

Growth Performance and blood profile of *Oreochromis niloticus* Fed *Syzygium aromaticum* (Clove) Buds Fortified Diets

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Abstract

Feed quality is central to aquaculture, as it directly influences growth, survival, and production efficiency. In recent years, plant-derived feed additives have gained attention as sustainable alternatives to synthetic growth promoters. Among them, *Syzygium aromaticum* (clove buds) is valued for its bioactive compounds with potential antimicrobial, antioxidant, and immune-boosting effects. However, its nutritional and physiological impacts in fish diets remain insufficiently explored. Nile tilapia (*Oreochromis niloticus*), a globally important aquaculture species, was selected to investigate the effects of dietary clove supplementation on growth and health performance. Treatments were arranged in one replicate group, and parameters monitored included growth indices (final weight, weight gain, specific growth rate, and feed conversion ratio), proximate body composition, haematological parameters, and serum metabolites. Results showed significant differences ($p < 0.05$) in growth across treatments. The control group achieved the highest weight gain and feed efficiency, while fish fed 50% clove powder recorded the lowest growth performance. Survival ranged from 81.25% to 100%, with the control and 5% groups maintaining the highest values. Proximate analysis revealed higher crude protein and fat in control fish compared with clove-supplemented groups. Haematological indices, including packed cell volume and haemoglobin, increased with clove supplementation, while white blood cell counts peaked at 25% inclusion before declining. Serum biochemistry showed mild variations in protein, albumin, and cholesterol, while AST activity increased across all supplemented groups. These findings suggest that moderate clove inclusion (5–10%) may enhance blood indices and survival, whereas higher levels (25–50%) negatively affect growth and metabolic functions. Further studies are recommended to determine optimal supplementation levels for sustainable tilapia production.

Keywords: *Oreochromis niloticus*, *Syzygium aromaticum*, feed efficiency, haematology, serum biochemistry

Running title: Clove Bud Diets and Performance of Nile Tilapia

Performance de croissance et profil sanguin d'*Oreochromis niloticus* nourris avec des régimes enrichis aux bourgeons de *Syzygium aromaticum* (girofle)



Résumé

La qualité des aliments est centrale en aquaculture, car elle influence directement la croissance, la survie et l'efficacité de la production. Ces dernières années, les additifs alimentaires d'origine végétale ont gagné en attention en tant qu'alternatives durables aux promoteurs de croissance synthétiques. Parmi eux, *Syzygium aromaticum* (clou de girofle) est valorisé pour ses composés bioactifs aux effets potentiellement antimicrobiens, antioxydants et immunostimulants. Cependant, ses impacts nutritionnels et physiologiques dans l'alimentation des poissons restent insuffisamment explorés. Le tilapia du Nil (*Oreochromis niloticus*), une espèce aquacole d'importance mondiale, a été sélectionnée pour étudier les effets de la supplémentation alimentaire en girofle sur la croissance et l'état de santé. Les traitements ont été organisés

en groupes avec une répétition, et les paramètres suivis incluaient les indices de croissance (poids final, gain de poids, taux de croissance spécifique et indice de conversion alimentaire), la composition corporelle globale, les paramètres hématologiques et les métabolites sériques.

Les résultats ont montré des différences significatives ($p < 0,05$) dans la croissance entre les traitements. Le groupe témoin a atteint le gain de poids et l'efficacité alimentaire les plus élevés, tandis que les poissons nourris avec 50 % de poudre de girofle ont enregistré la plus faible performance de croissance. La survie a varié de 81,25 % à 100 %, les groupes témoin et 5 % maintenant les valeurs les plus élevées. L'analyse globale a révélé une teneur plus élevée en protéines brutes et en lipides chez les poissons témoins par rapport aux groupes supplémentés en girofle. Les indices hématologiques, incluant l'hématocrite et l'hémoglobine, ont augmenté avec la supplémentation en girofle, tandis que les numérations de globules blancs ont atteint un pic à 25 % d'inclusion avant de diminuer. La biochimie sérique a montré des variations légères des protéines, de l'albumine et du cholestérol, tandis que l'activité de l'AST (ASAT) a augmenté dans tous les groupes supplémentés. Ces résultats suggèrent qu'une inclusion modérée de girofle (5-10 %) peut améliorer les indices sanguins et la survie, tandis que des niveaux plus élevés (25-50 %) affectent négativement la croissance et les fonctions métaboliques. Des études supplémentaires sont recommandées pour déterminer les niveaux de supplémentation optimaux pour une production durable de tilapia.

Mots-clés : *Oreochromis niloticus*, *Syzygium aromaticum*, efficacité alimentaire, hématologie, biochimie sérique.

Introduction

Feed quality is a critical determinant of fish performance in aquaculture, influencing growth, health, and production costs (White, 2013; Eriegha and Ekokotu, (2017). The quality of feed is affected by factors such as fish behaviour, stocking density, feed composition, daily ration size, feeding frequency, and water temperature (Shackleton, 2012 ; Ferdous *et al.*, 2014; Chambel *et al.*, 2015). Since feed accounts for about 70% of the production cost in aquaculture (Ansari *et al.*, 2021; David *et al.*, 2021), understanding the implications of feed quality on fish growth and economic returns is vital. *Oreochromis niloticus*, commonly known as Nile tilapia, is one of the most widely cultured fish species globally due to its high commercial value, adaptability to diverse environments, and aquaculture potential (Ng and Romano, 2013; Kumar and Engle, 2016a). For fish farmers, the goal is to produce healthy table-sized fish within the shortest time possible, and feed utilization plays a critical role in achieving this goal (Ofonime *et al.*, 2019). The introduction of new

feed additives, such as *Syzygium aromaticum* (clove), has garnered attention for its potential benefits in fish nutrition and health (Saeed *et al.*, 2022; Vallejos-Vidal *et al.*, 2016).

Feed additives are widely used in aquaculture to enhance growth performance, feed efficiency, health, and general well-being (Amenyogbe *et al.*, 2020; Doğukan *et al.*, 2022). Phytobiotics, derived from plants, are increasingly popular as feed additives due to the presence of bioactive compounds, such as alkaloids, terpenoids, flavonoids, and essential oils, which exhibit antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory properties (Chakraborty *et al.*, 2014). Studies have shown that aromatic plant feed additives can improve growth, feed efficiency, and disease resistance in fish (Sutili *et al.*, 2018). Essential oils from clove, basil, and ginger have been found to enhance growth and immune responses in Nile tilapia when infected with *Streptococcus agalactiae* (Brum *et al.*, 2017). *S. aromaticum*, in particular, shows promise as an anti-fertility feed additive, offering a potential method for controlling prolific

breeding in *O. niloticus*. Despite its promising applications, the safety of *S. aromaticum* at certain concentrations remains a concern, as some phytobiotics may exhibit toxicity.

Maintaining fish health is essential for aquaculture success, and the evaluation of blood parameters provides valuable insights into the physiological and nutritional status of fish (Kumar and Banerjee, 2016b; Ashaf-Ud-Doulah *et al.*, 2019). Blood analysis can serve as an early indicator of stress, nutritional deficiencies, or exposure to harmful substances, making it a crucial tool for assessing the impact of new feed ingredients (Zarejabad *et al.*, 2010; Shahjahan *et al.*, 2022). Haematological and serum biochemical analyses are therefore particularly useful in understanding how dietary additives affect fish health and immunity. While some studies on herbal feed additives have reported positive outcomes, such as improved growth and immune responses (Talpur *et al.*, 2013; Brum *et al.*, 2017), others have shown negative effects (Ekor, 2014; Idowu *et al.*, 2020). Although *S. aromaticum* is widely recognized for its antibacterial properties (Cortés-Rojas *et al.*, 2014), its effects on the haematological and serum biochemical profiles of fish remain largely unexplored.

Against this background, this study aimed to evaluate the impact of *S. aromaticum* as a feed additive on the growth, health, and immunity of *O. niloticus*. Specifically, it assessed the haematological parameters, serum biochemistry, and growth performance of fish fed diets supplemented with *S. aromaticum* to determine whether the inclusion of *S. aromaticum* in fish diets enhanced growth, boosted immunity, and promoted overall health or presented potential risks.

Materials and methods

Study Area

This study was conducted in the Fish Nutrition Laboratory of Department of Forestry, Wildlife, and Fisheries, under the Faculty of Agricultural

Production and Renewable Resources at Olabisi Onabanjo University, Ayetoro Campus, Ogun State, Nigeria.

Preparation of S. aromaticum powder

S. aromaticum buds were procured from a herbal market in Ibadan, Oyo State, Nigeria. Fresh samples were rinsed with water and air-dried at room temperature for 14 days. The dried samples were pulverized into powder using an electric blender.

Fish Feeding and Culture

A basal diet containing 42% crude protein was used (Table 1). The inclusion levels of *S. aromaticum* powder were determined based on the calculated LD₅₀ of 223.61 mg/kg as reported by Durojaiye *et al.* (2023). Inclusion levels were set at 0 (control), 25, 50, 75, and 100% of the LD₅₀ value. Each inclusion level was mixed with 500 g of the basal diet. The prepared diets were pelletized into 2 mm diameter pellets, air-dried, stored in labelled cellophane bags, and refrigerated.

A total of 100 adult male and female *O. niloticus* were acclimatized for two weeks in a 1000Litres capacity tank, during which they were fed a 2 mm fish feed at 5% body weight twice daily. Following acclimatization, the fish were divided into five groups corresponding to the dietary treatments (T0, T1, T2, T3, and T4). Each treatment group had one replicate, with a total of 80 fish. Feeding trials lasted 45 days, with fish fed twice daily (10 a.m. and 5 p.m.) at 5% body weight. Uneaten feed and faecal matter were siphoned daily, and mortalities were recorded. Fish weights were measured biweekly using a digital electronic balance. Water quality parameters, including temperature, dissolved oxygen (DO), and pH, were monitored and maintained as recommended by Boyd and Tucker (2012). The tanks were drained and refilled weekly.

Growth Performance

Growth performance was evaluated to analyse

feed utilization efficiency using the following formulae:

$$\text{Body weight gain (BWG)} = W_t - W_i$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{total feed intake (g)}}{\text{total wet weight gain (g)}}$$

$$\text{Specific growth rate (SGR)} = \frac{[\ln W_t - \ln W_i]/T}{\times 100}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{wet weight gain (g)}}{\text{total protein intake (g)}}$$

$$\text{Survival rate (\%)} = \frac{\text{final number of remaining fish}}{\text{initial number of fish}} \times 100$$

Where:

W_t = mean final weight

W_i = mean initial weight

T = feeding trial period in days

Economic Analysis

The economic efficiency of the feeding trial was evaluated using:

Incidence Cost (IC): Cost of feed used to produce 1 kg of fish (Ali *et al.*, 2024).

$$\text{IC} = \frac{\text{Cost of feed}}{\text{weight of fish produced}}$$

Profit Index (PI): Value of fish produced relative to feed cost (Ali *et al.*, 2024).

$$\text{PI} = \frac{\text{Value of fish produced}}{\text{cost of feed}}$$

Carcass composition

At the end of the 45-days feeding trial, carcass composition analysis was conducted to evaluate the proximate composition of the fish fed diets containing varied levels of *S. aromaticum* powder. Three fish samples per treatment group (T0, T1, T2, T3, and T4) were randomly selected. The fish were euthanized using clove oil (Hajek *et al.*, 2006) and dissected to remove the gut contents. The whole-body samples were homogenized using an electric blender to ensure uniformity for proximate analysis. Proximate analysis was carried out following the standard procedures of the Association of Official Analytical Chemists (AOAC, 2005). Parameters analyzed included moisture content, crude protein, crude lipid, ash content, crude fiber, and nitrogen-free extract (NFE). Moisture content

was determined by oven-drying the samples at 105°C until a constant weight was achieved. Crude protein content was estimated using the Kjeldahl method, where total nitrogen was determined and multiplied by a factor of 6.25. Lipid content was measured using the Soxhlet extraction method with petroleum ether as the solvent, while ash content was obtained by incinerating the samples in a muffle furnace at 550°C for six hours until a constant white or light gray ash was obtained. Crude fiber was determined by acid and alkali digestion followed by incineration, and NFE was calculated by subtracting the sum of moisture, crude protein, crude lipid, ash, and crude fiber percentages from 100.

Haematology and Serum Biochemistry Analysis

After the feeding trial, fish samples were sedated using clove oil, which is considered safe for fish anaesthesia (Hajek *et al.*, 2006). Blood was collected from three fish per treatment using sterile syringes via the vertebral blood vessels at the caudal peduncle.

Two millilitres of blood were collected into EDTA-treated bottles for haematological analysis, using standard procedures described by Svobodova *et al.* (1991). Parameters analysed included white blood cell count (WBC), red blood cell count (RBC), haemoglobin (Hb), and packed cell volume (PCV). Calculations of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) followed the method of Stockham and Scott (2008).

Another 2 mL of blood was transferred into heparinised tubes for plasma biochemical analysis. Plasma was separated by centrifugation and stored at -20°C until analysis. Biochemical parameters analysed included glucose, alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatinine, total protein, albumin,

cholesterol, and alanine aminotransferase (ALT), using modified methods from previous studies.

Data

Statistical analyses were conducted using SPSS 23.0. Data were subjected to one-way analysis of variance (ANOVA), and significant differences among treatment groups were determined using Duncan's multiple range test. Differences were considered significant at $p < 0.05$.

Analysis

Results

Proximate composition of basal diet

The proximate composition of the basal diet (Table 1) showed 42.73% crude protein, 4.86% crude fat, and 30.10% nitrogen-free extract, indicating a high protein-to-energy ratio. Moisture content was 10.58%, while crude fibre and ash were 3.63% and 8.10%, respectively

Table: 1 Proximate composition of basal diet

Parameter	Composition (%)
Moisture (%)	10.58±0.07
Crude Protein (%)	42.73±0.73
Crude Fat (%)	4.86±0.06
Crude Fibre (%)	3.63±0.03
Total Ash (%)	8.10±0.01
Nitrogen free extract (%)	30.10±0.10

Growth performance and economic analysis of *O. niloticus* fed varying levels of *S. aromaticum* powder fortified diets

The growth performance and economic analysis of *O. niloticus* fed *S. aromaticum* powder-fortified diets (Table 2) showed significant differences ($p < 0.05$) in several parameters. Final weight and mean weight gain were highest in the control (T0: 243.00 g; 91.00 g) and lowest in T2 (172.50 g; 19.50 g). Specific Growth Rate (SGR) was significantly higher in T0 (1.04) compared to

T2 (0.27). Feed Conversion Ratio (FCR) was lowest in T0 (2.81) and highest in T2 (11.40), with intermediate values in T1, T3, and T4. Survival was generally high, with T0 achieving 100%, T3 significantly higher than T2 and T4 (93.75%), and T4 the lowest (81.25%). Feed intake and feed cost varied but were not significantly different ($p > 0.05$). The profit index was highest in T0 (0.38), while T1 and T4 were the least profitable (0.32 and 0.31, respectively), and T2 and T3 showed moderate values

Table 2: Growth performance and economic analysis of *O. niloticus* fed varying levels of *S. aromaticum* powder fortified diets

Parameters	T0	T1	T2	T3	T4
Initial weight (g)	152.00±1.00	154.00±1.00	153.00±0.00	151.50±0.50	152.00±2.00
Final weight (g)	243.00±21.00	226.50±37.50	172.50±0.50	207.50±16.50	175.50±1.50
Mean weight gain (g)	91.00±20.00	72.50±36.50	19.50±0.50	56.00±16.00	23.50±3.50
SGR	1.04±0.18 ^a	0.83±0.36 ^{ab}	0.27±0.01 ^b	0.69±0.17 ^{ab}	0.32±0.05 ^{ab}
FCR	2.81±0.83 ^b	4.47±2.44 ^b	11.40±0.80 ^a	4.45±1.75 ^b	8.90±2.30 ^{ab}
Survival (%)	100.00±0.00	87.50±12.50	87.50±0.00	93.75±6.25	81.25±6.25

Feed intake (g)	235.00±3.00	246.50±5.50	219.00±11.00	226.00±9.00	207.50±20.50
Cost of feed (₦)	520.65±8.15	547.70±12.20	486.65±24.45	502.20±20.00	461.05±45.55
Profit index	0.38±0.02	0.32±0.05	0.33±0.02	0.36±0.00	0.31±0.09

Means with different superscripts along same row are significantly different ($p < 0.05$)

Proximate composition of *O. niloticus* fed varying levels of *S. aromaticum* powder fortified diets

The proximate composition of *O. niloticus* fed *S. aromaticum* powder-fortified diets (Table 3) showed significant ($p < 0.05$) differences across treatments. Moisture content was highest in T1 (6.81%) and lowest in T2 and T4 (5.72%). Crude protein was significantly higher in the control

(T0, 65.32%) than in all treatment groups (62.50–63.27%). Crude fat was also highest in T0 (18.12%) and significantly lowest in T2 (13.47%). Total ash was significantly increased in T1 (10.94%) and T4 (10.89%) compared to T0 (10.24%). Nitrogen-Free Extract (NFE) was significantly higher in treatments, with T2 (8.13%) recording the highest value, while T0 (0.12%) was the lowest.

Table 3: Proximate composition of *O. niloticus* fed varying levels of *S. aromaticum* powder fortified diets

Parameters (%)	T0	T1	T2	T3	T4
Moisture	6.11±0.01 ^b	6.81±0.01 ^a	5.72±0.02 ^d	5.81±0.01 ^c	5.72±0.02 ^d
Crude Protein	65.32±0.02 ^a	62.83±0.03 ^b	62.50±0.01 ^b	63.14±0.04 ^b	63.27±0.07 ^b
Crude Fat	18.12±0.02 ^a	15.05±0.05 ^d	13.47±0.07 ^e	16.68±0.02 ^c	17.21±0.01 ^b
Crude Fiber	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b
Total Ash	10.24±0.04 ^c	10.94±0.04 ^a	10.88±0.02 ^a	10.65±0.05 ^b	10.89±0.01 ^a
Nitrogen free extract	0.12±0.02 ^f	4.23±0.03 ^c	8.13±0.03 ^b	3.69±0.02 ^d	2.83±0.02 ^e

Means with different superscripts along same row are significantly different ($p < 0.05$)

Haematology profile of *O. niloticus* fed varying levels of *S. aromaticum* powder fortified diets

The haematological profile of *O. niloticus* fed *S. aromaticum* powder-fortified diets (Table 4) showed significant changes in key parameters.

Packed Cell Volume (PCV) was significantly higher ($p < 0.05$) in T1 (34.00%) and T4 (33.00%) compared to the control (28.00%). **Haemoglobin (Hb)** was also significantly elevated ($p < 0.05$) in all treated groups—T1 (11.10 g/dl), T2 (11.80 g/dl), T3 (11.00 g/dl), and T4 (11.95 g/dl)—relative to the control (9.10 g/dl). For differential counts, **neutrophils (NEUT)** were significantly reduced ($p < 0.05$) in T4 (25.50%) compared to the control (31.00%), while **lymphocytes (LYM)** were significantly higher ($p < 0.05$) in T4 (72.00%) than in the control (66.00%). **Eosinophils (EOS)** were significantly lower

($p < 0.05$) in T1 (0.00%) relative to the control (1.00%). Other parameters showed no significant differences ($p > 0.05$).

Table 4: Haematology profile of *O. niloticus* fed varying levels of *S. aromaticum* powder fortified diets

	T0	T1	T2	T3	T4
PCV %	28.00±0.00 ^b	34.00±0.00 ^a	31.00±0.00 ^{ab}	31.00±0.00 ^{ab}	33.00±2.00 ^a
Hb g/dL	9.10±0.20 ^b	11.10±0.20 ^a	11.80±0.30 ^a	11.00±0.70 ^a	11.95±0.25 ^a
RBC x10 ⁶	3.40±0.70	4.15±0.65	3.20±0.10	3.85±1.25	4.20±0.40
WBC x10 ³	11.20±0.00	14.10±0.50	14.25±1.45	13.40±1.50	13.20±0.80
NEUT. %	31.00±1.00 ^a	29.00±1.00 ^{ab}	28.00±1.00 ^{ab}	27.00±2.00 ^{ab}	25.50±0.50 ^b
LYM %	66.00±1.00 ^b	69.50±0.50 ^{ab}	69.00±0.00 ^{ab}	70.00±2.00 ^{ab}	72.00±1.00 ^a
EOS %	1.00±0.00 ^a	0.00±0.00 ^b	1.50±0.50 ^a	1.00±0.00 ^a	1.00±0.00 ^a
BAS %	1.00±0.00	1.00±0.00	0.50±0.50	0.50±0.50	0.50±0.50
MONO %	1.00±0.00	0.50±0.50	1.00±0.00	1.50±0.50	1.00±0.00
MCV (fl)	86.00±17.71	83.99±13.16	96.97±3.03	90.01±29.23	79.75±12.36
MCH (pg)	27.82±5.14	27.34±3.80	36.94±2.09	31.28±8.34	28.66±2.14
MCHC	32.50±0.71	32.65±0.59	38.07±0.97	35.49±2.26	36.39±2.96

g/dL

Means (±SE) with different superscripts along same row are significantly different (p<0.05).

PCV- Packed cell Volume, Hb- Haemoglobin concentration, RBC- Red blood cell, WBC- White blood cells or leukocytes, NEUT- Neutrophils, LYM- Lymphocytes, EOS- Eosinophil, BAS- Basophil, MONO- Monocytes, MCV- Mean corpuscular volume, MCH- Mean corpuscular haemoglobin, MCHC-Mean corpuscular haemoglobin concentration

Serum biochemistry of *O. niloticus* fed varying levels of *S. aromaticum* powder fortified diets

The serum biochemistry of *O. niloticus* fed *Syzygium aromaticum* powder-fortified diets (Table 5) showed few significant differences.

Total protein was significantly higher (p<0.05) in the control (T0, 5.85 g/dL) compared to T3 (4.90

g/dL). Urea levels were also significantly elevated (p<0.05) in T1 (2.79 mg/dL) relative to the control (1.58 mg/dL). Other parameters, including albumin, globulin, cholesterol, creatinine, and most glucose values, did not differ significantly (p>0.05) among treatments, though slight numerical variations were observed.

Table 5: Serum biochemistry of *O. niloticus* fed varying levels of *S. aromaticum* powder fortified diets

	T0	T1	T2	T3	T4
T.P (g/dL)	5.85±0.15 ^a	5.40±0.00 ^{ab}	5.45±0.15 ^{ab}	4.90±0.30 ^b	5.05±0.35 ^{ab}
ALB (g/dL)	3.20±0.10	3.25±0.25	3.15±0.05	2.85±0.05	2.90±0.10
GLOB (g/dL)	2.60±0.10	2.10±0.20	2.35±0.05	2.05±0.35	2.15±0.25
CHOL (mg/dL)	86.00±4.20	92.40±6.60	93.40±6.00	95.20±7.40	92.60±14.40
CREAT (mg/dL)	4.80±0.30	4.55±0.95	3.85±0.55	3.80±1.40	4.10±0.70
GLUC (mg/dL)	48.02±3.52	59.73±4.23	130.85±66.45	123.80±68.60	51.10±6.30
UREA (mg/dL)	1.58±0.23 ^b	2.79±0.31 ^a	2.62±0.19 ^{ab}	1.79±0.28 ^{ab}	1.93±0.39 ^{ab}

Means (±SE) with different superscripts along same row are significantly different (p<0.05).

TP- Total protein, ALB- Albumin, GLOB- Globulin, CHOL- Cholesterol, CREAT- Creatinine, GLUC- Glucose.

Serum enzymes of *O. niloticus* fed varying levels of *S. aromaticum* powder fortified diets

The serum enzyme activity of *O. niloticus* fed *S. aromaticum* powder-fortified diets (Table 6) showed significant variations. Aspartate aminotransferase (AST) levels were significantly higher ($p < 0.05$) in T1 (106.00 U/L), T2 (109.00 U/L), and T3 (120.50 U/L) compared to the

control (T0, 96.00 U/L), while T4 (103.00 U/L) was not significantly different. Alkaline phosphatase (ALP) was significantly reduced ($p < 0.05$) in T3 (20.50 U/L) and T4 (21.00 U/L) relative to the control (26.00 U/L). Alanine aminotransferase (ALT) showed no significant differences ($p > 0.05$) among treatments.

Table 6: Serum enzymes of *O. niloticus* fed varying levels of *S. aromaticum* powder fortified diets

	T0	T1	T2	T3	T4
AST (U/L)	96.00±2.00 ^c	106.00±4.00 ^b	109.00±1.00 ^b	120.50±1.50 ^a	103.00±2.00 ^{bc}
ALT (U/L)	14.50±3.50	19.00±0.00	17.00±3.00	22.50±5.50	12.00±4.00
ALP (U/L)	26.00±1.00 ^a	23.50±0.50 ^{ab}	24.00±1.00 ^{ab}	20.50±1.50 ^b	21.00±2.00 ^b

Means (±SE) with different superscripts along same row are significantly different ($p < 0.05$).

AST- Aspartate aminotransferase, ALT- alanine aminotransferase, ALP- Alkaline Phosphatases

Discussion

The determination of proximate composition in fish is crucial because of its role in human nutrition, offering essential proteins, amino acids, lipids, vitamins, and minerals (Mohanty *et al.*, 2019). Fish proteins, known for their high digestibility and complete amino acid profile, are vital for human health (Balami *et al.*, 2019). Biochemical composition of fish meat is influenced by numerous of endogenous and exogenous factors but depends mostly by the biochemical composition of feed administered (Hernández-Sánchez and Aguilera-Morales, 2012). In this study, the proximate composition analysis showed that fish in the control group exhibited significantly higher crude protein and crude fibre content compared to those fed diets supplemented with *Syzygium aromaticum* powder. This finding contrasts with reports by some researchers who observed an increase in carcass protein content of fish fed phytobiotics (Answer *et al.*, 2018; Ghanbary *et al.*, 2022). However, it aligns with the study by Antache *et al.* (2013), that documented a reduction in the protein content of Nile tilapia carcasses fed phytobiotics. However, the crude protein content in the control group was lower than the values reported by previous researchers (Kumar *et al.*,

2011; Bag *et al.*, 2012 Mabroke *et al.*, 2013). El-Zaeem *et al.* (2012) highlighted differences in the biochemical composition of Nile tilapia grown in natural environments compared to those raised in aquaculture systems. They observed that aquaculture-reared fish typically exhibited higher moisture and protein content. This observation may help explain the results of the present study, as the fish used were obtained from a semi-intensive aquaculture system. This indicates that the inclusion of *S. aromaticum* may alter nutrient retention in fish tissues, potentially due to changes in metabolism or nutrient bioavailability. Plant extracts are known to increase digestibility and bioavailability of nutrients that ultimately result in an increase of feed conversion, higher protein synthesis and growth of fish (Talpur *et al.*, 2013, Chung *et al.*, 2020). Growth performance analysis in the present study showed that fish fed diets with *S. aromaticum* exhibited reduced body weight gain, specific growth rate (SGR), and survival rate (SR) compared to the control. In addition, the feed conversion ratio (FCR) was higher across all treatments, indicating poor feed utilization and digestibility. This negative growth response is consistent with reports by Mahmoud *et al.* (2019), on poor growth in tilapia fed with diets containing phytobiotics like garlic and

ginger. The implications of these findings suggest that while *S. aromaticum* may have bioactive properties beneficial for health, its inclusion at high levels (25-100%) adversely affects growth and feed efficiency. This could be attributed to anti-nutritional factors or imbalances introduced by the phytobiotic, despite processing techniques aimed at reducing such effects. In contrast to the negative effects observed in this study, other studies have demonstrated positive outcomes with phytobiotic supplementation. For instance, Rostika *et al.* (2018), reported improved growth performance in pangasius fed diets containing papain, while Kusi *et al.* (2024) observed enhanced weight gain and feed efficiency in *O. niloticus* fed clove basil-based diets. The discrepancies between studies may be due to differences in species, phytobiotic type, inclusion levels, and experimental conditions.

Blood contains easily accessible information about the individual physiological state of a fish and is considered a useful diagnostic tool due to its non-destructive nature (Fazio *et al.*, 2019; Seibel *et al.*, 2021). Hence, haematology and serum biochemical evaluation can be used as diagnostic indicators to reflect alterations in blood constituents. Haematological indices, particularly PCV and Hb, serve as indicators of the oxygen-carrying capacity of blood in fish, maintain systemic acid/base equilibria, and they also serve as important interorgan communication systems with additional functions, including redox regulation, blood rheology, and viscosity (Ortega-Villaizan *et al.*, 2022; Witeska *et al.*, 2023). Generally, the results of the haematological parameters investigated in this study showed that fish were in good condition as most of the parameters were within desirable values. In this study, RBC did not show any significant variations across the treatments, and values were within the acceptable limits of 0.4 to 5.2 x10⁶/μL in healthy fish (Esmaeili, 2021). This observation aligns with findings by Adegbola *et al.* (2023) and Amao *et al.* (2023), who reported no significant

differences in the values of RBC when clove bud extract was administered to male Wistar rats and cocks, respectively. This finding further reinforces reports on the beneficial effects of phytobiotics on PCV in fish (Subramanian *et al.*, 2017; Yonar *et al.*, 2019).

White blood cells (WBCs), which play a crucial role in immune defense, showed no significant differences across the treatment groups in this study. WBC counts initially increased with the inclusion of *S. aromaticum* (clove) up to 50% in the feed but subsequently decreased at higher inclusion levels (>50%). This observation contradicts the findings of Shama *et al.* (2013), who noted an increase in WBC counts in Wistar rats fed an aqueous extract of *S. aromaticum* buds but supports the results of Olufayo and Ojo (2018), who reported a similar trend in *C. gariepinus* when clove oil was administered. Leukocytosis, which is a natural response to foreign substances, reflects an immune system activated to protect against infections, potentially triggered by the bioactive compounds in cloves (Kabir *et al.*, 2011). The significant reduction in neutrophils suggests a compromised phagocytic response, likely diminishing their ability to efficiently clear pathogens, as neutrophils are essential for pathogen elimination and tissue repair (Sharma *et al.*, 2022).

Albumin and globulin levels followed a similar pattern, with no significant increases in fortified diet groups compared to the control. These findings contradict the results of Abdel-Rahman *et al.* (2020), who reported that the inclusion of clove powder in the diet of Nile tilapia led to an increase in serum albumin levels. Similarly, Mani *et al.* (2012) noted an improvement in serum albumin concentration following clove administration in rats.

Adu *et al.* (2012), reported that diets fortified with clove leaves significantly reduced cholesterol levels in broiler chickens. In contrast, the elevated levels observed in the treated groups of this study, although not statistically significant, may be

linked to enhanced lipid metabolism, as clove contains essential oils known to influence lipid regulatory pathways (Said *et al.*, 2011). Nonetheless, elevated cholesterol levels could also signify a potential lipid disorder (Rader and Hobbs, 2014).

Creatinine and urea are by-products of muscle metabolism and serve as indicators of renal function (Saleh *et al.*, 2012). The relatively stable creatinine values in this study suggest that dietary treatments did not adversely impact kidney function. However, urea levels showed significant variation across the treatment groups. Elevated urea levels may indicate increased protein catabolism or impaired renal function (Ilesanmi and Ridwan, 2021). Despite this, the reported values, though somewhat elevated, remain within the physiological limits considered normal for fish.

Transaminases, such as AST and ALT, are involved in amino acid metabolism; therefore, elevated levels of these enzymes are often associated with liver cell damage or metabolic stress (Udenze *et al.*, 2012). The increase in AST observed in T3 may suggest a dose-dependent stress response to higher clove powder inclusion, which is indicative of liver stress or metabolic changes (Bai *et al.*, 2012). On the other hand, the observed reduction in ALP may indicate a stabilizing effect on liver enzyme regulation at moderate clove supplementation levels.

Conclusion

In conclusion, this study highlighted the influence of *Syzygium aromaticum* (clove) powder supplementation on the growth, biochemical, and immune responses of *Oreochromis niloticus*. While moderate inclusion had no significant impact on some haematological and biochemical parameters, higher levels of clove powder were linked to reduced growth performance and altered liver enzyme activity. The observed variations in immune and biochemical responses suggest a complex interaction between clove supplementation and fish metabolism. Although

clove's bioactive compounds may offer health benefits, high inclusion levels could negatively affect growth and immune function. Further research is needed to determine the optimal dosage for maximizing health benefits without compromising growth or metabolic processes.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

- Abdel-Rahman, A., El-Bouhy, Z., Wahbah, M. and Ahmed, S. 2020.** Effects of dietary turmeric and clove powder on growth and immune response of the Nile tilapia. *Egyptian Journal of Aquatic Biology & Fisheries* 24(5): 589 – 608
- Adegbola, M. V., Ayeleso, A. O., and Oyedepo, T. A. 2023.** *Biochemical Constituents and Toxicological Effects of Syzygium aromaticum on Selected Organs in Male Wistar Rats.* In Curative and Preventive Properties of Medicinal Plants. Apple Academic Press. Pp. 165-182
- Adu, O. A., Gbore, F. A., Oloruntola, O. D., Falowo, A. B. and Olarotimi, O. J. 2020.** The effects of *Myristica fragrans* seed meal and *Syzygium aromaticum* leaf meal dietary supplementation on growth performance and oxidative status of broiler chicken. *Bull Natl. Res. Cent.* 44: 149.
- Ali, M. E., Watafua, H., Maisheru, J. A., Salisu, A. S., Remkyes, M. S. and**

- Yakubu, M. 2024.** Cost-benefit of *Clarias gariepinus* (Burchell, 1822) hatchlings fed different levels of maca (*Lepidium meyenii* Walp.) root powder as feed additive. *Journal of Agriculture, Environmental Resources and Management*, 6(4):106-113.
- Amao, E. A., Amao, O. D., Adelegan, T. M., Tiamiyu, W. A., Busari, Z. O. and Yunus, M. O. 2023.** Haematological and serum biochemical indices of cocks drenched varying levels of clove powder (*Syzygium aromaticum*). *Adan Journal Of Agriculture*, 4(1).
- Amenyogbe, E., Chen, G., Wang, Z., Huang, B., Li, H. 2020.** The Exploitation of Probiotics, Prebiotics and Synbiotics in Aquaculture: Present Study, Limitations and Future Directions: A Review. *Aquac. Int.* 28:1017–1041.
- Ansari, F.A., Guldhe, A., Gupta, S.K., Rawat, I. and Bux, F. 2021.** Improving the Feasibility of Aquaculture Feed by Using Microalgae. *Environ. Sci. Pollut. Res.* 28: 43234–43257.
- Antache, A., Cristea, V., Grecu, I., Dediu, L., Mocanu, M., Ion, S. and Petrea, S. M. 2013.** The effects of some phytobiotics on biochemical composition of *Oreochromis niloticus* meat reared in a recirculating aquaculture system. <https://scholar.google.com/>
- Anwer, I., Hafeez-ur-Rehman, M., Abbas, F., Javid, A., Hussain, A., Khan, N. and Azmat, H. 2018.** Effect of onion powder as feed additive on the growth, haematology and biochemical profile of grass carp (*Ctenopharyngodon idella*). *Pakistan Journal of Zoology*, 13: 58-64.
- AOAC 2005.** Official methods of analysis of AOAC International. Association of Official Analytical Chemists, Washington DC. <https://scholar.google.com/>
- Ashaf-Ud-Doulah, M., Shahjahan, M., Islam, S. M., Al-Emran, M., Rahman, M. S. and Hossain, M. A. R. 2019.** Thermal stress causes nuclear and cellular abnormalities of peripheral erythrocytes in Indian major carp, rohu *Labeo rohita*. *Journal of Thermal Biology*, 86:102450.
- Bag, M. P., Mahapatra, S. C., Rao, P. S. and Pal, H. 2012.** Growth performance of Nile tilapia (*Oreochromis niloticus* L.) fed with two leguminous plant leaf meals. *International Journal of Pharmacy & Life Sciences*, 3(10):2010-2014.
- Bai, D., Wu, X., Zhu, G., Guo, Y., Yang, G. and Ning, B. 2012.** Astragalus polysaccharides enhance cellular immune response and disease resistance in yellow catfish. *The Israel Journal of Aquaculture-Bamidgeh* 65: 1-7.
- Balami, S., Sharma, A. and Karn, R. 2019.** Significance of nutritional value of fish for human health. *Malaysian Journal of Halal Research*, 2(2): 32-34.
- Boyd C.E. and Tucker C.S. 2012.** Pond aquaculture water quality management. Springer Science & Business Media, Berlin, Germany <https://scholar.google.com/>
- Brum, A., Pereira, S. A., Owatari, M. S., Chagas, E. C., Chaves, F. C. M., Mouriño, J. L. P. and Martins, M. L. 2017.** Effect of dietary essential oils of clove basil and ginger on Nile tilapia (*Oreochromis niloticus*) following challenge with *Streptococcus agalactiae*. *Aquaculture*. 468 (1): 235-243
- Chakraborty, S. B., Horn, P. and Hancz, C. 2014.** Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. *Reviews in Aquaculture* 5:1-19.
- Chambel, J., Severiano, V., Baptista, T., Mendes, S. and Pedrosa, R. 2015.** Effect

- of stocking density and different diet on growth of *Percula clown fish*, (*A. percula*) Marine and environment Science center, intituto politecnico de leirir, 2520–641, peniche, Portugal. <https://doi.org/10.1186/s40064-015-0967-x>
- Chung, S., Lemos, C.H.P., Teixeira, D.V., Fortes-Silva R. and Copatti, C.E. 2020.** Essential oil from *Ocimum basilicum* improves growth performance and does not alter biochemical variables related to stress in pirarucu (*Arapaima gigas*). *Annals of the Brazilian Academy of Sciences* **92** (1): 1-11. doi: 10.1590/0001-3765202020181374
- Cortés-Rojas, D.F., de Souza, C.R. and Oliveira, W.P. 2014.** Clove (*Syzygium aromaticum*): A precious spice. *Asian Pac. J. Trop. Med.* **4**: 90–96
- David, L. H., Pinho, S. M., Agostinho, F., Kimpara, J. M., Keesman, K. J. and Garcia, F. 2021.** Emery synthesis for aquaculture: A review on its constraints and potentials. *Reviews in Aquaculture*, **13**(2):1119-1138.
- Doğukan, K.A.Y.A., Hersi, M.A., Ercüment, G.E.N.Ç., Arslan, H.Ö. 2022.** Symbiotic Effect of *Bacillus clausii* and Galacto-Oligosaccharide on Growth and Survival Rates in Red Cherry Shrimp (*Neocaridina davidi*). *Mar. Life Sci.* **42**: 146–151
- Durojaiye, A.F., Obasa, S.O. and Olusesi, A. S. 2023.** Safety Assessment of Crude and Aqueous Extracts of *Syzygium aromaticum* in *Oreochromis niloticus* (Linnaeus, 1758) Juveniles. *Nigerian Journal of Fisheries* **20** (1): 2567-2572.
- Ekor, M. 2014.** The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol.* **4**: 10.3389/fphar.2013.00177.
- El-Zaeem, S. Y., Mohamed, M. M. A., Mohamed E.S. S., and Abd El-Kader, W. N. 2012.** Flesh quality differentiation of wild and cultured Nile tilapia (*Oreochromis niloticus*) populations. *African Journal of Biotechnology*, **11**(17): 4086-4089
- Eriegha, O. J., and Ekokotu, P. A. 2017.** Factors affecting feed intake in cultured fish species: A review. *Animal Research International*, **14**(2), 2697-2709
- Esmaeili, N. 2021.** Blood performance: a new formula for fish growth and health. *Biology*, **10**(12): 1236.
- Fazio, F., Saoca, C., Costa, G., Zumbo, A., Piccione, G. and Parrino, V. 2019.** Flow cytometry and automatic blood cell analysis in striped bass *Morone saxatilis* (Walbaum, 1792): a new hematological approach. *Aquaculture*. **513**:734398. doi:10.1016/j.aquaculture.2019.734398
- Ferdous, Z., Nahar, N., Hossen, M.S., Sumi, K.R. and Ali, M.M. 2014.** Performance of different feeding freq. on growth indices and survival of monosex tilapia fry. *Int J Fish Aquacult* **1**(5):80–83
- Ghanbary, K., Firouzbaksh, F., Arkan, E. and Mojarrab, M. 2022.** The effect of *Thymbra spicata* hydroalcoholic extract loaded on chitosan polymeric nanoparticles on some growth performances, hematology, immunity, and response to acute stress in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **548**,:737568.
- Hajek, G., Klyszejko, B. and Dziaman, R. 2006.** The anaesthetic effect of clove oil on common carp, *Cyprinus carpio* L. *Acta Ichthyologica Et Piscatoria*, **36**(2): 93-97.
- Hernández-Sánchez, F. and Aguilera-Morales, M. E. 2012.** Nutritional richness and importance of the consumption of tilapia in the Papaloapan Region, REDVET,

- 13(6)
http://www.veterinaria.org/revistas/redvet/n060612/061_205.pdf
- Idowu, A. A., Popoola, O. C., Alani, J. O., Ipadeola, A. and Nwekoyo, V. E. 2020.** Toxicity effect of *Kigelia africana* aqueous extract on the haematology and histopathology of juvenile Nile Tilapia (*Oreochromis niloticus*). *Journal of Tropical Agriculture, Food, Environment and Extension* **19(1)**: 37 – 42
- Ilesanmi, O. and Ridwan, L. 2021.** The beneficial effect of a phytonutrient-rich product against cadmium chloride-induced hepatorenal toxicity. *Ukrainian Journal of Nephrology and Dialysis*.**4(72)**: 26-35.
- Kabir, M., Akpa, G. N., Nwagu, B. I., Adeyinka, I. A. and Bello, U. I. 2011.** Sexual dimorphism, breed and age characteristics of rabbits in Zaria, Nigeria. Proceedings of the 16th Annual Conference of Animal Science Association of Nigeria. Pp133-137
- Kumar, G. and Engle, C.R. 2016a.** Technological advances that led to growth of shrimp, salmon, and tilapia farming. *Reviews in Fisheries Science & Aquaculture*, **24(2)**: 136–152. <https://doi.org/10.1080/23308249.2015.1112357>
- Kumar, R. and Banerjee, T. K. 2016b.** Arsenic induced hematological and biochemical responses in nutritionally important catfish *Clarias batrachus* (L.). *Toxicology Reports*, **3**:148-152.
- Kumar, V., Akinleye, A. O., Makkar, H. P. S., Angulo-Escalante, M. A. and Becker K. 2011.** Growth performance and metabolic efficiency in Nile tilapia (*Oreochromis niloticus* L.) fed on a diet containing *Jatropha platyphylla* kernel meal as a protein source. *Journal of Animal Physiology and Animal Nutrition*, Blackwell Verlag GmbH <https://scholar.google.com/>
- Kusi, I. O., Obirikorang, K. A., and Adjei-Boateng, D. 2024.** Diets Supplemented With Phytobiotics *Calopogonium mucunoides*, *Ocimum gratissimum*, and *Tridax procumbens* Improve Growth, Immunity, and *Oreochromis niloticus* Resistance to *Streptococcus agalactiae*, *Aquaculture International*. (2024) **32(6)**: 7215–7234, <https://doi.org/10.1007/s10499-024-01510-7>.
- Mabroke, R. S., Tahoun, A. M., Suloma, A. and ElHaroun, E. R. 2013.** Evaluation of meat and bone meal and mono-sodium phosphate as supplemental dietary phosphorus sources for broodstock Nile tilapia (*Oreochromis niloticus*) under the conditions of Hapain- Pond System. *Turkish Journal of Fisheries and Aquatic Sciences*, **13**: 11-18
- Mahmoud, R., Aziza, A., Marghani, B. and Eltaysh, R. 2019.** Influence of ginger and garlic supplementation on growth performance, whole body composition and oxidative stress in the muscles of Nile tilapia (*O. niloticus*). *Adv. Anim. Vet. Sci.*, **7(5)**: 397-404. <https://doi.org/10.17582/journal.aavs/2019/7.5.397.404>
- Mani, F., Braga, C.P., Barbosa-Novelli, E.L. and Sforcin, J.M. 2012.** Influence of Clove Tea (*Syzygium aromaticum*) on Body Weight and Biochemical Parameters of Rats Subjected to Ethanol Consumption and Abstinence. *Med chem* **2**:081-085.
- Mohanty, B. P., Mahanty, A., Ganguly, S., Mitra, T., Karunakaran, D. and Anandan, R. 2019.** Nutritional composition of food fishes and their importance in providing food and nutritional security. *Food chemistry*, **293**: 561-570.

- Ng, W.K. and Romano, N. 2013. A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. *Reviews in Aquaculture*, **5(4)**: 220–254.
- Ofonime, E. A., Gift, S. D. and Imefon, U. U. 2019. Studies on the effect of feeding levels on growth response and nutrient utilization of Heteroclarias (Hybrid Catfish). *Journal of Applied Sciences* **19**: 725-730.
- Olufayo, M. O. and Ojo, O. M. 2018. Anaesthetic effects and haematological responses of heterobranchus bidorsalis juveniles exposed to clove oil. *International Journal of Fisheries and Aquaculture*, **10(9)**: 116-121.
- Ortega-Villaizan, M. D. M., Coll, J. and Rimstad, E. 2022. The role of red blood cells in the immune response of fish. *Frontiers in immunology*, **13**: 1005546.
- Rader, D.J. and Hobbs, H.H. 2014. *Disorders of lipoprotein metabolism*. In: *Harrison's Principles of Internal Medicine*. Kasper, D., Fauci, A., Hauser, S., Longo, D., Jameson, J. and Loscalzo, J. (Eds.). McGraw Hill. Pp 3145-3161
- Rostika, R., Nurhayati, A., Buwono, I. D., Rizal, A., Dewanti, L. P. and Maulana, T. 2018. Papain and bromelain crude enzyme extract in commercial feed, effectiveness toward pisciculture production of striped catfish (*Pangasianodon hypophthalmus*) in aquaculture facility. *Aquaculture, Aquarium, Conservation & Legislation*, **11(5)**: 1598-1604.
- Saeed, M., Khan, M. S., Younas, U., Siddique, F., Niaz, K., Farooq, Z. and Ramadan, M. F. 2022. Clove (*Syzygium aromaticum*) and its derivatives in fish feed. In *Clove (Syzygium aromaticum)* (pp. 257-266). Academic Press.
- Said, M.M. 2011. The protective effect of eugenol against gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. *Fundamental and Clinical Pharmacology* **25**:708–716
- Saleh, I.A., Gamal, A.S., Awaad, A.S. and Abd El Raheim, M.D. 2012. Anti-inflammatory activity, safety and protective effects of *Leptadenia pyrotechnics*, *Haloxylon salicornicum* and *Ocharadenus baccatus* in ulcerative colitis. *Phytopharmacology* **2(1)**: 58-71.
- Seibel, H., Baßmann, B. and Rebl, A. 2021. Blood will tell: what hematological analyses can reveal about fish welfare. *Frontiers in Veterinary Science*, **8**: 616955.
- Shackleton, E. 2012. Effects of temperature and terrestrial carbon on fish growth and pelagic food web efficiency, Umeå University: 978–91–7459–412–6 <https://link.springer.com/article/10.1007/s00484-022-02347-6>
- Shahjahan, M., Islam, M. J., Hossain, M. T., Mishu, M. A., Hasan, J. and Brown, C. 2022. Blood biomarkers as diagnostic tools: An overview of climate-driven stress responses in fish. *Science of the Total Environment*, **843**: 156910.
- Shama, I. Y., Adam, S.M. and Warda, S. A. 2013. Effects of the Aqueous Extract of Clove (*Syzygium aromaticum*) on Wistar Rats. *British Journal of Pharmacology and Toxicology* **4(6)**: 262-266
- Sharma, S., Gupta, S., Gupta, D., Juneja, S., Gupta, P., Dhiman, G., Kautish, S. and Gupta, S. K. 2022. Deep Learning Model for the Automatic Classification of White Blood Cells. *Hindawi Computational Intelligence and Neuroscience* <https://www.hindawi.com>

- Stockham, S.L. and Scott, M.A. 2008.** *Leucocytes in Fundamentals of Veterinary Clinical Pathology*. 2nd edn. Blackwell Publishing Ltd., Nova Jersey. Pp 45–212
- Subramanian, D., Jang, Y. H., Kim, D. H., Balasundaram, C. and Heo, M. S. 2017.** Evaluation of the dietary effect of hallabong peel oil on growth, hematological, and immune gene expression in rock bream, *Oplegnathus fasciatus* challenged with *Edwardsiella tarda*. <https://scholar.google.com/scholar>
- Sutli, F.J., Gatlin, D.M., Heinzmann, B.M., Baldisserotto, B. 2018.** Plant Essential Oils as Fish Diet Additives: Benefits on Fish Health and Stability in Feed. *Rev. Aquac.*, **10**: 716–726.
- Svobodova, Z., Pravda, D. and Palackova, J. 1991.** *Unified methods of haematological examination of fish*. Research Institute of fish culture and hydrobiology. <https://scholar.google.com/>
- Talpur, .A.D, Ikhwanuddin, M. and Bolong, A.M.A. 2013.** Nutritional effects of ginger (*Zingiber officinale* Roscoe) on immune response of Asian sea bass, *Lates calcarifer* (Bloch) and disease resistance against *Vibrio harveyi*. *Aquaculture*. **400**:46-52
- Udenze, E.C.C., Braide, V.B., Okwesilieze, C.N., Akuodor, G.C. and Odey, M.O. 2012.** The effects of gavage treatment with *Garcinia kola* seeds on biochemical markers of liver functionality in diabetic rats. *Annals of Biological Research*, **3** (9):4601-4608
- Vallejos-Vidal, E., Reyes-López, F., Teles, M. and MacKenzie, S. 2016.** The response of fish to immunostimulant diets. *Fish Shellfish Immunol.* **56**:34-69. doi: 10.1016/j.fsi.2016.06.028.
- White, P. (2013).** Environmental consequences of poor feed quality and feed management. FAO Fisheries and Aquaculture Technical Paper, 583: 553 – 564
- Witeska, M., Kondera, E. and Bojarski, B. 2023.** Hematological and hematopoietic analysis in fish toxicology—a review. *Animals*, **13**(16): 2625.
- Yonar, M. E., Yonar, S. M., İspir, U. and Ural, M. S. 2019.** Effects of curcumin on haematological values, immunity, antioxidant status and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Aeromonas salmonicida* subsp. achromogenes. *Fish & Shellfish Immunology*, **89**: 83-90.
- Zarejabad, A. M., Jalali, M. A., Sudagar, M. and Poralimotlagh, S. 2010.** Hematology of great sturgeon (*Huso huso* Linnaeus, 1758) juvenile exposed to brackish water environment. *Fish physiology and Biochemistry*, **36**: 655-659

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