($p<0.05$) higher than the other three groups. The feed conversion ratio (FCR) was lowest for the groups receiving either MOS alone (FCR = 3.79±0.08) or MOS+VitE (FCR = 3.82±0.12); both were significantly lower than the control and the group fed 1% VitE. Regarding survival rate, it was apparent that supplementation of 1% MOS, 1% VitE, or 1% MOS+1% VitE in feed improved survival rate of Nile tilapia as compared to the control (Table 1). In conclusion, MOS and VitE improved the growth of Nile tilapia and a synergistic effect on growth enhancement was apparent, while each supplement improved survival rate but without any synergistic effect of the two supplements.

The length of the intestinal microvilli in the jejunum region was examined in each sampled fish. The results showed that Nile tilapia fed the experimental diets containing either 1% MOS or 1% MOS+1% VitE exhibited a significant ($p<0.05$) increase in villi length in the small intestine (mean villi length = 1,507.33±102.47 µm and 1,490.78±120.75 µm, respectively) as compared with the group fed with a diet supplemented with 1% VitE alone and the control (no supplement) (mean villi length = 1,230.01± 71.64 µm and 1,201.56±215.34 µm, respectively) (Table 1).

Table 1. Growth and survival rates of Nile tilapia after being fed with a diet containing prebiotics (mannan oligosaccharides, MOS), vitamin E (VitE) or their combination (MOS+VitE) for 120 days.

Growth parameter	Treatment			
	Control	**1%MOS**	**1%VitE**	**1%MOS+1%VitE**
Initial length (cm)	14.24 ± 0.02^{a}	14.20 ± 0.07^{a}	14.26 ± 0.07^{a}	14.11 ± 0.10^{a}
Final length (cm)	15.22 ± 0.21^{c}	15.81 ± 0.28^{b}	15.32 ± 0.17^{c}	16.10 ± 0.11^{a}
Initial weight (g)	101.57 ± 1.55^{a}	101.05 ± 1.19^{a}	100.94 ± 0.73^{a}	101.13 ± 0.89^{a}
Final weight (g)	242.08 ± 5.32^{d}	273.90 ± 3.53^{b}	257.95 ± 0.62^{c}	286.30 ± 1.39^{a}
Daily weight gain	1.17 ± 0.03^{d}	1.44 ± 0.03^{b}	1.30 ± 0.01^{c}	1.54 ± 0.01^{a}
Specific growth rate ($\% \cdot day^{-1}$)	0.72 ± 0.008^{b}	0.83 ± 0.015^{a}	0.78 ± 0.080^{b}	0.80 ± 0.011^{b}
Feed conversion ratio	4.12 ± 0.10^{a}	3.79 ± 0.08^{c}	4.09 ± 0.07^{b}	3.82 ± 0.12^{c}
Survival rate (%)	90.00 ± 8.66^{b}	97.12 ± 3.56^{a}	96.77 ± 5.77^{a}	98.33 ± 2.88^{a}
$Villi_{0day}$ (µm)	874.05 ± 12.74^{a}	884.10 ± 41.01^{a}	894.11 ± 13.46^{a}	856.20 ± 23.36^{a}
$Villi_{120day}$ (µm)	$1{,}201.56 \pm 215.34^{b}$	$1{,}507.33 \pm 102.47^{a}$	$1{,}230.01 \pm 71.64^{b}$	$1{,}490.78 \pm 120.75^{a}$

Note: Means±SD in the same row superscripted with different lowercase letters are significantly ($p<0.05$) different.

Mannan oligosaccharides have been widely used in aquaculture to increase growth and improve overall health. MOS improves fish growth by serving as a food source for beneficial bacteria in the gut and thus promoting their growth. This mechanism results in maintaining the balance of intestinal microflora. In this study, the fish fed a diet containing 1% MOS or 1% MOS+1% VitE improved digestion and absorption efficiency of nutrients, leading to better feed efficiency as shown by the improved FCR. MOS also helps fish utilize nutrients in food more efficiently and reduces the energy used to build the immune system, allowing more of the available energy to be used for growth. Notably, FCR was high for all experimental groups and may be explained by feed loss during the early experimental period. The fish used in this experiment were initially reared in an earthen pond and basically fed on natural food. As such, they did not readily accept the artificial feed during the early experimental period. In addition, changing the rearing environment from an earthen pond to cages might have caused stress and reduced the feed intake. Additionally, MOS has anti-inflammatory properties and reduces health deterioration caused by the stress response. However, prebiotics are recommended at a dose of not more than 0.20% for growth enhancement and promoting aquatic animal health (Miandare *et al*., 2016) in fishes such as tilapia fry (Jimoh *et al*., 2019) and turbot fry (*Psetta maxina*) (Tiengtam *et al*., 2015; Hoseinifar *et al*., 2016). Researchers have used MOS to improve intestinal balance in catfish and zebrafish; these studies demonstrated an increase in villi length which increased surface area for nutrient uptake. MOS rapidly affects expanding cells in a growing state, stimulating the epithelium of intestinal mucosa through cell renewal and enhancing mitosis in many villi bases (Zhang *et al*., 2018; Xia *et al*., 2022). In terms of intestinal morphology, prebiotics may increase the absorption area of the tilapia gastrointestinal tract by causing changes in microvillus height. A higher absorption surface area and a higher density of intestinal microvilli result in increased nutrient absorption, leading to weight gain and improved feed efficiency, ultimately improving FCR (Hoseinifar *et al*., 2016). A previous study demonstrated that dietary supplementation of sunflower kernels to tilapia larvae for eight weeks increased villi length in the proximal and middle parts of the intestinal tract (Denji *et al*., 2015).

The improvement of survival rates as a result of prebiotic (MOS) supplement in this study was also shown in previous research by Zhang *et al*. (2018), who credited increased immunity and enhanced disease resistance for the improved survival. The addition of vitamin E, a powerful antioxidant, has also been found to improve the immune response of fish (Boonanuntanasarn *et al*., 2018). These combined factors ultimately might have led to a higher survival rate of Nile tilapia in the present study. When both MOS and VitE are used together, their synergistic effects can further enhance immune system activation to fight off pathogens and diseases (Neu *et al*., 2016). Moreover, MOS can protect Nile tilapia from oxidative stress by scavenging free radicals and reducing lipid peroxidation, thereby improving the antioxidant defense system of the fish (Lazado and Caipang, 2014). The combination of a strengthened immune system and improved disease resistance provided by both substances can significantly boost the overall health and survival of fish (You *et al*., 2016).

Effects of diet supplementation on sperm quality and Gonadosomatic Index

The results showed that supplementation of vitamin E or MOS significantly improved reproductive performance of male (sperm viability, sperm concentration, and male GSI) and female (GSI) Nile tilapia (Table 2). The enhancement effects of vitamin E were significantly ($p<0.05$) higher than that of MOS alone, except for sperm viability. The results also showed significant synergistic effects of MOS and VitE in enhancing the reproductive performance of Nile tilapia (Table 2). The sperm viability was highest in Nile tilapia fed the experimental diets containing 1% MOS+1% VitE. The sperm concentration in this treatment was also highest ($2.48\pm0.30\times10^8$ cells·mL^{-1}) as compared to the group fed with a diet supplemented with 1% VitE alone, 1% MOS, or the control group (no supplement) ($2.16\pm0.12\times10^8$ cells·mL^{-1}, $1.70\pm0.14\times10^8$ cells·mL^{-1}, and $1.30\pm0.10\times10^8$ cells·mL^{-1}, respectively). Male tilapia fed the experimental diets containing 1% MOS+1% VitE exhibited the highest average sperm viability, concentration and GSI (90.67±3.51%, $2.48\pm0.30 \times 10^8$ cells·mL^{-1}, and 0.195±0.01%, respectively). These values were found to be statistically different from the other experimental groups ($p<0.05$). Additionally, females in the group fed the combination of MOS+VitE had a mean GSI of 0.80±0.06%, which was statistically different from the other experimental groups ($p<0.05$) (Table 2).

Gonad development, mediated through improved gut health and nutrient absorption, can be enhanced by increasing the availability of required nutrients with MOS. Vitamin E can protect the reproductive tissues from oxidative damage and further support their growth and maturation. The combined use of these substances may provide a more comprehensive approach to stimulate gonad growth by addressing both gut health and oxidative stress (Xu *et al*., 2022). This, in turn, can lead to higher fertilization rates, better egg survival, and increased overall reproduction. MOS functions as

Table 2. Effects of dietary prebiotics (mannan oligosaccharides, MOS), vitamin E (VitE) and their combination (MOS+VitE) on sperm quality and gonadosomatic index (GSI) of Nile tilapia examined over a period of 120 days.

Trait	Treatment			
	Control	1%MOS	1%VitE	1%MOS+1%VitE
Sperm viability (%)	79.00±3.61^b	80.00±7.00^b	80.67±4.51^b	90.67±3.51^a
Sperm concentration ($\times10^8$ cells·mL^{-1})	1.30±0.10^d	1.70±0.14^c	2.16±0.12^b	2.48±0.30^a
GSI male	0.078±0.01^d	0.125±0.01^c	0.151±0.01^b	0.195±0.01^a
GSI female	0.29±0.03^d	0.48±0.07^c	0.66±0.02^b	0.80±0.06^a

Note: Means±SD in the same row superscripted with different lowercase letters are significantly ($p<0.05$) different.

a metabolizer, utilizing nitrogen for the synthesis of purine and pyrimidine through the synthesis of carbamoyl phosphate. This process facilitates continuous cell renewal and clearly has a positive effect on the reproductive system (Miandare *et al*., 2016). Vitamin E supplementation positively affects the reproductive development of freshwater fish (Neu *et al*., 2016) including tilapia (Yousefi *et al*., 2018) by promoting progesterone synthesis in aquatic animals (You *et al*., 2016). Additionally, vitamin E plays a role in preventing the destruction of cell membranes and the accumulation of lipoproteins and fatty acids in the body, which are essential for the production of sex hormones in aquatic animals (Zhao *et al*., 2018).

Serum hormone levels

The results revealed that progesterone and estradiol hormone levels in females significantly increased over the control in the groups fed diets with MOS, vitamin E or both. Among the fish fed the experimental diets, those fed 1% MOS+ 1% VitE-supplemented feed exhibited the highest levels of progesterone and estradiol, measuring 2.348±0.05 ng·mL^{-1} and 2.620±0.06 ng·mL^{-1}, respectively. Notably, females solely fed with MOS-supplemented feed showed higher levels of progesterone and estradiol than the group fed with vitamin E-supplemented diet. Likewise, MOS performed better than vitamin E in enhancing testosterone in the male Nile tilapia (1,724±61.94 pg·mL^{-1} vs. 1,648±25.30 pg·mL^{-1}), while the highest testosterone level was observed in the treatment with both MOS and vitamin E (1,757±12.22 pg·mL^{-1}) (Table 3).

These findings are consistent with previous studies on silver catfish (*Rhamdia quelen*) that demonstrated how VitE supplementation enhances the reproductive performance of female fish, leading to increased egg production, improved hatchability, and higher levels of the hormone 17β-estradiol (Marques *et al*., 2014). Similarly, studies have indicated that MOS supplementation can enhance the effects of reproductive hormones in aquatic animals. For instance, in Nile tilapia, dietary MOS supplementation was found to increase the plasma levels of reproductive hormones such as testosterone, estradiol, and 11-ketotestosterone. In males in this study, supplementation with 1% MOS and 1% VitE resulted in higher levels of testosterone compared to the control group. However, the levels were not significantly different from those observed in the group that received 1% MOS alone, which were higher than the levels in the group that received 1% VitE and the control group. These findings align with results for Pacific white shrimp (*Litopenaeus vannamei*) (Tacon *et al*., 2006). Furthermore, dietary vitamin E supplementation was shown to increase the plasma levels of the sex hormone 17β-estradiol, which plays a role in reproduction, improves reproductive performance, and increases the number of eggs produced in rainbow trout females (Bobe *et al*., 2004). These studies indicate that MOS and vitamin E supplementation can

Table 3. Reproductive hormone levels in female and male Nile tilapia fed with diet supplemented with prebiotics (mannan oligosaccharides, MOS), vitamin E (VitE) or their combination (MOS+VitE) for a duration of 120 days.

Hormone Level/sex	Treatment			
	1 Control	2 1%MOS	3 1%VitE	4 1%MOS+1%VitE
Female				
Progesterone (ng·mL^{-1})	1.265±0.01^{d}	2.042±0.02^{b}	1.921±0.06^{c}	2.348±0.05^{a}
Estradiol (ng·mL^{-1})	1.221±0.03^{d}	1.945±0.02^{b}	1.452±0.04^{c}	2.620±0.06^{a}
Male				
Testosterone (pg·mL^{-1})	1,553.85±23.12^{c}	1,724±61.94^{a}	1,648±25.30^{b}	1,757±12.22^{a}

Note: Means±SD in the same row superscripted with different lowercase letters are significantly ($p<0.05$) different.

positively influence reproductive hormone regulation and improve reproductive performance in fish. However, it is important to note that the effects of MOS and vitamin E on reproductive hormones may vary among species and can be influenced by factors such as dosage, duration of supplementation, and environmental conditions.

CONCLUSION

The present study reveals that using either 1% MOS or 1% VitE can enhance the growth and improve the FCR of Nile tilapia broodstock. However, MOS appears to be more effective than VitE in this regard. Additionally, only MOS significantly increased villi length. Notably, these two supplements demonstrated significant synergistic effects on growth. Both supplements equally improved the survivability of the broodstock.

When it comes to reproductive performance, VitE outperforms MOS in terms of sperm concentration and GSI for both sexes, with significant synergistic effects observed. However, when considering sex hormones, MOS proves to be more effective than VitE in enhancing progesterone and estradiol levels in females and testosterone levels in males, with profound synergistic effects seen only in females.

In conclusion, a supplement containing both 1% MOS and 1% VitE is recommended to improve the reproductive performance, growth, and survivability of Nile tilapia broodstock.

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