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A MIXTURE OF GARLIC EXTRACT AND *SACCHAROMYCES CEREVISIAE* ENHANCED THE PRODUCTIVITY, HATCHABILITY, INTESTINAL HEALTH, AND BLOOD IMMUNITY OF NILE TILAPIA BROODSTOCKS

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ABSTRACT

The production of healthy and active seeds is one of the main challenges associated with aquaculture sustainability. Herein, we investigated the role of Alliforte, a source of *Saccharomyces cerevisiae* and garlic extract, on Nile tilapia broodstock performances and the seed production as well as the growth performance of the produced fry. Five test diets were incorporated with Alliforte at 0, 0.5, 1, 1.5, and 2 mg/kg and fed to the broodstocks for 36 days; then, after hatching and spawning, the fry were fed the same respective diets for 60 days. The final body weight (FW), body weight gain (BWG), weight gain rate (WGR), average daily gain (ADG), specific growth rate (SGR), protein efficiency ratio (PER), and productive protein value (PPV) were markedly higher, but FCR was lower in fish fed Alliforte at 1, 1.5, and 2 mg/kg than fish fed 0 and 0.5 mg/kg. The intestinal histological features (villi length, width, and branching of villi) of broodstocks fed dietary Alliforte are markedly improved. The lysozyme activity was markedly improved in broodstock fed dietary Alliforte at 0.5, 1, and 1.5 mg/kg. Dietary Alliforte markedly improved the phagocytic activity, and the highest level was in fish fed 1.5 mg/kg followed by 2 mg/kg. The average weight of females is increased meaningfully in fish fed 1, 1.5, and 2 mg/kg. The absolute fecundity (AF) and relative fecundity (RF) were higher in fish fed 1, 1.5, and 2 mg/kg than 0 and 0.5 mg/kg, while fish fed 1.5 mg/kg had the highest AF and RF. The FW, BWG, ADG, and total feed intake were markedly higher in fry treated with Alliforte than the control, and fish fed 1.5 mg/kg had the highest performances. The WGR, SGR, and PER were markedly improved in fish treated with 0.5, 1, and 1.5 mg/kg while the FCR was decreased in the same groups. The fry survival rate was markedly increased in groups treated with 1 and 1.5 mg/kg. In conclusion, dietary Alliforte has positively influenced the growth performance, health status, and seed production of Nile tilapia broodstocks. Further, dietary Alliforte improved the fry performances and survival rates.

Keywords: aquaculture; seed production; hatchability; maternal nutrition; feed additives

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INTRODUCTION

The aquaculture of Nile tilapia (*Oreochromis niloticus*) has faced several challenges that need better strategies to improve quality and maximize production (El-Sayed, 2019). The high mortality rate of seeds at the early life stages severely impacts the productivity of Nile tilapia (Dong *et al*., 2020). Infection with pathogenic invaders, low water quality, predators, high stocking density, and low-quality feed are the main reasons for high fry mortality (Little and Hulata, 2000). Traditionally, farmers use antibiotics and chemotherapy to enhance the resistance of seeds to biotic and abiotic stressors (Karal Marx *et al*., 2020). Although antibiotics are highly efficient, the absence of natural immunity and accumulating residuals pose serious risks to the ecosystem and public health (Mugwanya *et al*., 2021). Alternative approaches have been applied to improve the growth and well-being of aquatic animals (Kari *et al*., 2022; Ushakova *et al*., 2021). Probiotics and medicinal plants are highly recommended in the aquafeed industry for their environmentally friendly efficiency, safe use, and functionality (Dawood *et al*., 2018).

Saccharomyces cerevisiae is live yeast cells with high potential as growth-promoting and immunostimulant roles (Abu-Elala *et al*., 2020). *S. cerevisiae* has functional derivatives (e.g., glucans and mannooligosaccharides) that fish can well utilize (Divya *et al*., 2020). Studies have indicated the positive effect of *S. cerevisiae* on hatchability, survivability, and fry production (Abu-Elala *et al*., 2021; Bhujel *et al*., 2020). *S. cerevisiae* can improve intestinal health, wellbeing, and the entire body immunity (Dawood *et al*., 2020). Besides, *S. cerevisiae* enhances digestive enzyme activity and result in high feed utilization and digestibility (Yang *et al*., 2020). Several studies also indicated the positive effect of

dietary *S. cerevisiae* in reducing the impacts of pathogenic invaders on fry production (Abdel-Tawwab, 2012).

Garlic (*Allium sativum*) is a member of the Alliaceae family, which is a rich source of allicin (Valenzuela-Gutiérrez *et al*., 2021). Allicin is a bioactive molecule involved in multiple pharmaceutical roles (Lee and Gao, 2012). Garlic also contains several vitamins, minerals, prostaglandins, essential oils, fructan, and pectin, which are involved in various metabolic and physiological roles (El-Saber Batiha *et al*., 2020). The role of garlic is well investigated in aquaculture and showed potential effects on growth performance, feed utilization, digestibility, antioxidative, and immune responses (Mesalhy Aly *et al*., 2010; Yılmaz and Ergün, 2014).

The fortification of feed additives in broodstock feeding is regarded as a significant element that can impact fish reproduction and thereby fry quality (Soaudy *et al*., 2021). Several reports indicated the positive roles of *S. cerevisiae* or garlic extracts on aquatic animals' productivity and health status (Abu-Elala *et al*., 2021; Mekawey, 2019). However, the combination of probiotics and herbal plants can synergistically impact the growth performance, and wellbeing of aquatic animals (Newaj-Fyzul and Austin, 2015; Soltani *et al*., 2019). Herein, we investigated the effects of dietary Alliforte, a mixture of *S. cerevisiae* or garlic extract, on Nile tilapia hatchability, fry survivability, and broodstock health condition.

MATERIALS AND METHODS

Preparation of experimental diets: The basal or control diet was formulated with fish meal, soybean, yellow corn, wheat bran, rice bran, sunflower oil, vitamins, and minerals mixture (Table 1). The diets were prepared by mixing the dry ingredients thoroughly at first and with oil after that. The basal diet was used as a control diet (without the addition of Alliforte, Smarts Masr, Borg Elarab, Alexandria - Batch: 0243), while the other tested diets were prepared by adding 0 .5, 1, 1.5, and 2 mg Alliforte/kg diet. Each one liter of Alliforte contains *Saccharomyces cerevisiae* extract 100000 mg, garlic extract 25000 mg, zinc 50000 mg, iron 5000 mg - phosphorus 25000 mg, copper 1000 mg, propylene glycol 25000 mg, excipient up to 1000 mg. Alliforte was first suspended in oil and mixed through with the basal diets. Experimental diets were pelleted, dried, and stored at -20ºC in a freezer until use. Composition and chemical analysis of the basal diet and other experimental diets are done following AOAC (1995) and presented in table 1.

Table 1. Ingredients and chemical composition (%) of the basal diets for broodstocks and fry.

Ingredients	Broodstock	Fry
Fish meal (72%cp)	28	35
Soybean meal	35	35
Yellow corn	20	20
Wheat bran	7	3
Rice bran	6	3
Sunflower oil	3	3
Premix[1]	1	1
Proximate analysis of the experimental diets (% DM)		
Dry matter (DM %)	90.55	90
Crude protein (CP %)	39.1	44.2
Ether extract (EE %)	6.47	7.1
Ash (%)	8.9	7.9
Crude fiber (CF %)	6.9	6.5
Nitrogen free extract (NFE %)	38.63	34.3
GE (kcal/kg)[2]	4450.8	457.28
DE (kcal/kg)[3]	3338.8	376.11
ME (kcal/kg)[4]	3654.4	342.96
P/E (mg/kca DE)[5]	87.87	96.47

(1) Premix Composition: Each 3 kg contains, Vit A (1200000 i.u.), Vit D (300000 i.u.), Vit E (700 mg) Vit K3 (500 mg,) Vit B1 (500 mg), Vit B2 200mg, Vit B6 (600 mg), Vit B12 (3 mg), Vit C 450mg, Niacin 3000 mg, Methionine3000mg, Cholin chloride 10000 mg, Folic acid 300 mg, Biotin 6mg, Panthonic acid 670mg, Magnesiam salphate 3000 mg, Copper sulphate 3000 mg, Iron sulphate 10000mg, Zinc sulphate, 1800 mg, Cobalt sulphate 300 mg, Carrier upto 3000 mg.
(2) GE (Gross energy) was calculated by using factors of 5.65, 9.45 and 4.22 K cal per gram of protein, lipid and carbohydrate, respectively.
(3) DE (Digestible energy) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy.
(4) ME (Metabolism energy) was calculated using a value of 4.5 Kcal/g proteins, 8.51 Kcal/g fat and 3.48 Kcal/g carbohydrates.
(5) P/E (protein /energy ratio) = crude protein x 10000 / digestible energy.

Broodstock feeding trial: Males and females of Nile tilapia (*Oreochromis niloticus*) with an average weight of 300 g for males and 200 g for females/fish were used in the present study. The fish were obtained from a commercial fish farm in Motobas Kafrelsheikh Governorate. They were kept in hapas at a temperature of 25 ºC and fed for 15 days for adaptation and fed on the basal diet. Then, fish were weighed and randomly distributed into the five experimental groups. In each tested group, the number of 150 females and 60 males were divided into three replicates, where each replicate contained 50 females and 20 males. Fish fed the

experimental diets for 36 days. The fish were fed on tested diets at 2.5% of live body weight at 10:00 am and 2:00 pm.

Growth performance and feed utilization were determined at the end of broodstock feeding trial as follows:

Weight gain (WG) = W2 (g) – W1 (g)

Specific growth rate (SGR % /day) =100 × (lnW2−lnW1) / T

Where: ln = the natural log; W2 = final weight at a certain period (g); W1 = initial weight at the same period (g); T = period per day

Daily weight gain (DWG) = (W2 – W1) / t

Feed conversion ratio (FCR) = feed intake (g)/WG (g).

Protein efficiency ratio (PER) = WG (g)/protein intake (g).

Protein productive value (PPV%) =100× (protein gain (g)/protein intake (g))

Survival rate (SR %) = final number of fish × 100 / initial total number of fish

At the end of the experiment, three blood samples from every treatment were taken randomly from the caudal vein for blood analysis and differential leukocyte count. Serum was collected after centrifugation (3000 rpm for 15 min at 4 ºC) then kept at −20 ºC until use. Serum lysozyme activity (unit/ml) was determined by following the turbidimetric assay (Parry *et al*., 1965). Phagocytic activity was determined, according to Kawahara *et al*. (1991). To calculate the phagocytic index, the number of phagocytic cells was counted in the phagocytic cells according to the following equations:

Phagocytic activity= macrophages containing yeast/ total number of macrophages ×100; phagocytic index= number of cells phagocytized/ number of phagocytic cells.

Intestinal histology: The intestines were dissected from three fish per replicate and rinsed in Boin's solution for histology sections. Standard paraffin embedding procedures were used to stain each sample (three cross-sections) using hematoxylin-eosin (Bancroft *et al*., 1996). Intestinal morphology measurements were detected using ImageJ software. A total of six wells, and random villi and villus-associated crypts, were determined for each intestinal cross-section.

Fry feeding trial: The fish were manually selected, sexed, and transferred to conditioning concrete tanks measuring (7×3×1 m). The fish were divided into 5 groups; every group contained three replicate every one contained 50 females and 20 males. The fish was weighed at the beginning and the end of spawning. To study the effects of Alliforte on spawning performance and reproduction of Nile tilapia broodstock, fifteen concrete tanks were used in this study, measuring (7×3×1 m) to obtain five experimental treatments. Each tank was aerated continuously using electric aerator. Water flow was maintained at approximately 2 L/min. The photoperiod was 12 h light:12 h dark. Water temperature was maintained at 28 ºC.

Batches of fry production were collected from all concrete tanks and compared among the tested treatments. Female fish were checked for fry daily. In the early morning females carrying the fry were taken from the concrete tanks and returned after taking out the fry. All fry were weighed, fry samples were taken, weighed, and counted, then fry were placed in concrete basin in hatchery greenhouse. For each concrete tank, the number of spawned female/wk, and fry (after yolk sac absorption) were recorded. After all brood fish were spawning; fry production was quantified in four days.

The fries were weighed and counted after harvesting for every treatment and kept in separate aquaria and fed a ration containing 44.2% protein and 17 alpha methyltestosterone at the rate of 60 mg/kg diet.

Five thousand fry were taken from each group and put in cages with dimension of (7×3×1 m) for about 60 days, fed on 44.2% protein without any additives at a rate of 30% of live weight for about 10 days of fry's age, 20 % thereafter for 10 days and a rate of 10% of life body weight for about 40 days to know the final weight of fry.

The effect of Alliforte on the parameters of fry production that include fry parameters (the initial body weight, final body weight, total weight gain, average daily gain, specific growth rate and fry number at the end of experiment or survival rate) was also estimated.

Statistical analysis: The statistical analyses were determined using SPSS Ver. 16. The data are presented as the mean ± standard error (n = 3). To compare differences among individual means at a significance level of $P \leq 0.05$, all variables were calculated using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests.

RESULTS

Growth performance of broodstocks: The final body weight (FW), body weight gain (BWG), weight gain rate (WGR), average daily gain (ADG), specific growth rate (SGR), protein efficiency ratio (PER), and productive protein value (PPV) were markedly higher, but FCR was lower in fish fed Alliforte at 1, 1.5, and 2 mg/kg than fish fed 0 and 0.5 mg/kg (Table 2). Further, fish fed 1.5 and 2 mg Alliforte/kg had higher growth performance and protein utilization and lower FCR than fish fed 1 mg/kg.

Table 2. Growth performance and feed utilization parameters of Female Nile tilapia fed diets containing different levels of Alliforte (Mean± S.E).

Items	Alliforte (mg/kg)				
	0	0.5	1	1.5	2
Initial body weight (g)	205.66±4.70	205.66±2.96	205.66±0.88	205.66±2.60	205.66±2.64
Final body weight (g)	274.00±3.60^{c}	274.66±2.60^{c}	284.00±3.78^{b}	298.00±1.00^{a}	294.00±2.64^{a}
Body weight gain (g)	68.34±4.66^{c}	69.00±3.46^{c}	78.34±4.66^{b}	92.34±2.84^{a}	88.34±5.00^{ab}
Weight gain rate (WG %)	33.23±2.81^{c}	33.55±2.07^{c}	38.09±2.43^{b}	44.90±1.94^{a}	42.95±3.01^{a}
Average daily gain (g)	1.89±0.12^{c}	1.91±0.09^{c}	2.17±0.12^{b}	2.56±0.07^{a}	2.47±0.13^{a}
Specific growth rate (% /day)	0.79±0.05^{c}	0.80±0.04^{c}	0.89±0.04^{b}	1.03±0.03^{a}	1.00±0.05^{a}
Total feed intake (g/fish)	209.33±4.17	205.33±2.84	210.66±0.88	209.66±2.02	211.00±2.64
Feed conversion ratio[5]	3.06±0.25^{a}	2.98±0.14^{a}	2.69±0.16^{ab}	2.27±0.08^{b}	2.39±0.10^{b}
Protein intake (g/fish)	81.85±2.15	80.28±2.22	82.37±2.31	81.98±3.08	82.50±2.87
Protein efficiency ratio[6]	0.83±0.07^{c}	0.86±0.04^{c}	0.95±0.06^{b}	1.13±0.04^{a}	1.07±0.04^{a}
Protein productive value (%) [7]	14.77±0.07^{c}	17.23±0.74^{ab}	18.21±1.49^{b}	18.93±2.52^{a}	18.62±1.43^{a}
Survival ratio (SR %)[8]	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

Means within the same row with different superscripts are significantly different (P<0.05).

Intestinal histology: The intestinal histological features of Nile tilapia broodstocks fed dietary Alliforte are markedly improved. The villi length, width, and branching of villi indicate increased absorption area (Figure 1).

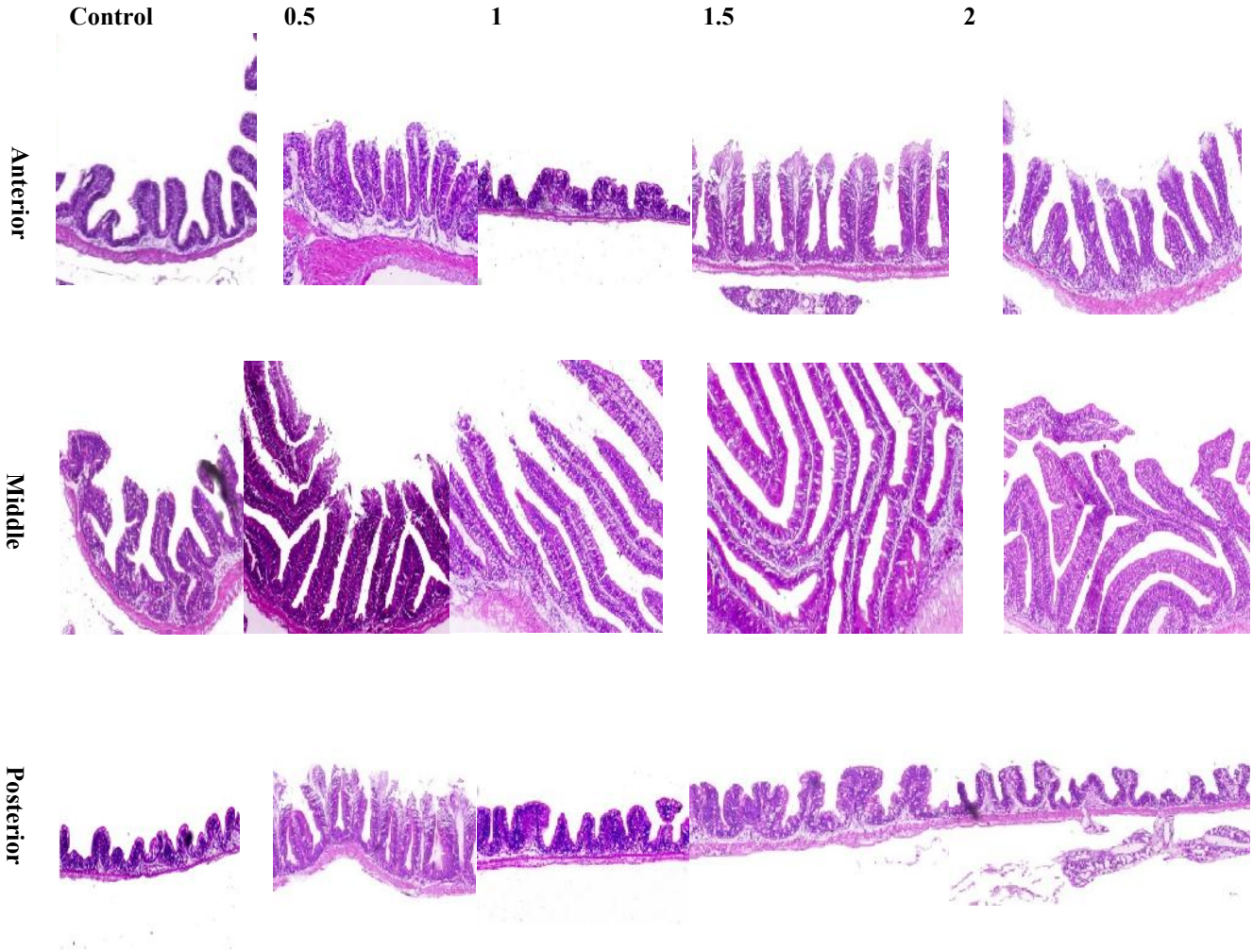

Figure 1. Intestine of Nile tilapia broodstocks fed dietary Alliforte for 36 days.

Broodstock immune response: The lysozyme activity was markedly improved in broodstock fed dietary Alliforte at 0.5, 1, and 1.5 mg/kg than those delivered 0 and 2 mg/kg. Further, fish fed 1 mg/kg had the highest lysozyme activity concerning the remaining groups (Table 3). Dietary Alliforte markedly improved the phagocytic activity, and the highest level was in fish fed 1.5 mg/kg followed by 2 then 1 and 0.5 mg/kg (Table 3).

Seed production: The seed production of Nile tilapia fed dietary Alliforte is presented in Table 4. The average weight of females is increased meaningfully in fish fed 1, 1.5, and 2 mg/kg and fish fed 1.5, and 2 mg/kg had higher females' weight than fish fed 1 mg/kg (Table 4). The absolute fecundity (AF) and relative fecundity (RF) were higher in fish fed 1, 1.5, and 2 mg/kg than 0 and 0.5 mg/kg, while fish fed 1.5 mg/kg had the highest AF and RF (Table 4).

Table 3. Blood immune parameters of Nile tilapia broodstock fed different levels of Alliforte.

Blood parameter	Alliforte (mg/kg)				
	0	0.5	1	1.5	2
Lysozyme activity (unit/ml)	33.540.34^{d}	38.32±0.04^{b}	39.55±0.21^{a}	35.39±0.16^{c}	33.68±0.17^{d}
Phagocytic activity (%)	23.41±0.08^{e}	25.32±0.06^{d}	27.46±0.11^{c}	30.33±0.09^{a}	28.32±0.57^{b}
Phagocytic index	2.60±0.01	2.37±0. 02	2.45±0.03	2.50±0.01	2.47±0.00

*Values expressed as means ± SE (n = 3). Means in the same row with different superscripts are significantly different at ($P < 0.05$).

Table 4. Seed production of Nile tilapia fed diets containing different levels of Alliforte (Mean± S.E)..

Alliforte (mg/kg)	N. of female	Mean weight per female (g)	Absolute Fecundity (AF)	Relative fecundity (RF)	
			Total fry No Per tank (x1000)	Seed No. / Female	Seed No. / g fish
0	50	274.66±2.60^{c}	24666.66±881.91^{d}	493.33±17.63^{d}	1.79±0.06^{d}
0.5	50	274.00±3.60^{c}	26000.00±577.35^{d}	520.00±11.54^{d}	1.90±0.06^{d}
1	50	284.00±3.78^{b}	29000.00±577.35^{c}	580.00±11.54^{c}	2.03±0.01^{c}
1.5	50	298.00±1.00^{a}	35333.33±333.33^{a}	706.66±6.66^{a}	2.36±0.01^{a}
2	50	294.00±2.64^{a}	32000.00±577.35^{b}	640.00±11.54^{b}	2.17±0.02^{b}

Means within the same row with different superscripts are significantly different ($P<0.05$).

Fry growth performance: The WGR, SGR, and PER were markedly improved in fish treated with 0.5, 1, and 1.5 mg/kg while the FCR was decreased in the same groups (Table 5). The fry survival rate was markedly higher in groups treated with 1 and 1.5 mg/kg than 0 and 2 mg/kg without significant differences with 0.5 mg/kg (Table 5).

Table 5. Growth performance and feed utilization parameters of Nile tilapia fry fed diets containing different levels of Alliforte (Mean± S.E).

Items	Alliforte (mg/kg)				
	0	0.5	1	1.5	2
Initial body weight (g)	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Final body weight (g)	1.16±1.01^{d}	1.28±0.02^{c}	1.36±0.02^{b}	1.62±0.03^{a}	1.23±0.01^{c}
Body weight gain (g)	1.14±0.00^{d}	1.26±0.02^{c}	1.34±0.02^{b}	1.60±0.03^{a}	1.21±0.01^{c}
Weight gain rate (WG %)	5700±12.21^{d}	6300±14.25^{c}	6700±14.60^{b}	8000±18.25^{a}	6050±13.21^{d}
Average daily gain (g)	0.019±0.00^{d}	0.021±0.00^{c}	0.023±0.00^{b}	0.027±0.00^{a}	0.020±0.00^{c}
Specific growth rate (% /day)	6.77±1.45^{d}	6.93±1.54^{c}	7.03±1.62^{b}	7.32±1.66^{a}	6.87±1.50^{d}
Total feed intake (g/fish)	1.81±0.04^{c}	1.84±0.04^{b}	1.88±0.05^{b}	2.09±0.05^{a}	1.87±0.04^{b}
Feed conversion ratio[5]	1.59±0.03^{d}	1.46±0.02^{c}	1.40±0.01^{b}	1.31±0.01^{a}	1.55±0.02^{d}
Protein intake (g/fish)	0.80	0.81	0.83	0.93	0.83
Protein efficiency ratio[6]	1.43±0.03^{d}	1.56±0.03^{c}	1.61±0.04^{b}	1.72±0.05^{a}	1.46±0.03^{d}
Survival ratio (SR %)[7]	85.22±3.54^{b}	89.54±3.22^{ab}	90.41±3.78^{a}	93.56±3.98^{a}	87.75±3.44^{b}

Means within the same row with different superscripts are significantly different ($P<0.05$).

DISCUSSION

The optimization of feeding is vital for improving the reproduction and well-being of fish broodstock and offspring (Kari *et al*., 2021; Karal Marx *et al*., 2020). Using feed additives is encouraged to strengthen digestion capacity, health status, immune response, and reproduction status in aquatic animals (Dawood *et al*., 2018). *Saccharomyces cerevisiae* and garlic additives have been incorporated in aquafeed and resulted in enhanced performances, health status, and hatchability of fish brooders (Abu-Elala *et al*., 2021; Mekawey, 2019). Consequently, high survivability, well-being, and productivity are associated with using dietary *S. cerevisiae* and garlic. Previously, it has been reported that using *S. cerevisiae* or garlic extracts can enhance the productivity, hatchability, and survivability of Nile tilapia broodstocks and offspring (Abu-Elala *et al*., 2021; Mekawey, 2019). Nevertheless, the present study exclusively investigates using a mixture of dietary *S. cerevisiae* or garlic extracts on the performances of Nile tilapia brooders and offspring.

The incorporation of Alliforte, a source of *S. cerevisiae* and garlic extract mixture, enhanced the growth performance of Nile tilapia brooders and offspring. Similarly, Abu-Elala *et al*. (2021) and Mekawey (2019) Nile tilapia fed *S. cerevisiae*, or garlic extract, resulted in increased growth performance of broodstocks and offspring. The enhancement of growth performance is related to the high feed utilization and efficient digestibility of nutrients in fish intestines (Dawood, 2021). Indeed, dietary *S. cerevisiae* can enhance the digestibility of nutrients in fish intestines by regulating intestinal microbial balance and diversity of beneficial microbiota (Gonçalves and Gallardo-Escárate, 2017; Haygood and Jha, 2018). In this sense, the elevated digestive enzymes regulate the feed utilization and led to well digestibility. On the other hand, garlic extracts, especially allicin, are involved in improving the anti-bacterial capacity in fish intestines and reduce the harmful impacts on intestinal digestion capacity (Valenzuela-Gutiérrez *et al*., 2021). The results also showed increased feed utilization and protein efficiency in fish brooders and offspring fed dietary *S. cerevisiae* and garlic extract.

The detected histomorphology indices in fish intestines indicated that Nile tilapia fed dietary Alliforte had enhanced intestinal features. The villi length, width, and absorption area were increased, explaining the enhanced feed utilization and growth performance in Nile tilapia brooders fed dietary Alliforte. The enhancement in intestinal features illustrates that absorption area in fish intestines increased by dietary Alliforte and thereby increased feed utilization and growth performance. In the same line, dietary *S. cerevisiae* enhanced the intestinal features as indicated earlier by Abu-Elala *et al*. (2021). Further, dietary garlic extracts enhanced the intestinal histological properties in Nile tilapia, as declared by Foysal *et al*. (2019). It cannot be ignored that the enhanced feed utilization and growth performance in Nile tilapia offspring are probably related to the maternal influence of dietary Alliforte (Little and Hulata, 2000). In this regard, Nile tilapia offspring's high feed utilization and growth performance can be related to efficient feed utilization, health status, intestinal digestion capacity, and the maternal effect resulting from Alliforte feeding.

The results also indicated high hatchability, seed production, and survivability of Nile tilapia offspring for brooders treated with dietary Alliforte. The results are in line with Abu-Elala *et al*. (2021) and Mekawey (2019), who stated that Nile treated with *S. cerevisiae* or garlic extracts had increased hatchability, seed production, and offspring survivability. It has been reported that efficient nutrition strategies may enhance the broodstocks' spawning capacity via the development of gonadal and seed quality (Bhujel *et al*., 2020). The improved intestinal morphology and reduced energy needed for cellular nucleotide synthesis, which also enables energy for the metabolic functions required to enhance the growth and survivability of fish larvae (Guo *et al*., 2017), is likely related to the promoted growth and feed utilization of brooders treated with Alliforte. The trained maternal immunity resulting from broodstock feeding with Alliforte is another reason for the increased seed production, hatchability, and offspring survivability (Ghaedi *et al*., 2015; Izquierdo *et al*., 2001).

The inclusion of *S. cerevisiae* and garlic extract in aquafeed are inducers for activating innate immunity in aquatic animals (Abu-Elala *et al*., 2021; Mekawey, 2019). The study showed enhanced lysozyme and phagocytic activities in Nile tilapia brooders fed dietary Alliforte. Similarly, the fortification of *S. cerevisiae* or garlic extracts enhanced the lysozyme and phagocytic activities in Nile tilapia (Abu-Elala *et al*., 2021; Mekawey, 2019). The activation of immunity in the broodstocks is probably related to the role of *S. cerevisiae* and its bioactive substances (e.g., β-glucan) in improving the local intestinal immunity, which entirely activates the whole-body immunity (Mugwanya *et al*., 2021; Valenzuela-Gutiérrez *et al*., 2021). Further, garlic extracts have antibacterial substances (e.g., allicin) with antioxidative potential (e.g., flavonoids and polyphenols) associated with activated intestinal immunity and thereby the whole-body immunity (Valenzuela-Gutiérrez *et al*., 2021). As long as lysozyme and phagocytic activities showed high levels, the brooders' resistance against pathogenic invaders increased, and the mortality rates reduced (Dotta *et al*., 2014). Hence, the trained maternal immunity may explain the high seed production survivability of Nile tilapia offspring's fed dietary Alliforte.

Conclusion: In conclusion, dietary Alliforte has positively influenced the growth performance, health status, and seed production of Nile tilapia broodstocks. Further, dietary Alliforte improved the fry performances and survival rates.

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