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The role of spent coffee ground extracts as natural antioxidant supplements in the diet of Nile Tilapia (*Oreochromis niloticus*)

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Abstract: This study evaluated the potential of spent coffee grounds extract (CGE) as an antioxidant supplement in the diet of Nile tilapia (*Oreochromis niloticus*). Four experimental diets with varying CGE levels were formulated: 0% (CGE0, control), 2% (CGE2), 4% (CGE4), and 6% (CGE6). Nile tilapia (initial mean body weight = 38.65 ± 0.85 g) were cultured in fiberglass tanks at a stocking density of 20 fish/m^2 under continuous aeration. The fish were fed three times daily at 5% of their total body weight. After an 8-week experimental period, the fish fed CGE-supplemented diets exhibited significantly higher growth rate compared to the control group, with CGE4 showing the most pronounced improvement in final body weight (P = 0.027) and weight gain (P = 0.050). However, no significant differences were observed in average daily growth (P = 0.054), specific growth rate (P = 0.256), survival rate (P = 0.487), and feed conversion ratio (P = 0.105) between the dietary treatments. Fish on the CGE6 diet exhibited the highest total plasma protein (P = 0.001) and immunoglobulin levels (P = 0.000). Moreover, CGE supplementation enhanced superoxide dismutase (P = 0.000) and glutathione peroxidase (P = 0.016) activities relative to the control group. The histopathological analysis showed significantly longer intestinal villi in fish fed CGE-supplemented diets, with the longest villi observed in the CGE6 group (P = 0.000). Fish fillets from the CGE6 group exhibited the highest springiness, while hardness was comparable between CGE6 and CGE4 but significantly higher than in CGE2 and CGE0. Additionally, CGE supplementation significantly influenced the colour expression, increasing lightness (L^*) while decreasing redness (a^*) and yellowness (b^*) values. These findings indicate that 4% CGE supplementation is the most effective concentration, as it significantly promotes growth as evidenced by the highest weight gain, while also enhancing blood biochemical parameters, flesh quality, and antioxidative responses in Nile tilapia.

Keywords: antioxidative activity; feed additive; fish feed; growth performance

Nile tilapia is one of the most widely farmed fish species globally, recognised for its rapid growth, adaptability to various environmental conditions, and high tolerance to diseases and stress (Van Doan et al. 2021). It is cultivated in over 85 countries, with major producers including China, Indonesia, Egypt,

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Bangladesh, Brazil, Thailand, and the Philippines (El-Sayed and Fitzsimmons 2023). The global production of tilapia has grown remarkably, from 5 tonnes in 1950 to approximately 4.5 million tonnes in 2020 (FAO 2022). By 2030, tilapia production is projected to rise by about 62% compared to current levels (FAO 2018). The growing demand has prompted the adoption of intensive aquaculture methods, which have been shown to increase commercial Nile tilapia production by more than 30% when combined with high-density farming and other farm management practices (Jewel et al. 2023). However, high-density farming can induce stress in fish, resulting in a range of physiological challenges such as reduced feed intake, compromised immune function, heightened vulnerability to diseases, and an increased risk of mortality. Consequently, effective management practices are crucial to maintain an appropriate stocking density, ensuring the health and survival of the fish (Ali et al. 2023). Accordingly, Nile tilapia was selected as the model species in this study due to its global economic importance, widespread use in intensive aquaculture systems, and well-documented physiological responses to nutritional and environmental interventions. The expansion of commercial Nile tilapia farming has heightened the need to investigate the nutritional requirements of this species and optimise feed formulations to ensure sustainable growth and production. A particular focus has been placed on enhancing the fish immune system. Several studies have shown that antioxidant supplementation can stimulate the immune system and improve the growth performance of aquatic animals (Li et al. 2023).

Coffee is one of the most widely utilised plant-based by-products for antioxidant supplementation of animal feed. The production of coffee for human consumption generates various by-products, which hold potential for use as feed ingredients in aquaculture and livestock nutrition (Roslan et al. 2024). In 2019, the global coffee production reached approximately 10.16 million tonnes (Van Doan et al. 2022), resulting in the generation of a substantial amount of spent coffee grounds, estimated at 6 million tonnes worldwide (Mata et al. 2018). Spent coffee grounds are rich in phenolic compounds (1–1.5% total polyphenols), particularly chlorogenic acid, which plays a crucial role in their potent antioxidant properties (Campos-Vega et al. 2015). These compounds protect cells from oxidative stress and delay lipid oxidation (Panzella et al. 2016). Additionally, the compounds in coffee can stimulate the expression of genes associated with antioxidant defence, thereby helping to protect the gastrointestinal system from oxidative stress (Liang and Kitts 2014). In terms of nutrition, spent coffee grounds are also a source of carbohydrates, proteins, and bioactive compounds. They contain approximately 10% protein, 11–17% fat, 38–42% carbohydrates, and 4.5–4.7% minerals (Esquivel and Jimenez 2012). Previous studies on the use of coffee by-products as antioxidant supplements in aquaculture have demonstrated their ability to improve growth performance and feed utilisation, elevate blood HDL levels, enhance antioxidant enzyme activity, reduce zinc bioaccumulation, and promote overall fish health (Abdel-Tawwab et al. 2017; Afriansyah et al. 2023). Additionally, polyphenolic extracts from spent coffee grounds have been found to increase antioxidant enzyme activity and reduce lipid peroxidation under hydrogen peroxide-induced oxidative stress (Leyva-Lopez et al. 2021). These findings highlight the potential of spent coffee grounds as a sustainable antioxidant source for aquatic animals. Therefore, the present study aimed to assess the effects of spent coffee grounds extract supplementation at different doses on the growth performance, blood biochemical parameters, antioxidative activity, flesh quality, and intestinal histopathology of Nile tilapia (*Oreochromis niloticus*).

MATERIAL AND METHODS

Preparation of spent coffee grounds extract (CGE)

Spent coffee grounds were obtained from a coffee shop located in Lopburi Province, Thailand. The collected grounds were dried at 65 °C for 24 h using an oven and subsequently weighed to prepare a 30% (wt/vol) solution. The dried material was then immersed in 40% ethanol as the solvent. To facilitate extraction, the mixture underwent microwave-assisted extraction for 8 minutes. The crude extract was filtered to separate solid residues, and the liquid supernatant was carefully collected. The extract was stored in a dark environment at 4 °C to preserve its integrity for future use. The antioxidant properties of CGE were analysed using the following methods: total phenolic content (TPC) was determined by the Folin-Ciocalteu phenol test,

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which was modified from the method of Luque-Rodriguez et al. (2007); antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, following the method of Nuengchamnong et al. (2009); and antioxidant capacity was assessed using the ferric reducing ability power (FRAP) assay according to Maier et al. (2009), as summarised in Table 1.

Experimental design and diet preparation

The experiment was conducted using a completely randomised design (CRD), with four treatment groups and three replicates per group. The experimental diets were formulated by incorporating the spent coffee grounds extract at concentrations of 0% CGE/kg diet (CGE0), 2% CGE/kg diet (CGE2), 4% CGE/kg diet (CGE4), and 6% CGE/kg diet (CGE6) into the basal diet. The concentration of the spent coffee grounds extract used in this study was based on the results of coffee silverskin supplementation in Nile tilapia (*Oreochromis niloticus*), as reported by Prakash and Doan (2022).

The CGE was applied by spraying it onto the feed pellets at the designated concentrations for each treatment. A double coating of soybean oil was then applied to the pellets to enhance the binding of the extract. The formulation and chemical composition of the basal diet were analysed following the procedures outlined by AOAC (2005), with the results presented in Table 2.

Fish and experimental setup

Sex-reversed Nile tilapia with an average initial body weight of 38.65 ± 0.85 g were procured from a reputable commercial farm in Surin Province, Thailand. A total of 300 healthy fish were randomly allocated into 15 plastic tanks (500 l each) at a stocking density of 20 fish/m^2. The tanks were equipped with aeration systems to ensure adequate oxygen levels throughout the study. Prior to the experimental period, the fish were acclimatised for 14 days, during which they were fed a basal diet. The experimental phase lasted for 8 weeks, with fish being fed at a rate of 5.0% of their body weight per day. Feeding was conducted three times daily at 08:30, 12:00, and 17:00, with feed quantities adjusted biweekly based on the changes in body weight. The dry matter weight of the feed provided was recorded throughout the trial to accurately calculate the feed conversion ratio (FCR) based on dry feed intake. To ensure optimal water quality, the tanks were cleaned weekly, and 20% of the water was replaced before each feeding. Throughout the study, the water temperature was maintained between 27 °C and 29 °C, the pH was regulated between 7.44 and 8.25, and dissolved oxygen levels were kept above 5 mg/l.

All fish handling and experimental procedures in this study adhered to the guidelines for the

Table 1. The antioxidant properties of spent coffee ground extracts (CGE)

Antioxidant properties	Amount
TPC (mg GAE/ml of CGE)	508.83
DPPH (%)	42.52
FRAP [mg Trolox equivalent (TE)/ml of CGE]	7 394.68

DPPH = 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay; GAE = gallic acid equivalents; FRAP = ferric reducing ability power; TPC = total phenolic content

Table 2. Formulation and chemical composition of basal diets

Feed ingredients	Amount (%)
Fish meal	25.00
Soybean meal	32.00
Defatted rice bran	15.00
Corn meal	12.00
Cassava meal	10.00
Soybean oil	3.00
Dicalcium-phosphate	1.00
Vitamin-mineral premix[a]	1.00
Binder	1.00
Chemical composition (% dry weight basis)	
Crude protein	32.50
Crude fat	5.97
Crude fiber	5.11
Crude ash	9.23
Moisture	9.57

[a]Vitamin premix provided per kg of diet: VA 12 000 000 IU; VD 2 000 000 IU; VE 6 000 IU; VK 2 000 mg; VB1 800 mg; VB2 2 500 mg; VB6 800 mg; VB12 10 mg; Minerals provided per kg of diet: Mn 18 mg; Mg 200 mg; Co 0.1 mg; I 0.25 mg; Fe 140 mg; Cu 2.5 mg; Zn 65 mg; Se 0.2 mg

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Care and Use of Animals for Scientific Purposes established by the National Research Council of Thailand (NRCT) (No. U1-05537-2559). The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee, ensuring compliance with NRCT ethical standards for the use of animals in scientific research (Certificate of Approval ID: 03-66-013).

Fish sampling and data collection

Growth performances, feed utilisation, and survival rate. At the end of the 8-week experimental period, the total feed intake was recorded, and individual fish were weighed using an electronic scale with a precision of 0.1 g. Subsequently, the growth performance, feed utilisation efficiency, and survival rate of the fish were calculated using standard formulae:

Weight gain (WG, g/fish) = final weight – initial weight (1)

Average daily growth (ADG, g/day) = (final weight – initial weight)/60 days (2)

Specific growth rate (SGR, %/day) = (Ln final weight – Ln initial weight) × × 100)/60 days (3)

Survival rate (SR, %) = (No. of fish harvested/ No. of fish stocked) × 100 (4)

Feed conversion ratio (FCR) = amount of feed given (g)/weight gain (g) (5)

Hepatosomatic index (HSI, %) = [liver weight (g)/body weight (g)] × 100 (6)

Viscerosomatic index (VSI, %) = [viscera weight (g)/body weight (g)] × 100 (7)

Carcass composition (%) = [body weight without viscera (g)/body weight (g)] × × 100 (8)

Plasma biochemical and antioxidative activity. Five fish from each experimental unit were anesthetised with clove oil at a concentration of 30 ppm. Blood samples were then collected from the caudal vein using a syringe fitted with a No. 24 hypodermic needle. The samples were transferred into tubes without anticoagulants and allowed to clot at 4 °C for 15 minutes. Plasma was separated by centrifuging the blood at 9 000 × *g* for 10 min at 4 °C, and the resulting serum was used for biochemical analysis.

Total protein concentration was determined using the method outlined by Lowry et al. (1951), while total immunoglobulin levels were assessed following the protocol described by Siwicki et al. (1994).

Superoxide dismutase (SOD) activity was measured using an ELISA assay kit (Product No. 19160; Sigma-Aldrich®, Darmstadt, Germany), and glutathione peroxidase (GPx) activity was assessed using a different ELISA kit (Product No. MAK437; Sigma-Aldrich®, Darmstadt, Germany).

Intestinal histopathology. Intestinal tissue samples, approximately 0.5 cm in length, were collected from the midgut of five fish per experimental unit for histological analysis. The tissue samples were initially fixed and then rinsed under running water for 24 h while wrapped in gauze. Following this, the samples were dehydrated in ethanol, embedded in paraffin, and sectioned to the thickness of 4 µm using a microtome. Standard histological procedures were employed, including haematoxylin and eosin (H&E) staining, as described by Peng et al. (2013). The stained tissue slides were examined and photographed under a light microscope (Olympus CX33) using a Canon EOS750D camera with EOS utility software. From each fish (n = 5 per replicate), all identifiable villi in the selected field were measured to determine the mean villus length per replicate.

Texture analysis and fillet colouration. After euthanasia by immersion in an ice bath (Robb and Kestin 2002), five fish were randomly selected from each tank. Dorsal muscle fillet samples were taken from the epaxial myotomes below the dorsal fin on one side of each fish (one fillet per fish), excluding the ventral section (Tian et al. 2024).

For texture analysis, the fillets were cut into 1.5 × 1.5 × 1.0 cm cubes and analysed for springiness and hardness using a texture analyser (TA.XT.Plus; Stable Micro Systems, Ltd., Godalming, UK), following Zhou and Xie (2021). The same five fillets were also used for colour measurement. Colour parameters including lightness (L^*), redness (a^*), and yellowness (b^*) were measured using a Colour Space CIF system with a Colour Reader Model CR10 (Konica Minolta Sensing, Inc., Tokyo, Japan).

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Statistical analysis

Descriptive statistics, including means and standard deviations, were calculated for all variables. One-way analysis of variance (ANOVA) was conducted to assess differences between treatment groups, followed by Tukey's Honest Significant Difference (HSD) test for post-hoc comparisons, with a significance level set at $P \leq 0.05$.

RESULTS

The experimental diets had varying effects on growth performance, feed utilisation, survival rate, and carcass composition, as presented in Table 3. Following an eight-week feeding trial, fish fed CGE-supplemented diets exhibited significantly higher growth rate compared to the control group, with CGE4 showing the most pronounced improvement in final body weight ($P = 0.027$) and weight gain ($P = 0.050$). However, no significant differences were detected in average daily growth ($P = 0.054$), specific growth rate ($P = 0.256$), or survival rate ($P = 0.487$) between the dietary treatments. Similarly, feed conversion ratios did not differ significantly between groups ($P = 0.105$). The hepatosomatic index also remained unaffected by dietary treatments ($P = 0.635$). In contrast, fish fed CGE-supplemented diets demonstrated a significantly higher viscerosomatic index ($P = 0.006$), which influenced carcass yield. Notably, fish fed CGE diets exhibited lower carcass yields compared to those in the control group.

The plasma biochemical parameters and antioxidative activity results are summarised in Table 4. Fish fed the CGE6 diet demonstrated the highest

Table 3. Growth performance, feed utilisation, survival rate, and carcass composition of Nile tilapia fed diets supplemented with spent coffee grounds extract over an 8-week period (mean ± SD; $n = 3$)

Parameters	Experimental diets				*P*-value
	CGE0	CGE2	CGE4	CGE6	
Growth performance					
Initial body weight (g/fish)	38.32 ± 0.46	38.55 ± 1.10	38.31 ± 0.94	39.43 ± 0.55	0.340
Final body weight (g/fish)	135.47 ± 2.01^{b}	137.83 ± 3.36^{ab}	142.56 ± 1.29^{a}	141.78 ± 2.96^{ab}	0.027
Weight gain (g/fish)	97.14 ± 1.64^{b}	99.28 ± 3.86^{ab}	104.26 ± 1.04^{a}	102.35 ± 3.25^{ab}	0.050
Average daily growth (g/day)	1.62 ± 0.03	1.65 ± 0.07	1.74 ± 0.02	1.70 ± 0.06	0.054
Specific growth rate (%/day)	2.11 ± 0.01	2.13 ± 0.07	2.19 ± 0.04	2.13 ± 0.05	0.256
Survival rate (%)	100.00 ± 0.00	97.78 ± 1.93	98.89 ± 1.92	98.89 ± 1.92	0.487
Feed utilisation					
Feed conversion ratio	1.44 ± 0.01	1.41 ± 0.05	1.35 ± 0.04	1.37 ± 0.05	0.105
Somatic indices (%)					
Hepatosomatic index	4.81 ± 0.90	5.38 ± 0.43	5.15 ± 0.30	5.22 ± 0.27	0.635
Viscerosomatic index	12.98 ± 0.10^{b}	14.31 ± 0.68^{a}	15.10 ± 0.19^{a}	14.67 ± 0.32^{a}	0.001
Carcass composition	82.21 ± 0.86^{b}	80.31 ± 0.82^{a}	79.74 ± 0.25^{a}	80.10 ± 0.43^{a}	0.006

^{a,b}Mean ± SD values with different superscript lowercase letters within each row indicate significant differences ($P \leq 0.05$)

Table 4. Plasma biochemical parameters and antioxidative activity of Nile tilapia fed diets supplemented with spent coffee grounds extract over an 8-week period (mean ± SD; $n = 3$)

Parameters	Experimental diets				*P*-value
	CGE0	CGE2	CGE4	CGE6	
Total plasma protein (g/l)	1.74 ± 0.03^{b}	1.69 ± 0.04^{b}	1.77 ± 0.07^{b}	1.97 ± 0.07^{a}	0.001
Total immunoglobulin (g/l)	0.12 ± 0.02^{c}	0.19 ± 0.00^{bc}	0.25 ± 0.04^{b}	0.41 ± 0.00^{a}	0.000
Superoxide dismutase (U/mg protein)	6.33 ± 0.09^{c}	6.75 ± 0.17^{b}	7.39 ± 0.18^{a}	6.94 ± 0.08^{b}	0.000
Glutathione peroxidase (U/mg protein)	10.54 ± 0.90^{b}	11.59 ± 0.33^{ab}	12.40 ± 0.15^{a}	12.04 ± 0.54^{a}	0.016

$^{a–c}$Mean ± SD values with different superscript lowercase letters within each row indicate significant differences ($P \leq 0.05$)

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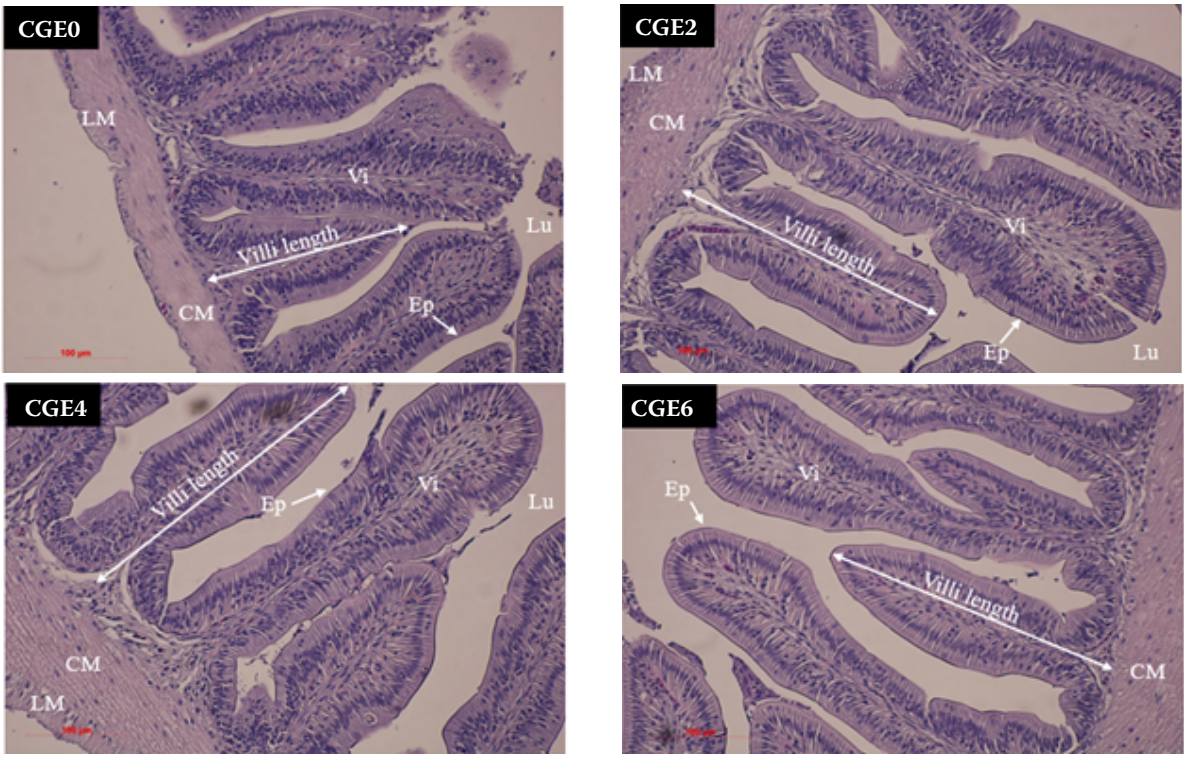

Figure 1. Intestinal histopathology of Nile tilapia fed diets supplemented with spent coffee grounds extract (CGE) over an 8-week period

Three samples per treatment group were examined at 40X magnification; the histological structure of the intestine is presented, which includes the longitudinal muscle (LM), circular muscle (CM), villi (Vi), and epithelial cells (Ep)

levels of total plasma protein (P = 0.001) and total immunoglobulin (P = 0.000). Notably, fish fed CGE-supplemented diets exhibited significantly higher SOD (P = 0.000) and GPx (P = 0.016) activity compared to those receiving the control diet.

The intestinal histopathological analysis revealed that the villus length in fish fed the experimental CGE diets was significantly greater than in those fed the control diet (Figures 1 and 2). The longest villi were observed in fish fed the CGE6 diet (618.01 ± 22.65 µm), followed by those receiving CGE4 (606.41 ± 16.76 µm), CGE2 (565.80 ± 38.76 µm), and the control diet (471.35 ± 40.96 µm) (P = 0.000).

The springiness and hardness of fish fillets were highest in fish fed the CGE6 diet, followed by fillets from fish fed the CGE4, CGE2, and CGE0 diets (P = 0.000). However, the hardness of fillets from fish fed the CGE6 and CGE4 diets did not differ significantly but it was significantly higher than that of fillets from fish fed the CGE2 and CGE0 diets (P = 0.000), as presented in Figure 3. The colour analysis of fish fillets demonstrated that CGE supplementation significantly influenced the colour expression. Increased levels of CGE in the

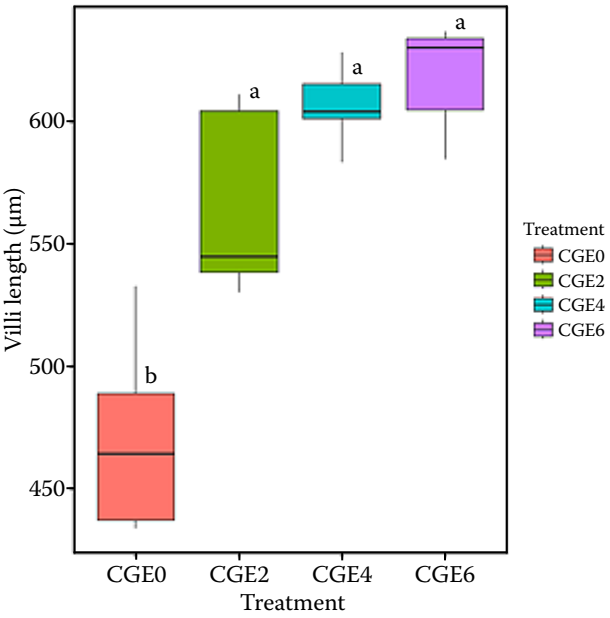

Figure 2. Villus length in the middle intestine of Nile tilapia after an 8-week feeding period with diets supplemented with spent coffee grounds extract

[a,b]Different superscript letters indicate statistically significant differences among groups (P < 0.05)

CGE = coffee grounds extract

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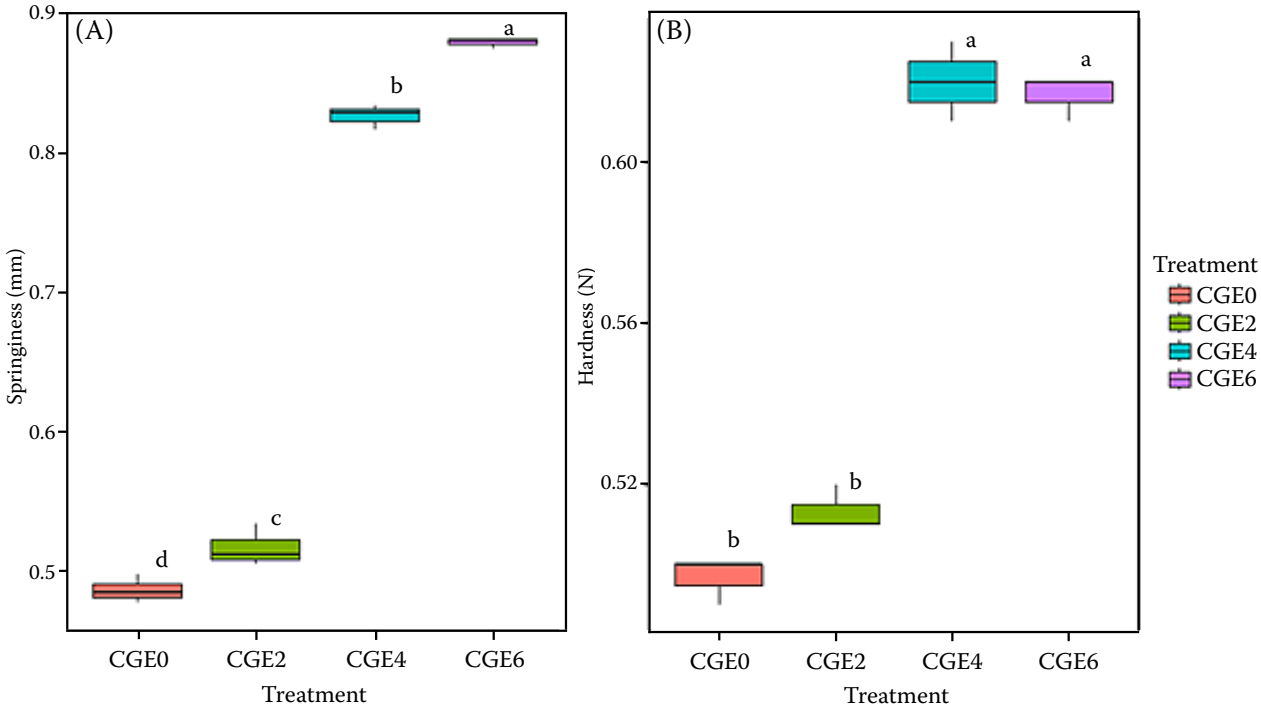

Figure 3. Springiness (A) and hardness (B) of Nile tilapia fillets after an 8-week feeding period with diets supplemented with spent coffee grounds extract

[a–d]Different superscript letters indicate statistically significant differences among groups ($P < 0.05$)

CGE = coffee grounds extract; mm = millimetres; N = Newton

diet resulted in higher lightness (L^*) values, while redness (a^*) and yellowness (b^*) values were significantly decreasing, as illustrated in Figure 4.

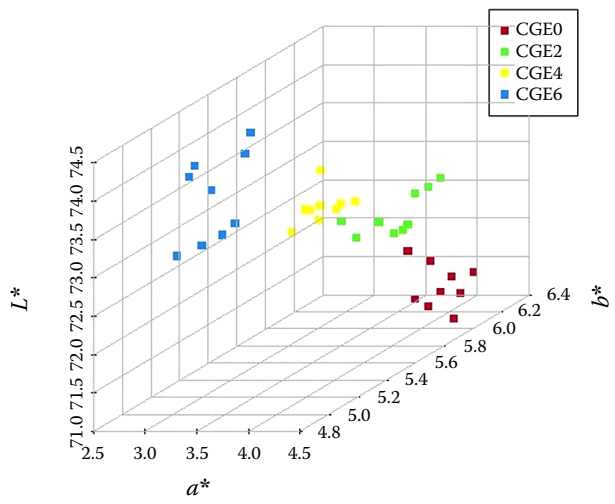

Figure 4. The colouration of Nile tilapia fillets after an 8-week feeding period with diets supplemented with spent coffee grounds extract

a^* = redness; b^* = yellowness; CGE = coffee grounds extract; L^* = lightness

DISCUSSION

This study demonstrated that dietary supplementation of 4% spent coffee grounds extract (CGE) improved the growth performance of Nile tilapia. These findings align with previous studies reporting that dietary supplementation of 4% coffee husk (Afriansyah et al. 2023) and 4% coffee silverskin (Prakash and Doan 2022) enhanced the growth rate of *Oreochromis* sp. In contrast, Abdel-Tawwab et al. (2017) reported that coffee bean supplementation of the diets of common carp (*Cyprinus carpio* L.) did not significantly affect the growth rate compared to the control group. Additionally, Abdel-Tawwab (2015) found that roasted coffee bean supplementation of tilapia larvae diets negatively impacted the growth performance. Specifically, increasing the level of coffee grounds in the diet significantly reduced weight gain in tilapia, although survival rates remained unaffected. Interestingly, Van Doan et al. (2022) observed that raising tilapia in a biofloc system supplemented with coffee grounds (10 g/kg) resulted in a higher specific growth rate and a lower feed conversion ratio compared to the control group. These contrasting results suggest that the ef-

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fectiveness of coffee grounds and coffee by-products as feed additives in aquaculture depends on various factors, including the level of supplementation, species, and size of the aquatic animals. Spent coffee grounds, a by-product of the coffee extraction process, are known to contain essential nutrients such as proteins, carbohydrates, fats, vitamins, and minerals (Afriansyah et al. 2023). Additionally, they are rich in bioactive compounds, including chlorogenic acid, caffeine, phenolic acids and flavonoids, which are known for their antioxidant properties (Glowacka et al. 2019; Angeloni et al. 2020; Fanali et al. 2020). These results are consistent with the findings of this study, which revealed that the spent coffee grounds extract contains phenolic compounds and exhibits high antioxidant activity. Additionally, the inclusion of spent coffee grounds in the diet improved feed utilisation, as evidenced by reduced FCR, with the trend to lower FCR observed in the CGE4 group. This result aligns with previous studies, which demonstrated that supplementing coffee silverskin at a 4% level similarly enhanced the feed efficiency (Prakash and Doan 2022).

The present study showed that the hepatosomatic index (HSI) of fish fed the experimental diet did not differ significantly from that of the control group. This contrasts with findings from studies on red tilapia, e.g. by Afriansyah et al. (2023), where the addition of coffee husk decreased the HSI and promoted glycogen accumulation in the liver. HSI is often used as an indicator of the health status of aquatic animals, as the liver plays a key role in glucose production through the breakdown of glycogen during glycolysis, which provides an important energy source for the brain and red blood cells. A decrease in HSI can indicate stress, illness, or malnutrition in aquatic organisms (Shoemaker et al. 2003). Additionally, Abdel-Tawwab (2015) reported that coffee grounds supplementation resulted in a reduction in protein accumulation in tilapia, while promoting higher fat and ash content. These findings are consistent with the present study, which observed that Nile tilapia fed the CGE4 diet exhibited the highest viscerosomatic index, suggesting that the supplementation influenced the fish body composition, particularly in terms of fat accumulation.

The present study also demonstrated that CGE supplementation enhanced the expression of plasma biochemical and antioxidative activity in Nile tilapia. Plasma protein levels are often used as indicators of the health status in aquatic animals. Under stressful conditions, infectious disease, or sudden environmental changes, plasma protein levels may decrease (Sala-Rabanal et al. 2003). Plasma proteins consist of various peptides, such as lysozyme and gamma-globulin, which play crucial roles in the immune system of aquatic organisms. This is consistent with the study by Roslan et al. (2024), who found that supplementation of fermented spent coffee grounds at a 10% inclusion level resulted in the highest total protein levels in plasma. Total immunoglobulin is especially important for humoral immune responses, facilitating immune defence through the blood circulation (Adams and Hamilton 1984). In the present investigation, an increase in plasma protein levels was observed, indicating enhanced health and immune responses in fish fed CGE. Moreover, the study found a significant increase in SOD and GPx activities in fish fed CGE, which is in agreement with the report by Afriansyah et al. (2023), where coffee grounds supplementation stimulated SOD activity in red tilapia and reduced malondialdehyde (MDA) levels. MDA is a toxin that damages cell membranes and proteins through lipid oxidation reactions. The antioxidant properties of CGE inhibit and delay oxidation processes, which is consistent with the findings of this study based on the DPPH radical scavenging assay and the ferric reducing ability power (FRAP) assay. Similarly, Abdel-Tawwab et al. (2017) reported that coffee grounds supplementation in common carp (*Cyprinus carpio* L.) stimulated SOD, GPx, and catalase activities. Consistently with the findings of Xu et al. (2022), chlorogenic acid was found to stimulate SOD, GPx, and catalase activities, while reducing MDA formation in koi carp (*Cyprinus carpio* L.). Wan et al. (2021) reported that the bioactive phenolic compounds, particularly chlorogenic acid, play a crucial role in activating the nuclear factor erythroid 2-related factor 2 (Nrf2) signalling pathway. This pathway serves as a key regulator of cellular responses to oxidative stress and the maintenance of redox homeostasis. Upon activation, Nrf2 translocates into the nucleus and upregulates the expression of various antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as components of the glutathione redox system. Beyond its antioxidant functions, Nrf2 also plays a vital role in modulating innate immune responses under both physiological and oxidative stress conditions (Xu et al. 2022). This mechanism likely underlies the enhanced immune parameters

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observed in Nile tilapia following the dietary supplementation of CGE in the present study.

The intestinal histopathological results indicated that CGE supplementation enhanced the villus length in the middle intestine of Nile tilapia. This is consistent with the supplementation of fermented spent coffee grounds at a 10% inclusion level in the diet of African catfish (*Clarias gariepinus*) (Roslan et al. 2024). An increase the in villus length provides a larger surface area for nutrient absorption, which can improve growth efficiency and feed utilisation in animals (He et al. 2017). CGE is rich in phenolic compounds, which are known for their antimicrobial and anti-inflammatory properties. These bioactive compounds contribute to the overall aquatic animal health by supporting the gut homeostasis through the modulation of gut microbiota and the reduction of inflammatory responses in the intestinal lining (Reverter et al. 2014). This is consistent with the study by Fontana et al. (2024), who reported that supplementation of mango (*Mangifera indica*) seed, a rich source of phenolic compounds, enhanced intestinal morphology, leading to increased villus height and crypt depth in Nile tilapia. These improvements contributed to enhanced growth performance and feed utilisation in the fish.

The analysis of fillet quality indicates that supplementing the diet with CGE, which possesses antioxidant properties, can improve fillet texture. This supplementation resulted in increased springiness and hardness of the fillets, while significantly reducing redness and yellowness and enhancing the lightness of the fish flesh. The enhancement of fillet texture is attributed to the protection of bio-membranes from oxidative damage (Brigelius-Flohe and Traber 1999). Muscle cell membranes, which are constantly subjected to physical stress, benefit from antioxidant supplementation. The inclusion of CGE, rich in antioxidant compounds, helps prevent the muscle degradation and preserve the integrity of fish flesh membranes. This finding is consistent with the study by Wu et al. (2017), who showed that dietary supplementation of vitamin E, a well-known antioxidant, improved the quality and structural integrity of Nile tilapia muscle. Spent coffee grounds exhibit significant antioxidant potency, providing protection of cells from oxidative stress and delaying lipid peroxidation (Panzella et al. 2016). Furthermore, reducing lipid oxidative reactions in fish flesh helps prevent the lipid degradation, a major cause of colour changes in fillets. Increased lipid oxidation can result in undesirable colour alterations, such as yellow, grey, pink, red, or other hues in fish fillets (NurSyahirah and Rozzamri 2022).

The overall improvements in growth, antioxidant defence, immune function, gut morphology, and fillet quality in Nile tilapia suggest that CGE confers benefits through multiple complementary mechanisms. Bioactive compounds, particularly phenolics, flavonoids, and chlorogenic acid likely enhance the physiological resilience by promoting cellular protection, immune regulation, and tissue integrity. These synergistic actions highlight the CGE potential as a functional feed additive in aquaculture. Despite these promising outcomes, certain limitations should be considered. The use of a single species, estimated group-level feed intake, and a relatively short experimental duration may have influenced the scope and precision of the results. Moreover, further research is needed to clarify the molecular mechanisms underlying the observed benefits. Addressing these aspects in future studies will help strengthen the evidence for the CGE potential as a functional feed additive.

CONCLUSIONS

This study demonstrates that dietary supplementation of spent coffee grounds extract (CGE) exerts beneficial effects on Nile tilapia. Among the tested levels, 4% CGE was the most effective, significantly enhancing the growth performance, as indicated by the highest weight gain. Furthermore, 4% CGE improved blood biochemical indices, antioxidative enzyme activities, and fillet quality. These findings suggest that 4% CGE is a promising feed additive to promote health and productivity in Nile tilapia aquaculture. However, further studies are warranted to explore its palatability, long-term effects, and underlying molecular mechanisms to fully establish its potential and application in diverse aquaculture settings.

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Conflict of interest

The authors declare no conflict of interest.

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