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The Impact of Interaction of Photoperiods and Vitamins Supplementation on the Hematological Parameters, Reproductive Hormones, Liver Function, and Reproductive Performance of the Female Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Three vitamin supplementations [non-supplemented, vitamin A (V.A), and vitamin D (V.D)] and three different photoperiods (8 light/ 16 dark, 12 light/ 12 dark, and 24 light/ 0 dark) were tested to evaluate their impacts on the hematological parameters, reproductive hormones, liver activity, and reproductive performance of the female Nile tilapia. Approximately, 810 females with an average-weight of 272.56± 37.5g were stocked inside 27 concrete tanks ($3m^3$), with 30 fish/ tank. Fishwere fed with a commercial diet (31.2% protein) at a daily rate of 3% for 60 days. The results revealed that the significant values of hematological parameters were observed under 8L/ 16D, while V.D could increase the mean cell volume, mean cell hemoglobin, and leucocytic count. The interaction effects of photoperiods and vitamins have a significant effect on the hematological parameters. Reproductive hormones were significantly increased under 8L/ 16D, while significant differences were observed by V.D followed by V.A. The interaction between 8L/ 16D and V.D enhanced the reproductive hormones. Transaminases activities were increased significantly under 8L/ 16D. For the interaction effects, the highest transaminases activities were observed under 8L/ 16D without any vitamin addition. The highest gonado and hepato somatic indexes and oocyte diameter were observed under 12L/ 12D, while the highest fecundity was recorded under 24L/ 0D. V.A could improve the gonadosomatic index and oocyte diameter, while the highest fecundity was observed with V.D. The interaction of photoperiods and vitamins A and D supplementation could improve the reproduction performance significantly. In conclusion, the interaction between photoperiod and vitamin A and D supplementation could positively affect the fish performance, hematological parameters, reproductive hormones, liver function, and reproductive performance of the Nile tilapia broodstock.

INTRODUCTION

One of the world's fastest-growing industries for providing food is aquaculture, which is expanding daily **(Syed *et al.*, 2021)**. Aquaculture, particularly the farming of tilapias, has the potential to be a major factor in the fight against starvation, malnutrition, and poverty in Africa. The Nile tilapia (*Oreochromis niloticus*) is one of the most significant fish species in tropical and subtropical aquaculture **(Prabu *et al.*, 2019)**. It is

the third most common species raised for its ability to provide high-quality fish protein to people across the world **(Syed *et al.*, 2021)**.

Since the Nile tilapia are exposed to climate change and nutritional deficiencies, some problems have arisen in fish reproduction. The effects of photoperiod on growth and reproduction are among the elements impacted by climate change and environmental factors **(Wu *et al.*, 2021; Li *et al.*, 2023)**. The photoperiod has a significant impact when fish go through puberty and reach sexual maturity. Fish's endogenous rhythms as well as the production and secretion of sex hormones, which are mediated by photoperiod, control gonadal development and reproduction. According to studies, prolonged photoperiod boosted tilapia fertility and accelerated top mouth gudgeon ovarian development, timing of gonadal maturation, and spawning frequency **(Shahjahan *et al.*, 2020)**. The literature has demonstrated that the brain-pituitary-gonad (BPG) axis controls ovary development in response to light. More specifically, it has been demonstrated that rhythmic signals prompted by light regulate ovary growth and reproduction cycle in the Nile tilapia **(Veras *et al.*, 2013; Medina-acosta & Tang, 2019))**.

On the other hand, all fish reproductive performance depends on proper nutrition in order to maintain the high energy requirements for the creation of gametes and reproductive behaviors. In most cases, the reproductive axis is inhibited by a negative energy balance and poor food intake **(Volkoff & London, 2018)**. Indeed, one of the most important nutrients for reproduction is vitamins. Vitamins are not synthesized by animals; therefore, they must obtain them from their diet to meet their needs. According to **Saleh *et al.* (1995)**, fat-soluble vitamins such as vitamins A and D are well recognized to be necessary for eyesight, metabolic processes, condition factors, growth, and reproduction in fish, and hence dietary supplementation in tilapia diets is crucial **(Hussein *et al.*, 2021)**. The function that vitamin A plays in a variety of physiological processes, such as vision, reproduction, embryogenesis, development and differentiation in addition to the maintenance of epithelial cells, makes it a vital nutrient for the normal growth of fish **(Hernandez & Hardy, 2020)**. Vitamin D plays a crucial role in fish reproduction, particularly in oocyte development and overall reproductive performance **(Lock *et al.*, 2010)**. Furthermore, research has indicated that vitamin D3 status can improve female reproductive performance in fish. The physiological function of vitamin D3 in fish is significant, emphasizing its role in supporting reproductive processes and overall health **(Grzesiak *et al.*, 2022)**.

Therefore, the present study aimed to evaluate the use of vitamin A and D supplementation diets under different photoperiods and the impact of their interaction on the hematological parameters, reproductive hormones, liver activity, and reproductive performance of the female Nile tilapia.

MATERIALS AND METHODS

The experimental procedures of the present study were carried out at the Fishery Research Center, Lake Nasser Development Authority, Aswan, Egypt.

1. Ethical approval

The guidelines of the Local Experimental Animal Care Committee were closely followed during the present study. Moreover, this study was also approved by the Scientific Research Ethics Committee of the Faculty of Agriculture, Suez Canal University (Approval No. 42/2021).

2. Broodstock collection, maintenance, and tanks preparation

Approximately, 810 healthy Nile tilapia broodstock (female) were collected from Lake Nasser Development Authority with an average initial weight of 272.56± 37.5g. The fish were weighed individually and manually sexed (by examining the genital papillae). Fish were starved for 24 hours before the experiment, and their body weight was recorded. Fish had homogeneous body weights and sizes, stocked inside 27 concrete tanks ($3m^3$), with 30 fish/ tank.

Tanks were washed, filled with water, and supplied with air hoses. The photoperiod regimes were established by manually controlling fluorescent lights suspended about 100cm above the water's surface. The fish were subjected to 9 treatments of 3 different photoperiods and 3 vitamins with 3 replicates for each, as shown in **Error! Reference source not found.**).

Table 1: The experimental design

Photoperiod (hour)	Vitamin
8 Light/16 Dark	Basal Diet
	Vitamin A Diet (1 mg/kg ≈ 10000 IU/kg)
	Vitamin D Diet (1 mg/kg ≈ 5000 IU/kg)
12 Light/12 Dark	Basal Diet
	Vitamin A Diet (1 mg/kg ≈ 10000 IU/kg)
	Vitamin D Diet (1 mg/kg ≈ 5000 IU/kg)
24 Light/0 Dark	Basal Diet
	Vitamin A Diet (1 mg/kg ≈ 10000 IU/kg)
	Vitamin D Diet (1 mg/kg ≈ 5000 IU/kg)

3. Water physico-chemical parameters

About 50% of the water in each tank was daily renewed with aerated fresh water. The daily water parameters were measured and controlled at the holding tanks to be as follows, as shown in **Error! Reference source not found.**). Water parameters were daily measured by dissolved oxygen (DO) meter (ExStik II D-0600, FLIR systems, Inc., USA), water pH meter, model: ph-009 and 3 digits total dissolved solids (TDS) Digital LCD TDS3/TEMP/PPM TDS Meter. Moreover, ammonia and nitrite were measured once a week.

Table 2: The water physico-chemical parameters of the experiment

Parameter	Value
DO mg/l	5.5-7.2
Temperature °C	28 - 30
TDS mg/l	115-130
pH	7.1-7.5
Ammonia mg/l	≤ 0.03
Nitrite mg/l	≤ 0.03

4. Feeding regime

The diet was provided at a daily rate of 3% of their body weight (BW), divided three times a day at 8:00, 12:00, and 17:00h for 60 days. Fish were fed with a commercial diet (Fishery Research Center, 31.2% protein, 3mm, Table 3). The experimental feed was provided by hand spreading. The daily diets were changed in accordance with the average weights of all the fish in each tank, which were measured at 15-day intervals. Vitamin A and D levels were added to the experimental diets carried out following the procedure described in the studies of **Chen *et al*. (2005)**, **Badran and Ali (2021)** and **Griesh *et al.* (2024)**.

Table 3. Ingredient composition and calculated chemical analysis of the basal diet

Ingredient	**(g/kg)**	**Proximate chemical analysis**	**%**
Wheat flour	*100*	*Dry matter*	*89.43*
Wheat bran	*150*	*Crude protein*	*31.2*
Soybean meal	*346*	*Crude fat*	*6.72*
Corn meal	*200*	*Ash*	*4.86*
Fish meal	*120*		
Corn oil	*30*		
Sunflower oil	*20*		
Ascorbic acid	*4*		

5. Blood sampling and analysis

After 60 days, 3 females from each replicate were anesthetized in diluted MS-222 at a concentration of 100mg/ l for sampling. Blood samples were quickly withdrawn by heparinize syringe (5000IU, Amoun Pharmaceutical Co.) from caudal vein. The collected blood was equally divided and poured into heparinized and non-heparinized Eppendorf tubes. There was one tube containing anticoagulants for hematology, while the other one and the second tube did not contain anticoagulants for hormonal and biochemical analyses, it was left to clot at 4°C and centrifuged at 3070xg for 5min at room temperature for separating serum. Red blood cell count (RBCs) and white blood cell count (WBCs) were counted under the light microscope using a Neubauer hemocytometer after blood dilution with phosphate-buffered saline (pH, 7.2) **(Badran & Ali, 2021)**. Hematocrit (PCV) and hemoglobin concentration (Hb) were determined according to **Jain (1993)** and **Řehulka and Adamec (2004)**. Mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were calculated according to **Griesh *et al.* (2024)**, as follows:

$$\text{MCV (fl)} = \frac{PVC\%x\ 1000}{RBCs\ \left(\frac{mill}{mm3}\right)}$$

MCH (pg) = Hb (g/dl) x10/RBCs (mill/mm^3)

MCHC (g/dl) = Hb (g/dl)/PVC% and transaminases (AST and ALT) activities were determined according to **Sarkheil *et al*. (2023)**.

6. Determination of hormonal levels

FSH, LH, and progesterone levels were determined using commercial assay ELISA kits (Invitrogen, Thermo Fisher scientific, Netherlands) as the manufacturer's instructions.

7. Biological measurements

After 60 days, females were prevented from feeding for 24 hours before sampling. They were netted & weighed, and 10 fish from each tank were dissected, and the liver and gonad were weighed. The following parameters were used to evaluate the Nile tilapia reproduction performance:

Gonadosomatic index % (GSI) is a technique used to study the spawning season by following the seasonal variations in the gonadal weight in relation to the total body-weight **(Badran *et al.*, 2019)**.

GSI %= (Gonad weight/Total body-weight) ×100.

The production of vitellogenin, a precursor to the yolk that is essential for egg development, by the liver. As it indicates the condition of energy stored in fish and is regarded as an excellent indicator of recent feeding activity of the fish; many researchers also believe the study of hepatosomatic index % (HSI) to be essential **(Jan *et al.*, 2017)**.

HSI %= (Liver weight/ Total body weight) ×100

Fecundity is the total number of mature oocytes produced by a female in one spawning season or year. It might also mean the number of eggs produced in a lifetime **(Badran *et al.*, 2019)**.

$$\text{Fecundity} = \frac{\text{no. of eggs} \times \text{gonad weight}}{\text{weight of gonad sample}}$$

Oocytes diameter: It's frequency helps to understand the reproduction. It gives the information on either the fish contributes once or several times in the spawning season. Hence, about 50 oocytes were separated from the ovarian tissues and put in saline solution (0.9 % NaCl) and then, taken on slide to measure the oocyte diameter. It was measured to the nearest 0.01µm by using an ocular micrometer on the binocular microscope (BioBlue lab) at a power magnification of 4X.50 **(Badran *et al.*, 2019)**.

8. Statistical analysis

The obtained results were presented as mean ± stander error, (SE) between all treatments. Statistically significant differences between treatments were evaluated using two-way ANOVA at 5% level of probability afterward *post-hoc* multiple comparison tests **(Duncan's 1955)**. SPSS program version 22 (Richmond, USA) was used in the present study's statistical analysis, as described by **Dytham (2011)**.

RESULTS

1. Hematological parameters

Table (4) displays significant ($P \leq 0.05$) differences between different photoperiods of hematological parameters. The peak values of HGB, RBCs, HCT, MCV, and WBCs were observed under 8 light and 16 dark treatments, followed by 24 light and 0 dark. Furthermore, no significant differences were observed by vitamins in HGB, RBCs, HCT, MCHC, and PLT values; while vitamin D could increase the values of MCV, MCH, and WBCs. The interaction effects of different photoperiods, and vitamins have a significant effect on the hematological parameters.

Table 4. The effect of photoperiods and vitamins supplementation on the hematological parameters of the Nile tilapia female

Treatment		HGB (g/dl)	RBCs (10^6 μL^{-1})	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLT (10^4 μL^{-1})	WBCs (10^4 /μL)
Main effects									
Photoperiod									
8 L/16 D		8.63± 0.68^{a}	0.91±0.08^{a}	28.43±2.32^{a}	266.40±10.61^{a}	82.27±3.29^{a}	30.21±0.05^{a}	246.31± 13.88^{a}	35.94±4.21^{a}
12 L/12 D		3.88± 0.49^{c}	0.54±0.03^{b}	12.79±1.57^{c}	227.74±16.39^{b}	68.13±4.50^{b}	30.11±0.14^{a}	218.11± 35.06^{a}	17.23±0.70^{c}
24 L/0 D		5.71± 0.68^{b}	0.84±0.08^{a}	18.83±2.52^{b}	228.37±17.71^{b}	71.24±4.65^{b}	30.21±0.06^{a}	196.44± 13.51^{a}	24.61±2.80^{b}
Vitamin									
Based Diet		5.62± 1.22^{a}	0.78±0.11^{a}	18.31±4.19^{a}	220.96±15.67^{b}	67.38±4.67^{b}	30.10±0.14^{a}	211.00± 21.54^{a}	25.28±4.73^{ab}
V. A Diet		5.88± 0.83^{a}	0.73±0.08^{a}	19.57±2.77^{a}	251.70±14.03^{a}	77.49±4.35^{a}	30.23±0.03^{a}	206.33± 15.17^{a}	21.91±2.02^{b}
V. D Diet		6.72± 0.60^{a}	0.78±0.07^{a}	22.18±1.91^{a}	249.86±17.34^{a}	76.78±4.22^{a}	30.20±0.06^{a}	243.53± 31.05^{a}	30.60±4.13^{a}
Interaction									
Photoperiod	**Vitamin**								
8 L/16 D	**Basal Diet**	10.4 ± 0.46^{a}	1.20 ± 0.05^{a}	34.97 ± 1.20^{a}	282.97 ± 3.34^{ab}	85.90 ± 0.91^{ab}	30.23 ± 0.07^{a}	295.00 ± 8.72^{ab}	43.33 ± 4.71^{a}
	V. A Diet	7.23 ± 0.69^{bc}	0.79± 0.07^{bc}	23.63 ± 2.22^{bc}	278.80 ± 10.44^{ab}	88.07 ± 1.03^{a}	30.27 ± 0.03^{a}	229.00 ± 17.62^{bc}	22.57 ± 1.89^{bc}
	V. D Diet	8.27 ± 1.51^{ab}	0.73 ± 0.08^{bcd}	26.70 ± 4.91^{b}	237.43 ± 24.45^{bc}	72.83 ± 7.74^{bc}	30.13 ± 0.12^{a}	214.93 ± 9.95^{bc}	41.93 ± 7.23^{a}
12 L/12 D	**Basal Diet**	2.90 ± 0.12^{d}	0.49 ± 0.02^{d}	9.87 ± 0.49^{d}	193.47 ± 2.28^{c}	58.57 ± 0.81^{d}	29.93 ± 0.42^{a}	166.33 ± 6.57^{c}	15.8 ± 0.64^{c}
	V. A Diet	2.97 ± 0.03^{d}	0.49 ± 0.01^{d}	9.63 ± 0.15^{d}	197.1 ± 1.70^{c}	60.47 ± 0.98^{cd}	30.20 ± 0.06^{a}	161.67 ± 6.36^{c}	16.07 ± 0.15^{c}
	V. D Diet	5.77 ± 0.38^{c}	0.65 ± 0.06^{cd}	18.87 ± 1.24^{c}	292.67 ± 7.02^{a}	85.37 ± 4.22^{ab}	30.20 ± 0.15^{a}	326.33 ± 76.69^{a}	19.83 ± 0.54^{bc}
24 L/0 D	**Basal Diet**	3.57 ± 0.59^{d}	0.65 ± 0.08^{cd}	10.10 ± 0.61^{d}	186.43 ± 5.71^{c}	57.67 ± 1.45^{d}	30.13 ± 0.17^{a}	171.67 ± 12.17^{c}	16.70 ± 0.92^{c}
	V. A Diet	7.43 ± 1.14^{bc}	0.92 ± 0.12^{b}	25.43 ± 3.49^{bc}	279.20 ± 3.95^{ab}	83.93 ± 1.92^{ab}	30.23 ± 0.07^{a}	228.33 ± 30.23^{bc}	27.10 ± 3.81^{bc}
	V. D Diet	6.13 ± 0.29^{bc}	0.94 ± 0.15^{b}	20.97 ± 1.26^{bc}	219.47 ± 38.80^{c}	72.13 ± 8.96^{bc}	30.27 ± 0.03^{a}	189.33 ± 16.92^{c}	30.03 ± 5.45^{b}

Means with various superscripts differ significantly at $P \leq 0.05$ within the same column.

2. Reproductive hormones

Table (5) exhibits significant ($P \leq 0.05$) differences between different photoperiods. The levels of FSH, LH, and progesterone were increased under 8 light/ 16 dark treatments. Significant ($P \leq 0.05$) differences were observed by vitamin D, followed by vitamin A in FSH, LH, and progesterone.

For the interaction effects of different photoperiods and vitamins, the highest level of FSH, LH, and progesterone were observed under 8 light and 16 dark and vitamin D treatments, followed by 24 light/ 0 dark and vitamins A and D treatments.

Table 5. The effect of photoperiods and vitamins supplementation on the reproductive hormones of female Nile tilapia

Treatment		FSH (IU/L)	LH (IU/L)	Progesterone (ng/mL)
Main effects				
Photoperiod				
8 L/16 D		0.29±0.03^{a}	0.77±0.04^{a}	0.52±0.07^{a}
12 L/12 D		0.20±0.01^{b}	0.55±0.02^{c}	0.24±0.03^{c}
24 L/0 D		0.28±0.02^{a}	0.66±0.04^{b}	0.41±0.06^{b}
Vitamins				
Based Diet		0.21±0.01^{b}	0.59±0.04^{b}	0.25±0.04^{b}
V. A Diet		0.26±0.02^{ab}	0.66±0.04^{b}	0.42±0.06^{a}
V. D Diet		0.29±0.03^{a}	0.74±0.05^{a}	0.50±0.07^{a}
Interaction				
Photoperiod	Vitamin			
8 L/16 D	Basal Diet	0.25 ± 0.02^{bcd}	0.73 ± 0.03^{b}	0.38 ± 0.04^{cd}
	V. A Diet	0.25 ± 0.01^{bcd}	0.69 ± 0.02^{b}	0.44 ± 0.06^{bc}
	V. D Diet	0.36 ± 0.07^{a}	0.90 ± 0.07^{a}	0.74 ± 0.12^{a}
12 L/12 D	Basal Diet	0.17 ± 0.01^{d}	0.49 ± 0.03^{d}	0.18 ± 0.03^{d}
	V. A Diet	0.19± 0.00^{cd}	0.53 ± 0.01^{cd}	0.22 ± 0.00^{d}
	V. D Diet	0.23 ± 0.03^{cd}	0.63 ± 0.04^{bc}	0.34 ± 0.05^{cd}
24 L/0 D	Basal Diet	0.21 ± 0.02^{cd}	0.54 ± 0.03^{cd}	0.22 ± 0.03^{d}
	V. A Diet	0.34 ± 0.03^{ab}	0.75 ± 0.05^{b}	0.59 ± 0.09^{ab}
	V. D Diet	0.28 ± 0.02^{abc}	0.68 ± 0.05^{b}	0.42 ± 0.06^{bc}

Means with various superscripts differ significantly at $P \leq 0.05$ within the same column.

3. Liver function

The present results of AST and ALT activities showed significant ($P \leq 0.05$) differences between different photoperiods. The AST and ALT activities were increased under 8 light and 16 dark treatments, while no significant differences were observed by different vitamins. About the interaction effects of different photoperiods and vitamins, the highest level of AST and ALT activities was observed under 8 light/ 16 dark and without the addition of any vitamin treatment (Table 6).

Table 6. The effect of photoperiods and vitamins supplementation on the liver function of the female Nile tilapia

Treatment		AST (U/L)	ALT (U/L)
Main effect			
Photoperiod			
8 L/16 D		162.09±5.74^{a}	57.17±4.74^{a}
12 L/12 D		138.08±1.49^{b}	38.16±0.94^{b}
24 L/0 D		147.11±3.41^{b}	44.39±2.35^{b}
Vitamin			
Based Diet		149.27±6.67^{a}	47.12±5.16^{a}
V. A Diet		147.16±3.57^{a}	44.37±2.44^{a}
V. D Diet		150.86±5.05^{a}	48.22±4.34^{a}
Interaction			
Photoperiod	Vitamin		
8 L/16 D	Basal Diet	175.37 ± 3.49^{a}	67.30 ± 3.40^{a}
	V. A Diet	147.20 ± 3.06^{cd}	44.90 ± 1.27^{cd}
	V. D Diet	163.70 ± 13.17^{ab}	59.30 ± 11.30^{ab}
12 L/12 D	Basal Diet	134.03 ± 1.71^{d}	36.07 ± 0.90^{d}
	V. A Diet	137.27 ± 0.49^{cd}	37.00 ± 0.20^{cd}
	V. D Diet	142.93 ± 1.82^{cd}	41.40 ± 1.27^{cd}
24 L/0 D	Basal Diet	138.40 ± 1.79^{d}	38.00 ± 0.53^{cd}
	V. A Diet	157.00 ± 6.76^{bc}	51.20 ± 4.38^{bc}
	V. D Diet	145.93 ± 1.79^{cd}	43.97 ± 1.77^{cd}

Means with various superscripts differ significantly at $P \leq 0.05$ within the same column.

4. Reproductive performance

In the current study, significant ($P \leq 0.05$) variations of reproductive performance of females were recorded for different photoperiods and vitamins supplementation. The highest gonad weight was recorded under 8 L/ 16 D, and the highest GSI, HSI, oocyte diameter was observed under 12 L/ 12 D, while the highest fecundity was recorded under 24 L/ 0 D, regardless of any vitamin supplementation. Vitamin A could improve the GSI % and oocyte diameter, while the highest fecundity was observed with vitamin D supplementation, regardless of the photoperiods. The interaction of different photoperiods and vitamins A and D supplementation could improve the values of gonad weight, GSI, oocyte diameter, and fecundity significantly ($P \leq 0.05$) (Table 7).

Table 7. The effect of photoperiods and vitamins supplementation on the reproductive performance of the female Nile tilapia

Treatment		Gonad weight (g)	GSI %	HSI %	Oocyte diameter (mm)	Fecundity
Main effect						
Photoperiod						
8 L/16 D		4.94±0.42 ^{a}	3.41±0.45 ^{ab}	1.54±0.06 ^{b}	0.96±0.08 ^{b}	1809.56±158.82 ^{b}
12 L/12 D		4.77±0.32 ^{ab}	4.06±0.22 ^{a}	1.83±0.06 ^{a}	1.16±0.05 ^{a}	1842.89±212.99 ^{ab}
24 L/0 D		4.50±0.71 ^{b}	2.28±0.17 ^{c}	1.47±0.16 ^{b}	0.79±0.05 ^{c}	2111.89±282.23 ^{a}
Vitamin						
Based Diet		2.91±0.31 ^{a}	2.15±0.27 ^{b}	1.69±0.10 ^{a}	0.83±0.08 ^{b}	1145.78±51.45 ^{c}
V. A Diet		5.30±0.18 ^{a}	3.81±0.28 ^{a}	1.59±0.17 ^{ab}	1.10±0.06 ^{a}	2130.00±144.40 ^{b}
V. D Diet		5.00±0.12 ^{a}	3.78±0.33 ^{a}	1.57±0.06 ^{b}	0.97±0.07 ^{ab}	2488.56±113.00 ^{a}
Interaction						
Photoperiod	Vitamin					
8 L/16 D	Basal Diet	3.33±0.29^{c}	1.63±0.15^{d}	1.32±0.02^{d}	0.70±0.10^{c}	1256.67±99.39^{d}
	V. A Diet	5.50±0.06^{a}	4.25±0.18^{a}	1.73±0.05^{b}	1.13±0.07^{ab}	1993.33±74.46^{bc}
	V. D Diet	6.00±0.17^{a}	4.36±0.12^{a}	1.58±0.06^{bc}	1.04±0.07^{ab}	2278.67±95.68^{abc}
12 L/12 D	Basal Diet	3.67±0.09^{c}	3.20±0.06^{b}	1.74±0.03^{b}	1.10±0.06^{ab}	1103.33±68.88^{d}
	V. A Diet	4.77±0.09^{b}	4.45±0.20^{a}	2.07±0.05^{a}	1.27±0.07^{a}	1846.67±129.14^{c}
	V. D Diet	5.87±0.18^{a}	4.54±0.13^{a}	1.69±0.04^{b}	1.10±0.10^{ab}	2478.67±221.39^{ab}
24 L/0 D	Basal Diet	1.73±0.03^{d}	1.63±0.04^{d}	2.00±0.05^{a}	0.70±0.12^{c}	1077.33±88.05^{d}
	V. A Diet	4.63±0.41^{b}	2.72±0.09^{c}	0.97±0.05^{e}	0.90±0.06^{bc}	2550.00±300.50^{a}
	V. D Diet	5.13±0.32^{b}	2.51±0.09^{c}	1.44±0.13^{cd}	0.77±0.08^{c}	2708.33±220.95^{a}

Means with various superscripts have differed significantly at $P \leq 0.05$ within the same column.

DISCUSSION

The current study found that the photoperiod and vitamins have a significant impact on the hematological parameters of the Nile tilapia broodstock. The HGB, RBCs, HCT, and WBCs were increased significantly ($P \leq 0.05$) in the treatment of 8L: 16D and fed with a control diet, and the lowest values were observed under 12L: 12D and fed with basal and vitamin A supplemented diet. This finding is in agreement with **Shahjahan *et al.* (2020)**, who showed that hemato-biochemical parameters decreased in longer photoperiod (18L: 6D) than 6L: 18D. In the studies of **Emelike *et al.* (2008)** and **Solomon and Okomoda (2012)**, the values of HGB, RBCs, HCT, and WBCs were reduced in 24L: 0D, which may be as a result of depletion of ATP caused by induced stress. **Inayat *et al.* (2020)** suggested that RBCs and associated metrics failed to exhibit any discernible pattern in relation to the vitamin D level. In the present study, 12L: 12D and supplemented vitamin D diet could increase the values of MCV and PLT, while those

values were decreased under 12L: 12D and 24L: 0D with basal diet. This finding is in line with that of **Zempleni *et al.* (2014)**, who approved that vitamin D regulates calcium and phosphorus balance, crucial for various biological activities like muscular contraction, nerve impulse transmission, blood coagulation, and membrane construction. The MCH values in the present study represented a significant increase under 8L: 16D and vitamin A-supplemented diet, and there are no significant differences between treatments for MCHC. The present results showed that, under a 12L: 12D cycle and fed with basal diet, all parameters of hematology significantly decreased. This suggests that hematological parameters are influenced by photoperiod and vitamin supplementation in the Nile tilapia broodstock. On the other hand, **Guimarães *et al.* (2014)** approved that vitamin A deficiency causes low HGB, RBCs, and HCT. The biological processes by which vitamin A deficiency may cause anemia can be divided into categories: stimulation of RBCs progenitor cell growth and differentiation, control of immunity to infections and reduction of anemia during infection, and mobilization of iron stored in tissues **(Semba & Bloem, 2002; Guimarães *et al.*, 2014)**.

FSH and LH are important hormones that control fish reproduction; FSH is mainly liable for gametogenesis start and gonadal growth, whilst LH controls gonadal maturation and ovulation **(Choi *et al.*, 2023)**. The present study showed that the concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone were significantly enhanced with vitamin D under 8L: 16D cycle. Hence, this indicates that photoperiod has an effect on hormone secretion in the Nile tilapia, as similar to the findings of **Li *et al.* (2023)** who showed that the expression level of FSH and LH were higher in the short light period than the long period (18L: 6D). Endogenous rhythms in addition to the creation and release of gonadal steroid hormones regulate the influence of photoperiod on fish gonad development and reproduction **(Elisio *et al.*, 2014, 2015)**. Changes in photoperiod trigger the hypothalamus to secrete several hormones, including GnRH, which stimulate the pituitary gland to produce FSH and LH, which are then carried to the gonads via circulation and linked to their receptors to control the development of gonadal cells, sexual maturity, and the release of sex hormones (such as progesterone) **(Ahmed *et al.*, 2016; Zahangir *et al.*, 2021; Wang *et al.*, 2022; Choi *et al.*, 2023; Li *et al.*, 2023)**. Therefore, the present value of progesterone increased with increasing FSH and LH under 8L: 16D photoperiod. Melatonin rhythms transmit these signals to the brain-pituitary-gonadal axis in response to changes in photoperiod. Melatonin levels are closely associated with photoperiod modification in fish, which results in spawning time advancement or delay, implying that melatonin acts as a modulator in reproductive performance **(Bromage *et al.*, 2001; Ahmed *et al.*, 2016)**. **Lock *et al.* (2010)** mentioned that fish include a vitamin D endocrine system which functions similarly to mammals. This is consistent with our study, as vitamin D influences the secretion of FSH and LH. The existence of VDR (Vitamin D receptor) protein in the ovary of various fish shows that vitamin D has an impact on the gonadal

gametogenesis **(Grzesiak *et al.*, 2022)**. VDR is present in the human pituitary gland and has a function in releasing the hormones and the expression of genes **(Pérez-Fernandez *et al.*, 1996)**.

The present levels of both ALT and AST activities were influenced by changes in photoperiod and vitamin supplementation. The highest values were achieved by using 8L: 16D photoperiod and non-supplementation diet, while the lowest levels were observed under 12L: 12D photoperiod and non-supplementation diet. This is in line with **Khanjani and Sharifinia, (2021)** who established that, the greatest values of the hepatic enzymes AST and ALT were found in 0L: 24D. This might be caused by a healthy diet and a decrease in the formation of harmful metabolites when light is abundant. Additionally, the AST and ALT increased activity is presumably attributable to their function in creating the circumstances for the process of gluconeogenesis and supplying the essential energy in stressful situations carried on by light limitation. Increased transaminases are an immunological response that happens in the early stages of stress and are caused by the enzymes AST and ALT, which are involved in the gluconeogenesis of amino acids **(Rastiannasab *et al.*, 2016; Wu *et al.*, 2018)**. Our results showed that vitamins have an effect on AST and ALT activities. In fish, a lack of vitamin A has been associated to liver inflammation and a reduction in liver size **(Guimarães *et al.*, 2014)**. Vitamin D deficiency in fish diet led to low growth, increased liver fat content, and decreased calcium balance **(George *et al.*, 1981; NRC, 2011)**.

In the production of some commercial species, such as the Nile Tilapia, the modification of photoperiods to enhance fish development has become more prevalent. Fish could find more food if the photoperiod is extended **(Casey *et al.*, 2020)**. The results of the current experiment indicated that the manipulation of the photoperiod and supplementation with vitamins A and D had a significant effect on the Nile Tilapia broodstock performance. Furthermore, the current study also revealed that the combination of optimal photoperiod and vitamin supplementation could potentially improve the overall performance and productivity of the Nile tilapia broodstock. The supplemented vitamin D diet under photoperiods 8L: 16D and 12L: 12D could increase the gonad weight and GSI%, and the lowest value was observed in 24L: 0D groups which alignes with the results of **Amal and Reisy (2005)**, who noted that, the GSI values are increasing in prolonged photoperiods (15L), and **Rad *et al.* (2006)**, who proved that, GSI was much lower in fish maintained under a continuous light regime (24L: 0D). Moreover, vitamin D has a positive effect on gonad weight and GSI. The existence of the vitamin D receptor (VDR) gene in fish ovaries shows that vitamin D3 has direct effects on gonadal gametogenesis and steroidogenesis. The expression of VDR suggests that fish gonadal tissues are a target for vitamin D activity, as indicated in the gonadosomatic index (GSI) **(Lock *et al.*, 2007; Craig *et al.*, 2008; Grzesiak *et al.*, 2022)**. As the main function of vitamin D is calcium metabolism, vitamin D is essential for the absorption of calcium in the intestines **(Gai, 2018)**. Calcium has an important role in the formation and upkeep of

the skeletal system, as well as in various physiological processes in fish **(Hossain & Yoshimatsu, 2014)**. On the other hand, the lowest gonad weight and GSI were observed under 24L: 0D and basal diet which may be due to the extended periods between feedings of fish over a long and consistent photoperiod that may allow for improved digestion, resulting in greater nutritional retention **(Biswas *et al.*, 2005, 2006; Veras *et al.*, 2013; Wang *et al.*, 2020)**. Prolonged photoperiods may indirectly influence fish growth by increasing muscle mass as a result of enhanced locomotor activity **(Boeuf & Le Bail, 1999; Veras *et al.*, 2013)**. The current study showed that vitamin A has a positive effect on HSI, which was high under 12L: 12D with vitamin A. This finding is consistent with the result of **Guimarães *et al.* (2014)** who proved that, vitamin A deficiency has been linked to liver inflammation and decrease size of the liver in fish. Fish fed an unsupplemented vitamin A diet had less liver weight, as demonstrated by a significantly lower HSI.

The findings of our study suggest that both photoperiod and vitamin supplementation, specifically vitamin A, have a significant impact on the reproductive performance of the Nile tilapia. Oocyte diameter (OD) was higher under the treatment 12L: 12D and fed with vitamin A supplemented diet, indicating that a combination of a 12-hour light/ dark photoperiod and vitamin A supplementation promoted better reproductive performance in the Nile tilapia. **Couto-Mendoza *et al.* (2014)** and **Ahmed *et al.* (2016)** suggested that considerably larger oocytes were observed when day length was normal (12L: 12D). Significantly, smaller oocytes were found in fish kept under a continuous photoperiod (24L: 0D) than natural photoperiod, which was proven by **Rad *et al.* (2006)** in their study. In the present study, vitamin A and vitamin D significantly improved the fecundity under 24L: 0D. Increasing egg production, fecundity, and the number of spawns can be obtained by exposing the fish to long light periods (18L: 6D) **(Campos-Mendoza *et al.*, 2003)**. Moreover, the addition of both vitamins A and D to the diet of the Nile tilapia had a positive influence on gonadal development and reproductive performance. These findings are supported by previous studies that have also reported the positive influence of vitamins on the reproduction and performance of fish species **(Shiau and Lin, 2006; Martins *et al.*, 2016)**. Additionally, longer and brighter photoperiods were found to have a positive effect on the reproductive cycle of the Nile tilapia **(Medina-acosta & Tang, 2019)**. This is evident from the report by **Ridha and Cruz (2000)**, which found that longer and brighter days resulted in increased fry production and improved spawning synchrony in the Nile tilapia compared to shorter days and lower light intensity. Furthermore, the present results indicated that the importance of vitamin A for reproductive performance is similar to the results of **Hernandez and Hardy (2020)**, who found that vitamin A has vital effects on reproduction, egg production, egg hatchability, and early larval survival and development. **Wang *et al.* (2014)** proved that vitamin A could accelerate the

development of the gonad, enhance the maturity of the eggs, and extend the spawning period.

CONCLUSION

This study provides details on the impact of photoperiods and vitamin supplementation on the female Nile tilapia performance and reproductive performance. The interaction between photoperiod and vitamin A and D supplementation could be an effective intervention that can positively affect fish performance, hematological parameters, reproductive hormones, liver function, and reproductive performance of the Nile tilapia broodstock. Therefore, vitamins A and D supplementation could be positively used under different photoperiods as a result of climate changes to stimulate reproduction in the Nile tilapia hatcheries.

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