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Original Article

Chasteberry, *Vitex agnus-castus*, extract as a natural enhancer for growth, chemical composition and hematological parameters in Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Integrating natural plant-based additives into aquafeeds has gained growing interest as a sustainable strategy to to improve growth and health in aquaculture. *Vitex agnus-castus*, commonly known as chasteberry, is recognized for its bioactive compounds with antioxidant, anti-inflammatory, and endocrine-modulating properties. This study aimed to evaluate the effects of dietary supplementation with chasteberry tree extract (CTE) on growth performance, muscle chemical composition, and hematological parameters in Nile tilapia. A total of 72 pre-market-sized tilapia (average weight 139.6±0.46 g) were randomly distributed into 12 net cages (1 m^3). Four experimental groups were designed to supplement the basal diet with 0, 0.25, 0.5, and 1.0 g CTE per 100 g diet in triplicate. Fish were fed 3% of body weight, twice a day, six days a week, for 45 days. Results revealed that fish fed diets supplemented with 0.5% CTE exhibited significantly higher final body weight, weight gain, specific growth rate and improved feed conversion ratio compared to other groups ($P<0.05$), with no significant difference in survival rate. Chemical analysis of muscle tissue showed that 0.25% and 0.5% CTE groups had significantly increased moisture and ether extract content ($P<0.05$), while crude protein, ash and gross energy showed no significance. Hematological analyses showed that the 0.5% CTE group had significantly elevated white blood cells, hemoglobin, hematocrit, glucose, and total protein levels, indicating an enhanced immune and metabolic response. These findings clearly recommend the use of 0.5% CTE as a promising natural feed additive to enhance tilapia growth and physiological status, contributing to more sustainable aquaculture systems.

Key words: Chasteberry, *Vitex agnus-castus*, Nile tilapia, growth rate, haematological parameters

1. INTRODUCTION

Aquaculture is one of the most important modern agricultural activities that contributes effectively to achieve global food security, especially in light of increasing population growth and declining the dependence on natural aquatic and fishery resources (FAO, 2022). This sector represents a major source of low-cost animal protein and is highly efficient in converting food into growth, compared to other livestock (Naylor *et al.*, 2021).

The world has witnessed remarkable development in aquaculture production in recent decades, with global production increasing from approximately 5 million tons in the 1980s to more than 122 million tons in 2021, representing more than 50% of total

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global fish production (FAO, 2022). This increase is due to advances in aquaculture technologies, improved management of rearing systems, and the use of natural feed additives and biological enhancers to improve growth and disease resistance (Tidwell, 2023).

Nutrition is one of the most influential economic and environmental factors in aquaculture systems, representing about 60% of the total production cost in aquaculture (Responsible Seafood Advocate, 2021). This significant finance necessitates improving feed utilization efficiency to maximize productivity and minimize waste. Beyond the economic implications, the environmental footprint of aquaculture feed is considerable. For instance, feed loss or improper use leads to increased organic accumulation in water, negatively impacting water quality and contributing to environmental problems such as eutrophication and ecosystem degradation (Boyd and Chatvijitkul, 2017).

Therefore, improving feed utilization not only enhances farm profitability but also contributes to reducing the environmental impact. To mitigate these environmental concerns, there is a growing emphasis on developing cost-effective and sustainable feed alternatives. *Vitex agnus-castus*, commonly known as chasteberry, is a medicinal plant traditionally used in human medicine for its hormonal balancing properties in females (Sirotkin, 2025). Its pharmacological potential is attributed to a diverse phytochemical profile, including flavonoids, diterpenes, iridoids, and essential oils (Ulusoy *et al.*, 2024; Kızılbey *et al.*, 2024).

Additional constituents such as ketosteroids and progestins further contribute to its endocrine-modulating effects (Brakat *et al.*, 2025).

Historically, *V. agnus-castus* was also used to reduce libido and modulate male hormones, possibly through the suppression of prolactin and testosterone levels (Webster et al., 2002; Azadbakht *et al.*, 2005). These properties make it a compelling candidate for further investigation in reproductive and endocrine-related applications, including its potential role in aquaculture.

Recent studies have highlighted the multifaceted biological benefits of chasteberry tree extract (CTE) in aquaculture, particularly its role in enhancing fish immunity, growth performance, and overall health. For instance, Salem and Barkah (2025) reported that dietary inclusion of a methanol–water extract from *Vitex agnus-castus* seeds significantly improved hematological parameters in juvenile Rainbow trout (*Oncorhynchus mykiss*). In a related study, Salem and Mohamed (2025) found that dietary supplementation with CTE at 50–200 mg/kg enhanced growth performance and digestive enzyme activity in the same species, further supporting its physiological benefits. Moreover, in carp, Khoris *et al.* (2024) demonstrated that dietary supplementation with CTE at 1 g/kg not only improved survival rate and immune function but also enhanced antioxidant capacity when fish were challenged with *Vibrio anguillarum*, with notable antibacterial activity confirmed through in vitro assays. Similarly, in goldfish (*Carassius auratus*), Rashmeei *et al.* (2020) showed that dietary inclusion of CTE at levels up to 15 g/kg enhanced both mucosal and systemic immune responses, upregulated key immune- and growth-related genes (e.g., *TNF-α*, *IGF-1*, *ghrelin*), and improved resistance to *Aeromonas hydrophila*. Among the tested doses, the 1.5% inclusion level yielded the most favorable outcomes for growth and immune modulation.

In addition to its immunostimulatory and growth-promoting properties, the reproductive effects of *V. agnus-castus* have also been investigated. Zamani *et al.* (2018) reported that dietary supplementation at 1250 mg/kg significantly improved fecundity, gonadosomatic index (GSI), and larval output in *Xiphophorus helleri*. Furthermore, Gholampour *et al.* (2020) observed feminization effects in zebrafish larvae at a 15 g/kg inclusion level, while Hosseinzadeh *et al.* (2018) demonstrated that intramuscular injections of 30–50 mg/kg in female goldfish led to reduced estradiol and testosterone levels, along with suppressed ovarian development. Collectively, these findings position CTE as a promising natural alternative to antibiotics and synthetic additives, offering a functional and sustainable feed strategy to support health, productivity, and reproductive regulation in intensive aquaculture systems.

Nile tilapia (*Oreochromis niloticus*) is a prominent aquaculture species, ranking third in the world during the past five years (2015–2019) after grass carp and silver carp (Deng *et al.,* 2023). Its 2019 production was 6.5 million metric tons, up 4% from 2018 (Fujaya *et al.,* 2023). Tilapias are regarded as essential food fish in most developing nations due to their rapid growth rates, toughness, and ability to grow in a wide range of environmental conditions, as well as their high marketability and preferences (Abdel-Aziz *et al.,* 2023). However, there is insufficient information about the effect of graded levels of CTE on the productive performance and physiological health of Nile Tilapia on the molecular level. Therefore, the current study conducted to investigate the effects of dietary CTE on the growth performance, chemical composition, and hematological parameters of Nile tilapia (*O. niloticus*) at the pre-market size stage.

2. MATERIALS AND METHODS

The current study was carried out at Desert Aquaculture Research Unit, Faculty of Marine Aquaculture and Fisheries, Arish University, Arish, North Sinai, Egypt. In addition to having both indoor and outdoor ponds, this unit occupies five feddans and is mainly dependent on groundwater from wells. The coordinates are 31°07'48''N, 33°49'40''E.

2.1. *Ethical standards*

The experiment was carried out in accordance with the protocol and ethical guidelines for animal care that were authorized by Suez Canal University, Ismailia, Egypt.

2.2. *Design of trial and experimental fish*

Nile tilapia (*Oreochromis niloticus*) fish were obtained from private fish farms of Kafr El-Sheikh Governorate at a pre-market stage. The fish were quarantined for two weeks, disinfected, and acclimated gradually upon arrival. Subsequently, an additional two-week acclimatization period was conducted in the experimental environment before the trial started. Dead fish during this time were replaced by healthy ones, and they were fed pellets that contained 30% crude protein on an *ad libitum* basis (Skretting Egypt Co., Belbies, Sharkia, Egypt). Once the fish had acclimated, they were gathered, anesthetized with clove oil, collectively weighed, and then randomly assigned to 12 net cages (1 x 1 x 1 m, each with a volume of 1 m^3/ hapa) with a stocking density of six fish per cubic meter. A 30% CP basal diet was locally formulated. Table 1 displayed the chemical proximate analysis and ingredient composition of the basal diet. Every treatment was triplicated.

2.3. *Rearing conditions*

72 Nile tilapia fish with an initial average body weight of 139.6±0.46 g were randomly stocked into 12 hapa (1 m^3 / hapa) installed inside a concrete pond. Water was sourced from 40 meters deep well with 30% daily exchange rate. Hapas were well aerated using an air blower (Model: XGB-3800, 380 V, 7.8 A, 3.8 KW, 2800 RPM).

2.4. *Diet formulation and feeding*

Synthetic chasteberry tree extract (CTE) was provided from Shana Herbal Company, German and shipped by Amazon (online shopping) in dried powdered. Four experimental diets were prepared by adding various levels of CTE —0.0%, 0.25%, 0.5% and 1.0%— to the basal diet biweekly. Practical diets were prepared by mixing ground dry ingredients with cool water, then pelleted using a meat mincer (KENWOOD MG51). The pellets were dried at 60°C for 24 hours in an oven (CONNECTION OVEN/CO-150) and kept in plastic bags for use. Each diet was hand-fed to three replicate hapas at a rate of 3% of the total biomass twice a day at 9:00 and 3:00 PM, six days a week, for 45 days. Fish were individually weighed every 14 days and feed amounts were readjusted according to the new weights.

2.5. *Measurements of growth*

At the end of the experiment, fish were fasted for 24 hours. Then, fish from each hapa were sedated using clove oil at a concentration of 100 µL/L of water (El-Dakar et al., 2021), individually weighed and their body lengths were recorded.

Table (1). Ingredients and a proximate composition (%) of basal diet

Ingredient	**Amount (g/100 g diet)**
Fishmeal (65%)	10
Corn gluten	10
Soybean meal (44%)	28
Yellow corn	10
Wheat bran	10
Wheat milling by products	25
Soybean oil	2.5
Linseed oil	2.5
Vit. & Min. Premix[1]	2
Total	**100**
Chemical composition of basal diet on dry matter basis (DM %)	
Dry matter (g/100g diet)	93.38
Crude protein (CP)	30.69
Ether extract (EE)	7.45
Crude fiber (CF)	6.97
Ash	5.40
Nitrogen free extract (NFE)[2]	49.49
Gross energy (kcal/100 g diet)	452
Digestible energy (kcal/100 g diet)	388
P/E ratio (mg CP / kcal DE)[3]	79

1- One kilogram of premix contain: 40.00g, 125.00g, 171.40g, 61.40g, 55.00g, 2.50g, 1.50g, 0.50g, 0.05g, 0.24g, 0.30g, 11.90g, 0.04g, 0.50g, 0.02g, 0.40g, 8.00g, 1.70g, 3.00gm, 4.00gm, 0.40g, 1.00g, 210.50g, 11.40g and up to 1000 g per kg of molasse, sodium bicarbonate, magnesium oxide, monocalcium phosphate, Triticum durum, Vitamin E, A, D3, B1, B2, Ca-D- pantothenate, manganese sulphate, vitamin B6, B12, K3, copper sulphate, *calcium iodate*, *organic selenium*, *sodium* selenite, cobalt chloride, antioxidants (BHT), phosphorus, zinc and sodium chloride, respectively.
2- Nitrogen free extract (calculated by differences) (NFE) = 100- (CP% + EE% + CF% + Ash%)
3- P/E; Protein energy ratio.

Growth performance and feed utilization efficiency parameters were calculated according to Ricker (1979) as follows:

1. **Body weight gain (WG)** = Final body weight (g) – Initial body weight (g)
2. **Average daily weight gain (ADG)** = WG (g) / Duration of feeding trial (days)
3. **Specific growth rate (SGR, %/ day)** = 100 $(\ln W_f - \ln W_i)/t$), where W_i and W_f are the initial and final weights (g), and *t* is the time of the experiment (days).
4. **Survival rate (SR%)** = (number of fish at the end of the trial/ number of fish at the initial stock) × 100
5. **Feed conversion ratio (FCR)** = Dry feed intake (g) / Fish weight gain (g)

2.6. *Fish and diet chemical composition analysis*

Dry matter, ether extract, crude protein, ash, and nitrogen-free extract (NFE) followed the AOAC (2023) methodologies to analyze the chemical composition of the diet ingredients and fish samples. The energy coefficients of 4.2, 5.65, and 9.45 kcal/g for carbohydrates, proteins, and lipids, respectively, were used to determine the gross energy content of the fish samples and experimental diets (NRC, 1993). Standard physiological fuel values of 4 kcal/g for proteins and carbohydrates and 9 kcal/g for lipids were used to determine digestible energy (DE) (Garling and Wilson, 1976).

2.7. *Water quality*

During the period of the trial, water quality measures were periodically checked.

Table (2). Physicochemical water quality parameters recorded during the 45-day feeding trial in Nile tilapia culture tanks.

Parameters	Treatments				SEM[1]
	0%	0.25%	0.5%	1%	
Water temperature (°C)	27.8[a]	27.6[a]	27.7[a]	27.8[a]	0.520
pH[2]	8.5[a]	8.5[a]	8.5[a]	8.5[a]	0.303
DO[3] (mg/L)	6.4[a]	6.5[a]	6.6[a]	6.7[a]	0.482
TAN[4] (mg/L)	0.40[a]	0.41[a]	0.40[a]	0.39[a]	0.964
Salinity (g/L)	4.0[a]	4.0[a]	4.0[a]	4.0[a]	1.000

* Values in the row having a common superscript letter are not significantly different (P>0.05).

1. Standard error of the means derived from the analysis of variance.
2. Potential of Hydrogen.
3. Dissolved Oxygen.
4. Total Ammonia Nitrogen.

Water temperature and dissolved oxygen (DO) were recorded daily using a waterproof portable dissolved oxygen meter (HANNA, HI98193). The pH was measured daily by a multiparameter benchtop meter capable of assessing pH, ORP, EC, TDS, and salinity (HANNA, HI2550-02, Romania). The concentration of total ammonia was measured using the usual chemical methods outlined by APHA (2000). These parameters were monitored to ensure optimal water quality conditions for Nile tilapia during the feeding trial.

2.8. *Blood collection and hematobiochemical indices*

At the end of the experiment, fish from each treatment group (in triplicate) were sedated using clove oil. Blood samples were collected individually from the hearts of sedated fish, after which the fish were euthanized. The collected blood was transferred to tubes containing ethylenediaminetetraacetate (EDTA) as an anticoagulant for hematological analysis. Hematological parameters, including white blood cell count (WBCs), red blood cell count (RBCs), hemoglobin concentration (Hb), and hematocrit (Hct), were measured using an automated hematology analyzer (Dymind DH 36, Made in China). Serum biochemical parameters such as glucose, urea, total protein, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined using a Rayto RT-9700 semi-automated chemistry analyzer and commercial diagnostic kits.

2.9. *Statistical analysis*

Statistical analysis was conducted using one-way analysis of variance (ANOVA) (Steel and Torrie, 1960) to determine differences among treatment groups. Significant differences between means were identified by Duncan's multiple range test at a significance level of 0.05. All analyses were conducted using SPSS software version 22 (IBM Corp., Armonk, NY, USA). Data are presented as mean ± standard error of the mean (SEM).

3.RESULTS

3.1. *Growth criteria and body indices*

Fish fed diets containing 0.5% CTE exhibited significantly higher weight gain (WG), average daily gain (ADG), and specific growth rate (SGR) compared to other treatment groups (P<0.05). No significant differences (P>0.05) were observed among the other groups for these growth parameters. Survival rates (SR) did not differ significantly across all treatments (P>0.05). Notably, the group fed 0.5% CTE displayed the lowest feed conversion ratio (FCR), indicating enhanced feed efficiency (Table 3).

Table (3). Growth criteria, survival rate, and feed utilization of pre-market stage Nile tilapia fed on diets containing varying amounts of CTE for 45 days.

Item	Treatment				
	0%	0.25%	0.5%	1%	SEM[1]
Initial weight, g	139.67[a]	140.67[a]	138.33[a]	139.73[a]	0.46
Final weight, g	217.57[b]	218.69[b]	224.23[a]	216.04[b]	1.14
Weight gain, g	77.90[b]	78.03[b]	85.93[a]	76.30[b]	1.46
ADG[2], g	1.73[b]	1.73[b]	1.91[a]	1.70[b]	0.03
SGR[3], %/day	0.98[b]	0.98[b]	1.07[a]	0.97[b]	0.02
Survival rate%	83.30[a]	83.30[a]	83.30[a]	77.70[a]	3.77
FI[4] (g/fish)	124.12[a]	122.00[a]	123.32[a]	125.53[a]	0.669
FCR[5]	1.87[c]	2.30[b]	1.76[d]	2.71[a]	0.12
PI[6] (g/fish)	38.09[a]	37.44[a]	37.85[a]	38.53[a]	0.669
PER[7]	2.05[a]	2.08[a]	2.28[a]	1.99[a]	0.122

* Values in the row having a common superscript letter are not significantly different (P>0.05).
1.Standard error of the means derived from the analysis of variance. 2. ADG: Average daily gain. 3. SGR: Specific growth rate. 4. FI: Feed intak. 5. FCR: Feed conversion ratio. 6. PI: Protein intake. 7.PER: Protein efficiency ratio.

3.2. *Chemical compositions of the fish muscle*

The proximate composition (on a wet weight basis) and gross energy content (on a dry matter basis) of Nile tilapia muscle were significantly affected by the dietary inclusion of CTE over the 45-day feeding trial (Table 4). Moisture content varied significantly among treatments (P<0.05), with the highest value recorded in fish fed 0.25% CTE (84.62%) and the lowest in the 1% group (63.82%). Crude protein content increased significantly with rising CTE levels, peaking at 29.51% in the 1% group, while the lowest value (12.80%) was found in the 0.25% group (P<0.05).

Table (4). Proximate chemical composition (wet weight basis) and gross energy (dry matter basis) of Nile tilapia muscle fed dietary CTE for 45 days.

Treatments	Moisture %	Crude protein %	Ether extract %	Ash %	Gross energy kcal/100 g diet
0%	67.63[c]	26.77[b]	0.61[b]	1.68[a]	485[a]
0.25%	84.62[a]	12.80[d]	0.45[c]	1.14[a]	497[a]
0.5%	78.82[b]	17.54[c]	0.66[b]	1.38[a]	497[a]
1%	63.82[d]	29.51[a]	1.10[a]	1.98[a]	489[a]
SEM[1]	3.16	0.00	0.00	0.54	0.15

* Values in the row having a common superscript letter are not significantly different (P>0.05).
1. Standard error of the means derived from the analysis of variance.

Ether extract followed a similar trend, reaching the highest level (1.10%) in the 1% CTE group compared to 0.45% in the 0.25% group. Ash content showed no significant differences across treatments (P>0.05). Interestingly, gross energy (on a dry matter basis) remained statistically unchanged across all dietary treatments, with values ranging between 485 and 497 kcal/100 g diet (P>0.05). Overall, the inclusion of 1% CTE in the diet enhanced muscle protein and lipid deposition without negatively affecting energy content, suggesting a potential role for CTE in improving muscle quality in Nile tilapia.

3.3. *Blood hematology indicators*

As presented in Table (5), significant differences (P<0.05) were observed among the fish groups in hematological parameters. The highest red blood cell (RBCs) count (1.72 × 10^6 /µL) was recorded in the control group, followed by the group fed 0.5% CTE (1.65 × 10^6 /µL).

In contrast, white blood cell (WBCs) count, hemoglobin (Hb) concentration, and hematocrit (Hct) values were highest in the 0.5% CTE -fed group, with values of 56.66 × 10^3 /µL, 8.8 g/dL, and 24.6 vol. %, respectively. These findings suggest that dietary inclusion of CTE at 0.5% may enhance certain hematological indicators in Nile tilapia.

Table (5). Hematological indicators of pre-market stage Nile tilapia fed on dietary CTE for 45 days.

Item	Treatment				SEM[1]
	0%	0.25%	0.5%	1%	
RBCs[2], 10^6 uL	1.72[a]	1.15[d]	1.65[b]	1.31[c]	0.07
WBCs[3], 10^3 uL	46.18[b]	37.12[d]	56.66[a]	39.00[c]	2.31
Hb[4], g/dL	8.20[b]	7.90[c]	8.80[a]	6.00[d]	0.32
Hct[5], vol. %	21.40[b]	18.70[c]	24.60[a]	15.50[d]	1.01

* Values in the row having a common superscript letter are not significantly different (P>0.05).

1. Standard error of the means derived from the analysis of variance. 2.RBCs: Red blood cells. 3.WBCs: White blood cells. 4. Hb: Haemoglobin. 5.Hct: Haematocrit.

3.4. *Serum chemical analyses*

As shown in Table (6), no significant differences (P>0.05) were observed among the treatment groups in the serum levels of AST, ALT, urea, creatinine, and cholesterol. However, significant variations (P<0.05) were detected in glucose and total protein concentrations. The group fed 0.5% CTE exhibited the highest glucose level (159.0 mg/dL), while the 1% extract group showed the highest total protein concentration (8.81 g/L), followed by the 0.5% group (7.10 g/L). The control group had the lowest values for both parameters. These results indicate that dietary supplementation with chasteberry extract may influence glucose metabolism and protein synthesis in female Nile tilapia.

Table 6. Blood biochemical parameters of pre-market stage Nile tilapia fed on dietary CTE for 45 days.

Item	Treatment				SEM[1]
	0%	0.25%	0.5%	1%	
Glucose, mg/dL	133.5[b]	139.0[ab]	159.0[a]	78.0[c]	11.61
Total protein, g/L	6.1[c]	7.74[b]	7.10[b]	8.81[a]	0.38
AST[2], U/L	44.35[a]	40.95[a]	31.70[a]	48.05[a]	4.78
ALT[3], U/L	28.20[a]	28.40[a]	22.15[a]	19.90[a]	2.36
Urea, mg/dL	15.85[a]	16.70[a]	16.35[a]	16.80[a]	0.18
Creatinine, mg/dL	0.48[a]	0.56[a]	0.47[a]	0.51[a]	0.02
Cholesterol, mg/dL	123.0[a]	150.5[a]	121.0[a]	186.0[a]	12.95

* Values in the row having a common superscript letter are not significantly different (P>0.05).

1. Standard error of the means derived from the analysis of variance. 2. AST: Aspartate aminotransferase.
2. ALT: Alanine aminotransferase.

4. DISCUSSION

Growth is achieved when the nutrients provided by feed intake exceed the body's basal energy demands and activity levels (Cho and Bureau, 1995). Several research have studied the use of herbal extracts and plant-derived compounds as growth promoters, for example the use of Chinese herbs in hybrid grouper *Epinephelus fuscoguttatus*♀×*E. lanceolatu* ♂ (Cai *et al.*, 2023), *Curcuma longa*, *Rosmarinus officinalis*, and *Thymus vulgaris* in Nile tilapia *O. niloticus* (Hassan *et al.*, 2018), figwort (*Scrophularia striata*) in common carp *Cyprinus carpio* (Hosseini *et al.*, 2022), *Mentha piperita* in Caspian white fish *Rutilus frisii kutum* (Adel *et al.*, 2015), mixture of *Zingiber officinale* and curcumin in rainbow trout *Oncorhynchus mykiss*, and fenugreek *Trigonella foenum graecum* in gilthead seabream *Sparus aurata* (Awad *et al.*, 2015*)*.

The findings of the present study revealed that the growth parameters of Nile tilapia fed with 0.5% *Vitex agnus-castus* extract (CTE) were significantly improved. Notably, the weight gain in the CTE-fed group was approximately 1.1 times greater than the control, suggesting that this concentration enhances growth performance at the pre-market stage. This improvement may be attributed to the physiological properties of CTE, which potentially enhance metabolic processes, digestive function, and nutrient utilization efficiency (Khoris *et al.*, 2024). According to Khoris *et al.* (2024), phytochemical screening of CTE revealed the presence of bioactive compounds including flavonoids, diterpenoids, and phenolic acids, all of which are known to exert antibacterial, antioxidant, and metabolic regulatory effects. These compounds, particularly flavonoids and iridoids, may stimulate digestive enzyme activity and modulate endocrine pathways involved in growth and feed efficiency. Similar classes of bioactives have been reported by Ramezani et al. (2010), confirming the presence of essential oils, fatty acids, and other metabolites with therapeutic potential. Furthermore, these constituents may enhance metabolic activity, nutrient absorption, and growth hormone secretion in fish (Rashmeei *et al.*, 2022), thereby contributing to improved weight gain. It may also be assumed that CTE's hormonal regulatory action (Hamood *et al.*, 2024) indirectly supports metabolic and growth processes. These findings are consistent with previous studies demonstrating the growth-promoting potential of various medicinal plant extracts in aquaculture species (Kannan *et al.*, 2022; Fujaya *et al.*, 2023; Abdel-Aziz *et al.*, 2023).

The higher SGR and lower FCR values in the 0.5% CTE group provide additional evidence for the growth-promoting properties of CTE. The higher the SGR, the faster the growth rate, while the lower the FCR, the better nutrient utilization efficiency. Similar findings had been observed in studies investigating the effects of medicinal plant additives on FCR and SGR in fish (Yilmaz et al., 2023; Abu-Zahra *et al.*, 2024). Rashmeei *et al.* (2022) indicated that two gram-goldfish (*Carassius auratus*) fed a 1.5% CTE-supplemented diet had significantly higher FCR and SGR among groups.

The consistent survival rates across all fish groups indicate that CTE does not adversely affect fish survival. This is an important factor in determining the safety of herbal feed additives in aquaculture. Enayat Gholampour *et al.* (2020) and Rashmeei *et al.* (2022) reported that fish fed with varied levels of CTE (0, 0.5%, 1%, and 1.5%) had no negative impact on survival between treatments. In the current study, dietary inclusion of CTE markedly influenced the proximate composition of Nile tilapia muscle after 45 days of feeding. Most notably**,** a dose-dependent increase in crude protein content was observed, with the highest value (29.51%) recorded in fish fed the 1% CTE diet. This enhancement may be attributed to improved feed utilization efficiency or the upregulation of digestion-related genes, consistent with mechanisms previously reported for phytogenic additives (Moyo *et al.*, 2025). Conversely, fish fed the 0.25% CTE diet exhibited a paradoxical reduction in protein content (12.80%), possibly due to insufficient metabolic stimulation or inefficient nutrient assimilation at suboptimal doses. In addition to protein, the 1% CTE group also demonstrated a moderate increase in ether extract ontent (1.10%), suggesting enhanced lipid dposition. This trend could be linked to the anabolic activity of chasteberry's bioactive flavonoids, which have been shown to modulate lipid metabolism and promote energy storage (Khoris *et al.*, 2024). Interestingly, moisture content exhibited an inverse pattern, with the lowest value (63.82%) observed in the 1% CTE group and the highest in the 0.25% group. This inverse correlation between moisture and protein/lipid contents may reflect the formation of denser, more nutrient-rich muscle tissue in fish receiving optimal levels of phytogenic supplementation (Rashmeei *et al.*, 2020). These findings are further supported by previous research on the effects of phytogenic additives on fish body composition. For instance, Maghawri *et al.* (2024) demonstrated that dietary inclusion of 2% dandelion (*Taraxacum officinale*) significantly increased muscle protein and lipid contents while reducing moisture and ash in *O. niloticus*, echoing the results observed with high-dose CTE in the present study. Similarly, Nyadjeu (2021) reported that supplementation with 1% garlic in *Clarias gariepinus* juveniles significantly enhanced crude protein (27.10%), lipid (3.52%), ash, and energy retention, emphasizing the potential of certain medicinal plants to improve tissue nutrient deposition. However, it is important to note that not all phytogenic additives exert such effects. Abdel-Tawwab and Abbass (2017), for example, found no significant changes in the whole-body composition of *Cyprinus carpio* fed turmeric powder (*Curcuma longa*), highlighting that the response to phytogenic additives is likely influenced by plant species, bioactive compound profiles, and inclusion levels. In contrast to these studies, Lee *et al.* (2021) reported that dietary supplementation with vegetable and fruit juice processing by-products - such as garlic, ginger, and yacon- enhanced growth performance and immune responses in juvenile black rockfish (*Sebastes schlegelii*) without affecting their proximate body composition. In their findings, crude protein ranged from 17.9% to 18.4%, lipid from 7.4% to 7.8%, and moisture from 69.2% to 70.3%, with no statistically significant differences among treatment groups. This suggests that certain phytogenic may deliver physiological benefits without necessarily altering muscle nutrient profiles, depending on their functional properties and target species.The health status of fish can be assessed through hematological and blood biochemical analyses, particularly when introducing new feed formulations, ingredients, and additives (Chen and Luo, 2023).

Our findings of hematological analysis illustrate significant differences among the various fish groups examined. Specifically, the control group exhibited the highest red blood cell count, whereas the group supplemented with 0.5% CTE demonstrated the second-highest count. Additionally, regarding white blood cells, hemoglobin, and hematocrit levels, the fish fed with 0.5% CTE displayed the highest values which may indicate a stimulatory effect of CTE on erythropoiesis and immune response. Similar findings were reported by Rashmeei *et al.* (2022), who found that dietary inclusion of *Vitex agnus-castus* extract in goldfish significantly elevated red blood cell count, hemoglobin concentration, and hematocrit values. Moreover, a related study by Rashmeei *et al.* (2020) demonstrated that goldfish (*Carassius auratus*) fed diets containing chasteberry extract (up to 15 g/kg) showed significantly increased lymphocyte and monocyte counts, compared to the control group.

Furthermore, the observed differences align with the known pharmacological properties of chasteberry extract, which has been shown to possess immunomodulatory effect in vitro and in vivo models (Ibrahim, et al., 2021). The present study demonstrates that dietary inclusion of CTE significantly influenced the serum biochemical parameters of female *Oreochromis niloticus* in terms of glucose and total protein. Notably, the group fed with 0.5% CTE exhibited elevated glucose levels (159.0 mg/dL), suggesting enhanced carbohydrate metabolism. This aligns with findings by Soleymanzadeh *et al.* (2020), who reported that CTE supplementation can modulate glucose metabolism in diabetic models of rats. Total protein levels were significantly higher in the 1% CTE group (8.81 g/L), suggesting improved protein synthesis or reduced catabolism. This observation is consistent with the work of Khoris *et al.* (2024), who found that CTE supplementation enhanced protein levels in carp.

In summary, the incorporation of CTE at 0.5% in the diet of Nile tilapia fish optimally enhanced growth performance, feed efficiency, muscle composition and overall health status, suggesting its potential as a functional feed additive in modern aquaculture nutrition.

CONCLUSION

The dietary inclusion of CTE at a level of 0.5% significantly improves growth performance, feed utilization, and muscle chemical composition in pre-market size of Nile tilapia fish. Additionally, 0.5% CTE positively influenced key hematological parameters, indicating enhanced physiological and immune status. These results recommend the inclusion of 0.5% CTE as an effective and natural dietary additive to promote the growth and health of Nile tilapia in aquaculture systems.

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Conflict of interest

The authors declare no conflict of interest.

Data availability statement

Data will be available upon request from authors

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