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Performance of *Ganoderma lucidum* Biomass and β-Glucan as Functional Feed for African Catfish (*Clarias gariepinus*) on Immuno-Oxidative Defenses

(Prestasi Biojisim *Ganoderma lucidum* dan β-Glukan sebagai Makanan Fungsian untuk Ikan Keli Afrika (*Clarias gariepinus*) terhadap Pertahanan Imuno-Oksidatif)

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ABSTRACT

This study evaluated the effects of dietary *Ganoderma lucidum* biomass and β-glucan on growth, organ function, and immune response in African catfish (*Clarias gariepinus*) over a 60-day feeding period. The experiment consisted of five groups: T1 (10 g biomass/3 g β-glucan), T2 (15 g biomass/3 g β-glucan), T3 (20 g biomass/3 g β-glucan), C1 (formulated feed without biomass/β-glucan), and C2 (commercial feed), respectively. Post-feeding analyses were performed on various liver function parameters, including serum total protein, albumin, globulin, bilirubin, phosphate, magnesium, cholesterol, and glucose levels, as well as liver metabolic enzymes. The kidney function was assessed via serum sodium, potassium, chloride, total carbon dioxide, anion gap, and urea, while the immunomodulatory effects of the diet were investigated on white blood cell counts (WBC) and absolute leucocyte counts. Results demonstrated that the combined diet of *G. lucidum* biomass and β-glucan significantly increased body weight and length, improved kidney and liver function (as indicated by favorable changes in serum biochemical markers and liver enzymes), and enhanced immune parameters by stabilizing white blood cell and leukocyte counts of African catfish. Group T2, containing 15 g *G. lucidum* biomass and 3 g β-glucan, exhibited the best performance in providing higher growth, effective liver and kidney functioning, and enhanced immunity in African catfish (*C. gariepinus*), making it a recommended concentration in this study.

Keywords: African catfish; β-glucan; *Ganoderma lucidum*; immunity; kidney function; liver function

ABSTRAK

Penyelidikan ini menilai kesan diet biojisim *Ganoderma lucidum* dan β-glukan terhadap pertumbuhan, fungsi organ dan tindak balas imun ikan keli Afrika (*Clarias gariepinus*) sepanjang tempoh pemakanan 60 hari. Uji kaji ini dibahagikan kepada lima kumpulan: T1 (10 g biojisim/3 g β-glukan), T2 (15 g biojisim/3 g β-glukan), T3 (20 g biojisim/3 g β-glukan), C1 (formula makanan tanpa biojisim/β-glukan) dan C2 (makanan komersial). Analisis selepas pemberian makanan dilakukan terhadap pelbagai parameter fungsi hati, termasuk jumlah protein serum, albumin, globulin, bilirubin, fosfat, magnesium, kolesterol dan tahap glukosa, serta enzim metabolik hati. Fungsi buah pinggang dinilai melalui natrium, kalium, klorida, jumlah karbon dioksida, jurang anion dan urea di dalam serum, manakala kesan imunomodulator diet dikaji dengan kiraan sel darah putih (WBC) dan kiraan leukosit mutlak. Keputusan menunjukkan bahawa gabungan diet biojisim dan β-glukan *G. lucidum* meningkatkan berat dan panjang badan dengan ketara, meningkatkan fungsi buah pinggang dan hati (seperti yang ditunjukkan oleh perubahan yang menggalakkan dalam penanda biokimia serum dan enzim hati)

dan meningkatkan parameter imun dengan menstabilkan jumlah sel darah putih dan leukosit ikan keli Afrika. Kumpulan T2, yang mengandungi 15 g biojisim dan 3 g β-glukan menunjukkan prestasi terbaik dengan menyokong pertumbuhan yang lebih baik, fungsi hati dan buah pinggang yang lebih berkesan dan meningkatkan imuniti dalam ikan keli Afrika (*C. gariepinus*), menjadikannya kepekatan yang disyorkan dalam kajian ini.

Kata kunci: β-glukan; fungsi buah pinggang; fungsi hati; *Ganoderma lucidum*; ikan keli Afrika; imuniti

INTRODUCTION

African catfish (*Clarias gariepinus*) is recognized as a promising species for aquaculture due to their unique characteristics, such as rapid growth and resilience to challenging environments (Ewane et al. 2024; Zhan et al. 2024). Nutritionally, African catfish has high protein and essential fatty acids, making it a valuable food choice for consumers (Scheld et al. 2024). As a result of this, African catfish has become the most widely farmed fish in Africa and Asia, highlighting their potential to enhance food security and serve as an affordable source of high-quality protein for humans (Langi et al. 2024). Particularly in Nigeria, in which the contribution to the global production was more than 67% (Olagunju et al. 2024).

Despite advancements in the mass production of African catfish using various functional ingredients, there are still challenges to address (Barasa & Ouma 2024; Otoh et al. 2024). These include poor nutrition, stress, genetic predisposition, and infectious diseases, which continue to impede growth and lead to deformities, particularly affecting kidney and liver function (Shahid et al. (2020). Dietary supplements play a crucial role in addressing these challenges by enhancing growth, immunity, and antioxidant activities, as well as renal and hepatic functions (Shinde et al. 2025). They also improve growth, enable detoxification, reduce oxidative stress, support metabolism, and increase disease resistance in fish (Lee et al. 2023; Ofori-Attah et al. 2024). In aquaculture, mushroom extracts, especially β-glucan, are known for their strong immunomodulatory and antioxidant properties, which help protect against liver and kidney damage (Venturella et al. 2021). Moreover, mushroom fruiting bodies and mycelial biomass are rich in phenolics and flavonoids that scavenge reactive oxygen species and safeguard vital organs from oxidative stress (Saravanan et al. 2024; Venturella et al. 2021).

The mushroom *G. lucidum* contains several bioactive compounds, including triterpenoids, lipids, flavonoids, lignans, polysaccharides, peptidoglycans, proteins, and sterols such as lanosterol, ergosterol, and ergosterol peroxide, which are suitable to enhance growth, immune, kidney, and liver function in fish (Martínez-Montemayor et al. 2019). They also contain important vitamins and minerals to enhance feed digestibility and palatability (Singh 2023). Utilizing the *G. lucidum* diet on African catfish can effectively enhance immunity to fight illness, promote growth, and improve antioxidant capacity and hematological status (Shahin et al. 2023; Yao et al. 2023). Besides its immunomodulatory effect, *G. lucidum* inhibits tumor growth and has anti-inflammatory, anti-allergic, and hypoglycemic effects (Chen et al. 2024; Mahmoud et al. 2020).

Several studies reported the potential benefits of incorporating mushroom fruiting body, mycelial biomass, or their bioactive compounds into a fish diet. However, the combined effects of both mushroom biomass and its bioactive compounds, such as β-glucan, on fish remain underexplored. This study aimed to investigate the combined effects of *G. lucidum* (biomass and β-glucan) on African catfish, with a focus on improving growth, immunity, kidney, and liver function.

MATERIALS AND METHODS

ETHICAL STATEMENT

The study protocol was approved by the Animal Care Committee of Universiti Malaya (ethical approval no. S/28102023/09082023-02/R). All experimental procedures involving animal use were conducted following the institution guidelines approved by the Committee of Experimental Animals, Universiti Malaya, Malaysia.

PRODUCTION AND PROCESSING OF *G. lucidum* BIOMASS AND β-GLUCAN

The mycelium of *Ganoderma lucidum* was obtained from the Functional Omics and Bioprocess Development Laboratory at the Universiti Malaya, Malaysia. The mycelium was activated on potato dextrose agar according to methods described by Alsaheb et al. (2020) and Nur Raihan et al. (2020). The sub-cultured mycelium was used for the liquid stage fermentation using potato dextrose broth in 250 mL flasks at 25 °C, pH 5.5, and 100 RPM for 10 days, then the culture was scaled up in a 4L ALSB-bioreactor to boost the biomass, and β-glucan (Sugenendran et al. 2023). After harvesting the culture, the β-glucan was precipitated using cold ethanol, centrifuged, dried, (Figure 1) and processed into powder for feed formulation (Alsaheb et al. 2020; Bae et al. 2000).

FEED FORMULATION

The feed was formulated according to the nutritional requirements of African catfish, as shown in Table 1. Three different concentrations of biomass and standardized β-glucan for the treatment groups were formulated: T1 (10 g biomass/3 g β-glucan), T2 (15 g biomass/3 g β-glucan), T3 (20 g biomass/3 g β-glucan), along with two

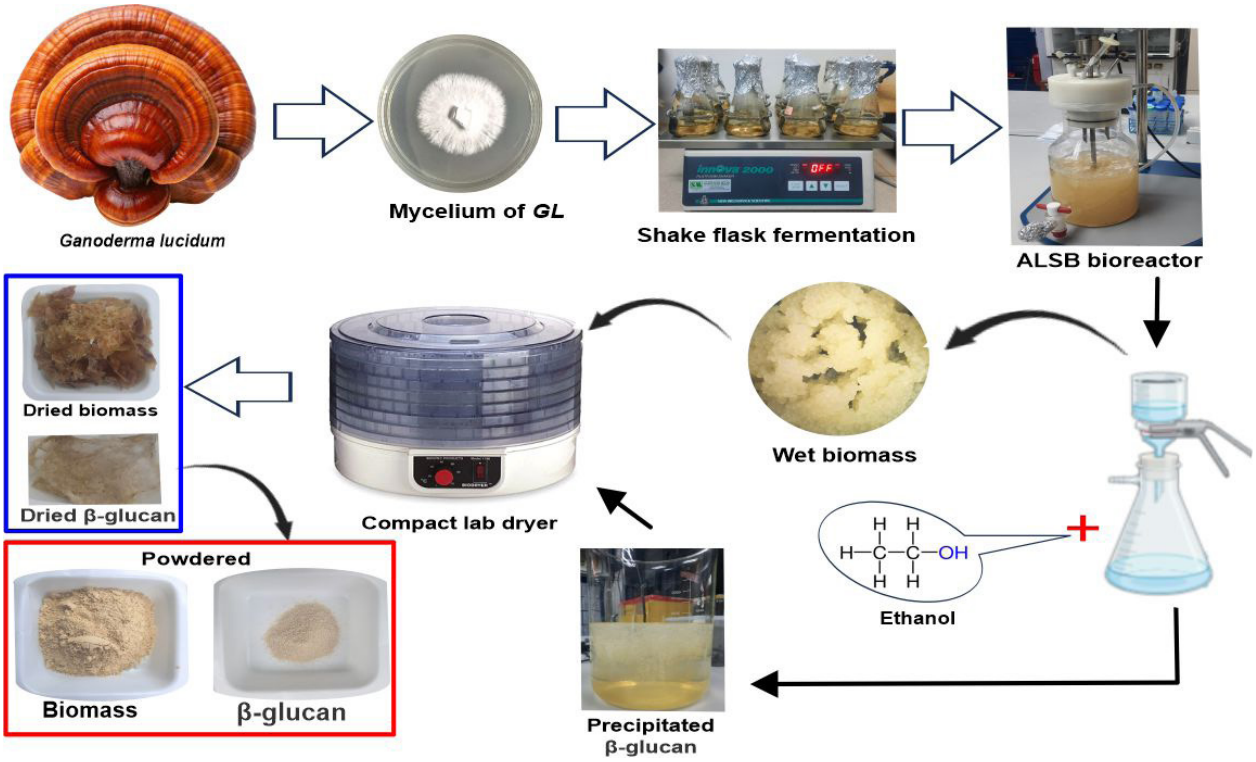

FIGURE 1. Production of experimental diet (*G. lucidum* biomass and β-glucan)

TABLE 1. Formulation and nutritional constituent (proximate analysis) of fish feed

Ingredients (g/kg)	C1	C2	T1	T2	T3
Fishmeal	Commercial feed	300	300	300	300
Cornmeal		193.9	192.6	192	188
Rice bran		199.3	197.4	196.4	194
Soymeal		236.8	230	226.6	228
Mycelial biomass		0	10	15	20
Vitamin		2	2	2	2
Mineral		3	3	3	3
Lysine		10	10	10	10
Methionine		5	5	5	5
Fish oil		40	40	40	40
Dicalcium Phosphate		10	10	10	10
EPS (standard)		0	3	3	3
Total		1000	1000	1000	1000
Nutritional composition (g/100 g)					
Ash		10.8	10.5	11.0	10.6
Moisture		7.4	7.6	7.8	7.5
Protein		28.6	28.9	28.2	29.2
Total fat		8.7	9.0	8.2	8.6
Carbohydrate		44.5	44.0	44.8	44.1
Energy		371	373	366	371

control groups: C1 commercial feed (negative control) and C2: formulated feed without biomass/β-glucan (positive control). The 3 g of β-glucan was selected as standard due to its superior performance in our previous study (Wan et al. 2021) against 10 g, 15 g, and 20 g of biomass to evaluate the synergistic influence on African catfish. All the feed for the experimental groups and C2 was formulated using the same basal ingredients, such as fishmeal, rice bran, soybean, cornmeal, mineral/vitamin premix, di-calcium phosphate (DCP), fish oil, L-lysine, and methionine. The feed formulas were generated using the latest version of Winfeed software (2.8.4) (Mohammad et al. 2019; Norhidayah et al. 2016).

EXPERIMENTAL DESIGN, FISH, AND FEEDING TRIAL

The experiment was conducted in a blue plastic tank (Guppy-L1060 model) with dimensions of 1065 × 720 × 310 mm and a water capacity of 120 L. Each tank was equipped with an SD-4200 aquarium pump and a filter box to ensure effective water filtration, circulation, and aeration (Taufek et al. 2016). Approximately 300 healthy African catfish fingerlings were purchased from the Aquaculture Resources Centre in Kampung Kubu Gajah, Sungai Buloh, Selangor, Malaysia. The fingerlings measured 7 - 8.5 cm and weighed 4.5 - 5.0 g. Before the feeding trial began, the fish were acclimatized for two weeks at the Universiti Malaya Aquarium Center (Seyyed et al. 2025). The fish were then randomly assigned into five groups, each in triplicate, with 15 fish per tank. The feeding trial was conducted for 60 days. During this period, the fish were given feed measuring 3% of their body weight twice daily (8:00 AM and 3:00 PM) (Norhidayah et al. 2016).

GROWTH PERFORMANCE

The initial body weight (g) and length (cm) of the African catfish fingerlings were determined by measuring the length with a ruler (cm) and the weight using a weighing balance (g). To monitor feed intake, the body weight of the fish was recorded weekly. The final weight gain and length gain were calculated as the difference between the final and initial body weight and length, respectively, following the methods described by Lee et al. (2024) and Zhang et al. (2025). i.e., weight gain (g*) = final body weight – initial body weight.* Length gain (cm) = *final body length – initial body length.*

WATER PARAMETERS

Dechlorinated tap water was used in this study, and the water parameters were carefully monitored throughout the experiment time, according to Table 2. Dissolved oxygen was measured using a DO9100 analyzer, while ammonia and nitrate levels were estimated with API® reagent kits. Chlorine concentration was tested using an Aquadine chlorine tester. Additionally, temperature, pH, total dissolved solids (TDS), oxidation-reduction potential (ORP), and salinity were measured using the YY-1010 analytical instrument (Tutul Kumar et al. 2023).

WHITE BLOOD CELL COUNT, KIDNEY AND LIVER FUNCTION TEST

At the end of the feeding trial, the fish were fasted overnight, and five fish from each tank were randomly collected for blood collection. They were anesthetized in 1 L of water with 200 µL of clove oil, and the blood samples were obtained from the caudal vein using a 1 mL syringe and a 24G needle. The collected blood was transferred into BD Vacutainer® tubes (K2 EDTA and SSTTM II Advance) and then transported to the Universiti Malaya Teaching Hospital for further analysis (Mobasshsirin et al. 2023). The samples in BD Vacutainer® (K2 EDTA) were used to analyze White Blood Cell (WBC) and differential blood counts (leucocyte counts) using a SYSMEX XN series machine. While those in BD Vacutainer® (SSTTM II Advance) were used for the liver and kidney function tests using an ADVIA 2400 clinical chemistry machine, following the methods described by Song et al. (2024) and Ye et al. (2024). Blood glucose was assayed using ACCU-CHECK® Active glucometer and strip (REF: 07124112) (Saeed et al. 2024).

TABLE 2. Range of water parameters

Parameters	C1	C2	T1	T2	T3	Reference interval
DO	5.61±0.14	5.81±0.09	5.62±0.18	5.65±0.16	5.65±0.15	≥5 mg/L
T	27.10±0.48	26.88±0.43	27.24±0.39	26.94±0.42	27.08±0.44	26 °C-28 °C
NO_3	214.8±1.59	214.1±1.73	212.6±1.65	213.7±1.50	213.9±1.46	<250 mg/L
NH_4	<0.05mg/L	<0.05 mg/L	<0.05 mg/L	<0.05 mg/L	<0.05 mg/L	<0.05 mg/L
Cl	Base on color changes	Base on color changes	Base on color changes	Base on color changes	Base on color changes	Base on color changes
pH	6.53±0.18	6.54±0.20	6.78±0.18	6.74±0.17	6.93±0.15	6.5 to 7.5
TDS	205.3±7.02	203.4±4.24	191.3±12.88	192.3±8.71	193.4±15.18	<250 ppm
ORP	299.9±8.67	303.4±12.62	311.3±9.34	293.0±7.43	286.5±11.74	250-350 mV

TABLE 3. The effects of dietary *G. lucidum* biomass and β-glucan (EPS) on serum liver function of African catfish (*C. gariepinus*)

Parameters	Experimental groups					ANOVA
	C1	C2	T1	T2	T3	P-value
Total protein (g/L)	34.67±0.33^{ab}	32.00±0.57^{ab}	36.67±1.85^{a}	31.33±0.66^{b}	35.67±1.73^{ab}	0.038
Total albumin (g/L)	1.00±0.00^{a}	1.33±0.33^{a}	1.00±0.00^{a}	1.33±0.33^{a}	1.66±0.33^{a}	0.382
Total globulin (g/L)	40.00±1.86^{a}	30.67±0.66^{b}	35.67±1.96^{ab}	30.00±0.44^{b}	34.00±2.23^{ab}	0.001
Total bilirubin (/L)	5.67±0.66^{a}	1.67±0.33^{b}	1.33±0.33^{b}	2.00±0.00^{b}	1.33±0.33^{b}	0.000
Calcium (mmol/L)	0.79±0.15^{b}	2.20±0.60^{ab}	1.22±0.47^{ab}	2.95±0.02^{a}	2.40±0.65^{ab}	0.041
Phosphate (mmol/L)	4.19±1.32^{ab}	3.85±0.08^{ab}	5.36±0.61^{a}	2.84±0.07^{b}	3.50±0.48^{ab}	0.001
Magnesium (mmol/L	0.28±0.08^{b}	1.27±0.11^{a}	0.51±0.20^{b}	1.19±0.03^{a}	0.88±0.19^{ab}	0.003
Cholesterol (mmol/L)	3.27±0.06^{a}	2.93±0.20^{a}	3.10±0.40^{a}	2.77±0.12^{a}	3.20±0.26^{a}	0.606
Glucose (mmol/L)	1.77±0.06^{b}	1.83±0.19^{b}	2.77±0.10^{a}	2.77±0.07^{a}	2.97±0.09^{a}	0.010

C1 = Control one (Commercial feed), C2 = Control two (formulated feed without biomass & EPS), T1 = Treatment one (10 g biomass/3 g EPS), T2 = Treatment two (15 g biomass/3 g EPS), and T3 = Treatment three (20 g biomass/3 g EPS). All data are presented as mean±SEM (n= 5, triplicate per group). Data in the same line with different superscripts are significantly different ($P < 0.05$)

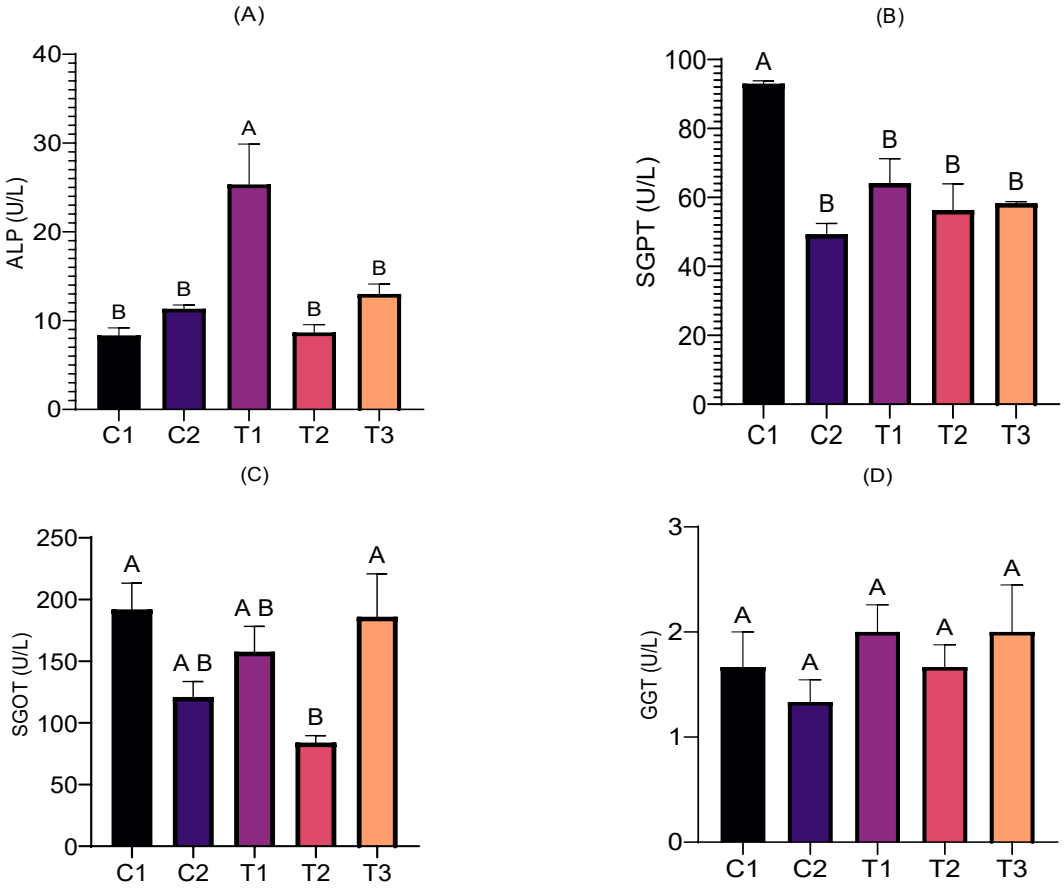

FIGURE 2. The effect of *G. lucidum* biomass and β-glucan on African catfish (*C. gariepinus*) serum biochemistry. (A) Alkaline phosphatase (ALP), (B) Serum glutamate pyruvate transaminase (SGPT)/ alanine aminotransferase (ALT), (C) Serum glutamic oxaloacetic transaminase (SGOT)/ aspartate aminotransferase (AST), (D) Gamma-glutamyl transferase (GGT). All data are presented as mean ± SEM. (n=5, triplicate per group), bars with different letters are significantly different

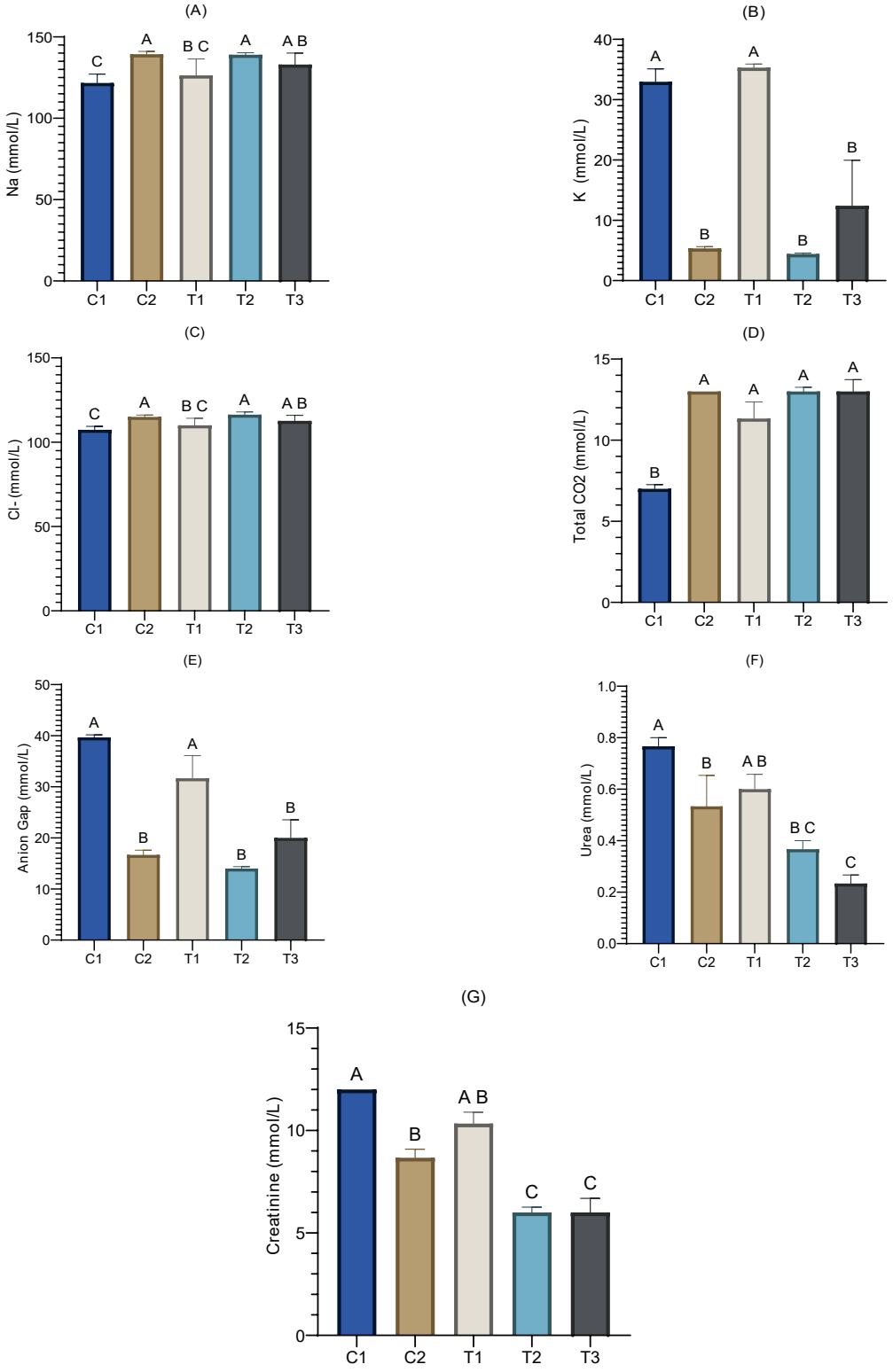

FIGURE 3. Combined effects of (biomass and β-Glucan) from a medicinal mushroom *G. lucidum* on serum kidney function of African catfish (*C. gariepinus*). (A) Sodium (Na^+), (B) Potassium (K^+), (C) Chloride (Cl^-), (D) Total Carbon Dioxide (CO_2), (E) Anion Gap, (F) Urea, and (G) Creatinine. Results are presented as mean ± SEM. (n=5, triplicate per group), bars with different letters are significantly different

STATISTICAL ANALYSIS

The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess the normality and homogeneity of variance (Gaussian distribution) of the data. For variables that passed the normality test, a one-way ANOVA was conducted to analyze the effects of dietary *G. lucidum* biomass and β-glucan on African catfish. Multiple comparisons were performed to identify mean differences across groups, with significance set at $P < 0.05$, using Tukey's post hoc test for pairwise analyses. All statistical analyses were performed using GraphPad Prism (version 10.2.1). Data are presented as mean ± SEM.

RESULT

LIVER FUNCTION TEST

The results of the liver function are presented in Table 3 and Figure 2. Serum protein levels were significantly higher in T1, among the treatment groups and controls ($P < 0.05$). Total albumin levels remained stable across all groups ($P > 0.05$), whereas globulin and bilirubin levels were significantly elevated in C1 ($P < 0.05$). Calcium levels were enhanced in the T2 group ($P < 0.05$), while phosphate levels were higher in the T1 group ($P < 0.05$). C2 and T2 exhibited higher magnesium levels ($P < 0.005$). Meanwhile, the cholesterol levels showed no significant difference across all the groups ($P > 0.05$), whereas glucose levels showed an increase in all treatment groups as the concentration increased ($P < 0.05$). Among the liver enzymes, alkaline phosphatase (ALP) activity was significantly higher in T1 ($P < 0.05$). Serum glutamate pyruvate transaminase (SGPT)//alanine aminotransferase (ALT) was elevated in C1 ($P < 0.05$), with no significant differences observed in other groups. However, serum glutamic oxaloacetic transaminase (SGOT)/aspartate aminotransferase (AST) showed no significant differences between C1 and T3, as well as between C2 and T1, but was reduced in the T2 group ($P < 0.05$). Gamma-glutamyl transferase (GGT) levels remained consistent across all groups ($P > 0.05$).

KIDNEY FUNCTIONS

The results of the kidney function showed that the kidney actively performed in eliminating excess waste as summarized in Figure 3. Sodium and chloride levels were significantly impacted in the C2 and T2 groups ($P < 0.05$) compared to the remaining groups. Similarly, the potassium and anion Gap were notably higher in the C1 and T1 groups ($P < 0.05$). Total CO_2 levels showed a significant decrease in C1, while no significant differences were observed in the other groups ($P > 0.05$). Urea and creatinine levels decreased in the treatment groups as the concentration increased ($P > 0.05$).

LEUCOCYTE COUNT (IMMUNE SYSTEM)

Table 4 presents the effects of dietary *G. lucidum* (biomass and β-glucan) on the immunological parameters of African catfish. WBC count was significantly impacted in the T2 and T3 groups ($P < 0.05$) compared to the other groups. However, neutrophil, lymphocyte, and monocyte levels showed no significant differences across all groups ($P > 0.05$). Furthermore, the detected basophils were not significant across all groups ($P > 0.05$), whereas eosinophils were completely undetected across all the groups ($P > 0.05$).

DISCUSSION

Dietary intervention plays a crucial role in the health and well-being of fish by ensuring balanced nutrition, promoting growth, supporting the immune system, enhancing antioxidant enzyme activity, and improving fertility and digestion (Saba et al. 2024). A key method for evaluating the impact of a new diet on fish welfare is by assessing kidney and liver function, as these organs directly influence the fish's overall physiological response (Fawole et al. 2024). In view of this, the serum total protein, albumin, and globulin are proteins that provide cellular nutrition, fight inflammation, transport biomolecules, build muscle, repair tissue, prevent blood clotting, and fight infections to enhance effective liver functioning (Ugoeze & Odeku 2025; Wang et al. 2024). Lower levels of these parameters indicate inadequate protein absorption, while excessively high levels may reflect a failure in the physiological response to maintain stability (homeostasis), a situation linked to liver or kidney dysfunction in fish (Long et al. 2024). In this study, serum total protein levels were adequate across all groups, with treatment groups T1 and T3 performing better. This supports previous studies that showed *G. lucidum* mushrooms provide digestible protein that supports growth and enhances physiological responses in fish (Mohammed Sharif et al. 2023; Sevdan et al. 2023). Similar results were reported in African catfish given dietary *Rynchophorus phoenicis* (Fawole et al. 2024). The total albumin content remained stable across all groups, staying within accepted limits for African catfish; albumin's primary role is to prevent fluid leakage into tissues (Muhammad Umar et al. 2024). In contrast, a higher serum globulin level was observed in the C1 group, likely due to variations in feed protein levels and environmental stress, which can disrupt the balance between albumin and globulin (Alfonso et al. 2024; Fazio et al. 2014). In the C2 and treatment groups, globulin levels remained within the normal range (Adegbesan & Abdulraheem 2020).

Bilirubin, a yellowish pigment produced by the breakdown of old red blood cells, is excreted through hepatic pathways into the bile to aid digestion (Rabia et al. 2024; Shayeganpour 2024). Excess bilirubin is eliminated through bodily fluids to maintain homeostasis (De Souza

TABLE 4. Influence of dietary *G. lucidum* biomass and β-glucan (EPS) on serum immune parameters of African catfish (*C. gariepinus*)

Parameters	Experimental groups					ANOVA
	C1	C2	T1	T2	T3	*P*-Value
WBC (10^9/L)	134.43±3.29^b	127.83±6.63^b	109.47±5.51^{ab}	136.37±3.95^a	134.50±6.48^b	0.001
Neutrophil (10^9/L)	1.11±0.97^a	2.01±0.56^a	1.16±0.65^a	1.80±0.21^a	1.09±0.54^a	0.757
Lymphocyte (10^9/L)	110.6±10.82^a	124.4±4.42^a	107.1±8.91^a	131.6±7.01^a	133.4±4.58^a	0.873
Monocyte (10^9/L)	3.02±1.97^a	1.33±0.15^a	1.02±0.39^a	2.91±1.83^a	0.31±0.25^a	0.470
Basophil (10^9/L)	0.03±0.02^a	0.02±0.01^a	0.01±0.00^a	0.03±0.01^a	0.02±0.00^a	0.794
Eosinophil (10^9/L)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.000

C1 = Control one (commercial feed), C2 = Control two (formulated feed without biomass & EPS), T1 = Treatment one (10 g biomass/3 g EPS), T2 = Treatment two (15 g biomass/3 g EPS) and T3 = Treatment three (20 g biomass/3 g EPS)

All data are presented as mean±SEM (n= 5, triplicate per group). Data in the same line with different superscripts are significantly different (*P<0.05*)

et al. 2024). An increase in bilirubin level was noted in C1, which is linked to stress situations associated with the distortion of serum biochemistry (Tanko et al. 2024), while C2 and all treatment groups were not negatively impacted. Similarly, calcium and magnesium levels were lower in the C1 group, suggesting deficiencies that could contribute to bone and kidney disorders (Liang et al. 2018). In the treatment groups, however, the absorption of dietary *G. lucidum* appeared to enhance serum mineral levels, thereby supporting kidney and liver function (Elhassaneen, Ragab & Salman 2016; Yang et al. 2020) and maintaining homeostasis (Bai et al. 2015).

Glucose levels were more stable in the treatment groups, ranging from 2.77 to 2.97 mmol/L, compared to the lower levels (1.77-1.83 mmol/L) observed in the control groups, which were associated with reduced pancreatic α- and β-cell activity (Polakof et al. 2012). The *G. lucidum* diet demonstrated better glucose maintenance compared to the *Fiscus deltoidea* diet, which resulted in glucose levels of 4.47 mmol/L in African catfish (Zulhisyam et al. 2024), Additionally, the *G. lucidum* diet outperformed metformin-treated African catfish, which had glucose levels of 4.52 mmol/L (Taher et al. 2022). These findings support the work of Aramabašić Jovanović et al. (2021), who noted that polysaccharides and triterpenoids in mushrooms are linked to glucose regulation. Furthermore, Ekiz et al. (2023) found that polysaccharides and triterpenoids from *G. lucidum* enhance glucose metabolism and improve insulin sensitivity. *G. lucidum* also modulates inflammatory pathways and protects pancreatic beta cells, thereby enhancing their response to insulin production (Md Faruque et al. 2024).

The ALP, SGPT, SGOT, and GGT are liver enzymes found in various tissues and organs, including the liver, kidneys, gills, intestines, blood, and bones. They collectively play a crucial role in phagocytosis, detoxification, nutrition, digestion, and absorption (Ibrahim et al. 2024; Yao et al. 2021). Elevated levels in the blood indicate symptoms of liver damage or physiological dysfunction (Seyedeh et al. 2024). In this study, the ALP activity showed no significant enzymatic changes across most of the groups, except for T1, which suggests a disruption in nutritional metabolism under stress conditions (Yu et al. 2024). An increase in SGPT was noted in C1, which is also linked to stress and metabolic ailments. According to Siddique and Kowdley (2012), stress-induced cellular changes to elevate SGPT, which is associated with cholestasis. The increase in bilirubin and SGPT recorded in C1 indicates a significant reduction or complete cessation of bile production. Several studies reported that stress contributes to increased SGPT levels in fish (Dagoudo et al. 2023; Sajjad et al. 2024; Zare et al. 2023; Zhou et al. 2024). In African catfish, stress leads to hepatocellular injury, resulting in rising SGPT levels (McGill 2016). The SGOT was also elevated in C1 and T3. At the same time, GGT activities were not impacted across all the diets, mushrooms improve lipid metabolism, reduce lipid deposition, and enhance liver enzyme activities to mitigate stress and prevent liver damage (Xu et al. 2024). The activities of these enzymes demonstrated enhanced performance in the C2 and T2 groups, highlighting the potential of the given diet in maintaining effective liver functioning.

The serum sodium levels recorded in this study varied across all the groups. However, all the recorded values are consistent with the reference interval reported by Tanko, Bilbonga, and Sati (2023) and Tanko et al. (2024). Notably, the C2 and T2 groups showed higher sodium performance; hence, the diet in all groups did not adversely affect sodium balance. In contrast, potassium levels were elevated in the C1 and T1 groups, suggesting hyperkalemia likely induced by environmental stressors such as pH, ammonia, and oxygen fluctuations (Samuel & King 2024). The C2 and T2 groups maintained optimal potassium levels, ranging between 4.40 and 5.33 mmol/L, consistent with the findings reported by Nabi, Ahmed and Wani (2022). Similarly, serum chloride levels in the C2 and T2 groups

demonstrated effective osmoregulation, while values in the other groups also remained within normal ranges (Boonsanit, Chanchao & Pairohakul 2024).

A total CO_2 level of 13.00 mmol/L was recorded in C2, T2, and T3, signifying effective blood pH maintenance through the chloride-bicarbonate exchange to regulate acid-base balance and intestinal homeostasis (Md. Ibrahim 2022). In contrast, the C1 group exhibited acid-base imbalances with lower CO_2 levels (7.00 mmol/L), which can disrupt overall health and metabolic stability (Wang, Poopal & Ren 2024; Zawisza et al. 2024). An elevated anion gap was observed in the C1 and T1 groups, indicating ketoacidosis, lactic acidosis, metabolic acidosis (Leyden et al. 2024), or hyperglycemic conditions (Sayed, Mekkawy & Mahmoud 2011). C2, T2, and T3 showed consistent levels that align with a reference study by Mohamed et al. (2023). Furthermore, urea and creatinine levels were higher in the C1 and T1 groups, indicating a reduced ability to eliminate nitrogenous waste. Improved kidney function, reflected by lower urea and creatinine levels, and was observed in the C2, T2, and T3 groups, confirming better nitrogenous waste elimination (Ellsaesser & Clem 1987; Higgins 2016; Seyyed et al. 2024; Yuniarti et al. 2024).

Regarding immune function, white blood cells (WBCs) and leukocytes play key roles in recognizing inflammation, promoting healing, and eliminating pathogens (Ritam et al. 2024; Zhu et al. 2025). In this study, WBC counts significantly increased in the C1, T2, and T3 groups, with the highest counts recorded in T2. This enhancement is attributed to the immunomodulatory effects of dietary *G. lucidum* biomass and β-glucan, which strengthen the fish's immune system against pathogens and stress (Karunarathna et al. 2024). Although the neutrophil, which is the essential component of the innate immune system responsible for rapid response and phagocytosis, remained stable across all groups, their consistency reflects a healthy immune status (Costantini 2024; Klak et al. 2024; Li et al. 2023). Lymphocytes, comprising T cells, B cells, and natural killer (NK) cells that play a role in adaptive immunity, showed a gradual increase in all treatment groups with rising concentrations, which was associated with enhanced immune system function (Lin et al. 2023; Ramasamy et al. 2021). In contrast, monocyte and basophil counts were not significantly affected by the experimental diets, indicating that these components of the innate immune response remained stable and effective in phagocytosis, antigen presentation, and cytokine production (Wu et al. 2024). Eosinophils were undetectable in all groups, suggesting the absence of allergenic responses (Tabaru et al. 2024).

CONCLUSION

The results of this study conclude that the combination of *G. lucidum* biomasst and β-glucan significantly improved growth (Figure 4), liver and kidney function, and the immune responses of African catfish. The recommended concentration in this study is 15 g of biomass combined with 3 g of β-glucan. This concentration was considered safe, with no adverse effects observed that would impede the production of healthy African catfish (*C. gariepinus*). Future research should explore the synergistic effects of the combined diets in outgrower pond systems to assess their long-term sustainability, economic viability, and impact on growth performance, intestinal histology, digestive enzyme activities, immunity, and antioxidant activities. Such studies will be essential for developing evidence-based guidelines to enhance commercial acceptability in aquaculture practices.

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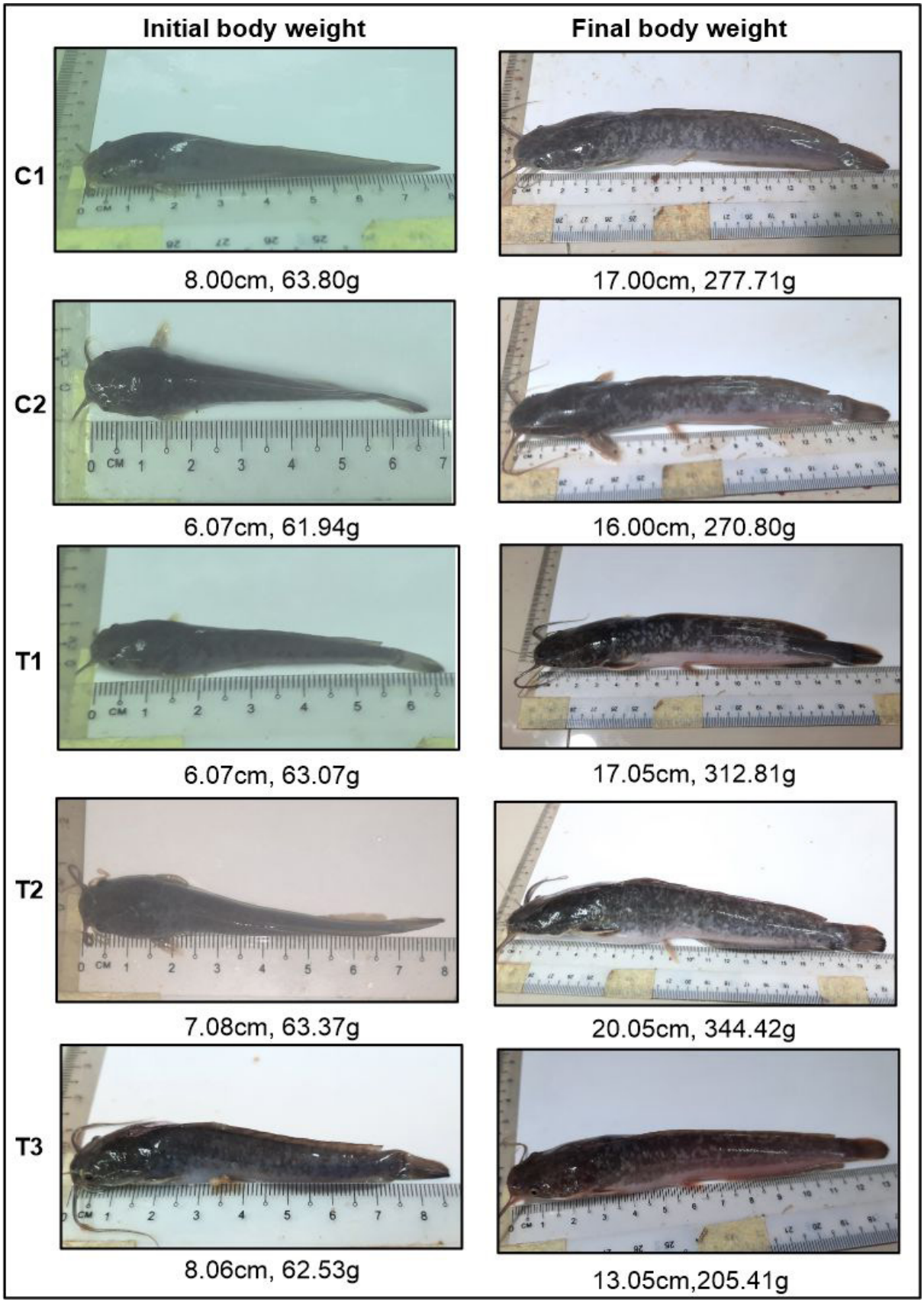

FIGURE 4. The initial and final body weight and length of African catfish over a 60-day feeding trial using *G. lucidum* biomass and β-glucan

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