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Effect of Phyto meal (Guinea grass) and Bacterial additives on growth Performance, Feed Utilization and Physiological Parameters of Grass carp (*Ctenopharyngodon idella*)

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Abstract

This study aims to evaluate the partial replacement of yellow corn with dried Gunia grass, with or without ZAD®, Bacti Silage, or their combination, on various growth and health parameters of Grass Carp (*Ctenopharyngodon idella*). The parameters assessed include growth performance, feed utilization, water quality, biometric measures, blood profiles, digestive and liver enzyme activities, body composition, organ histology, and economic feasibility. Thirteen experimental diets were formulated to contain 25% crude protein and mean gross energy 442.8 kcal/100 g. The control diet (D_1) contained no dried Gunia grass, while diets D_2, D_3, and D_4 included 10%, 20%, and 30% dried Gunia grass (DGG), respectively. Diets D_5, D_6, and D_7 incorporated 10%, 20%, and 30% DGG with ZAD® (GGZ), while diets D_8, D_9, and D_{10} included the same levels of DGG with Bacti Silage (GGBS). Diets D_{11}, D_{12}, and D_{13} combined both ZAD® and Bacti Silage (GGZB) at similar inclusion rates. The study was conducted in 39 hapa nets (120 cm L x 120 cm W x 100 cm H), with 15 Grass Carp fingerlings (average weight 0.97 ± 0.02 g) stocked per net. Each treatment had three replicates, and fish were fed at 3% of their body weight three times daily (six days per week). Synergistic use of ZAD® and Bacti Silage, especially at higher inclusion levels of dried Gunia grass (20% and 30%) consistently improves feed intake, feed efficiency, protein and energy utilization, and growth performance making GGZB as a superior alternative to yellow corn in Grass carp diets.

Keywords: dried grass, fibrolytic enzymes, Grass Carp, probiotics, growth performance.

INTRODUCTION

In the context of aquaculture, feed composition plays a critical role in determining the growth performance, health, and overall productivity of farmed fish species. The feed provided must not only meet the nutritional requirements of the fish but also be cost-effective and readily available. Feed constitutes 40%-50% of the total production costs in aquaculture, but despite this, fish feed technology is still underdeveloped, especially in regions such as Africa (Gabriel *et al*., 2007). In Egypt, the high cost of fish feed poses a significant barrier to the expansion of aquaculture and impacts profitability (Badrey *et al*., 2019). As a result, there is an increasing focus on identifying alternative ingredients that are both cheaper and more accessible. These alternatives can partially replace traditional feed sources or be used as feed additives to enhance growth and survival. Feed additives can be classified into living and non-living categories. Living feed additives include probiotics, algae, and plant-based materials such as leaves, extracts, and oils (Ogunkalu, 2019). While probiotics are typically used in small amounts, plants, algae and silage can be incorporated in larger quantities. Traditionally, yellow corn has been a staple ingredient in aquafeeds due to its high carbohydrate content and energy yield. However, the increasing cost and variable availability of yellow corn, especially in regions where it is not abundantly cultivated, have prompted the need for alternative locally-sourced by-products as feed ingredients. Some of these by-products are already being fully utilized by fish farmers but not on large scale (El-Shinnawy, 1990; Hathout and El-Nouby, 1990). These by-products are low protein content, high in fiber, poor palatability, low in minerals and vitamins content and have low digestibility, which due to their high content of cellulose (30-40%), hemicellulose (10-15%) (Theander and Aman, 1984). In nature, cellulose is the most abundant organic compound, but endogenous cellulase enzymes are notably absent in most vertebrates, with few exceptions such as freshwater crayfish, which produce their own cellulases distinct from those of their gut microbiota (Watanabe and Tokuda, 2001). Grass carp (*Ctenopharyngodon idella*), primarily herbivorous and consuming various aquatic plants (Křišťan *et al*., 2018), lack significant endogenous cellulase enzymes and rely instead on cellulolytic gut bacteria for cellulose digestion (Wu *et al*., 2012). These bacteria, including strains such as *Bacilli* and *Sphingomonas*, play a critical role in cellulose breakdown (Zhou *et al*., 2013; Li *et al*., 2009). While grass carp can tolerate dietary fiber levels of 14-16%, depending on fiber source and age, high fiber levels can negatively impact growth and nutrient absorption (Shao *et al*., 2020). Studies suggest cellulase supplementation could improve digestibility in aquaculture as endogenous enzymes are insufficient for complete fiber digestion (Zhou *et al*., 2013). Exogenous cellulase supplementation has shown to enhance other digestive enzymes, such as amylase and protease, though not lipase, supporting improved dietary fiber utilization (Zhou *et al*., 2013). Additionally, cellulolytic microorganisms exhibit highly efficient cellulase systems that convert cellulose into soluble sugars, facilitating nutrient absorption (Tomme *et al*., 1995). Overall, Gunia Grass (*Panicum maximum*) has attracted attention as alternative feed ingredient for herbivorous fish. (Aganga and Tshwenyane, 2004; Pedreira *et al*., 2015). This perennial herb from the Poaceae family is known for its high nutritional value, dense foliage, and soft texture, used as an energy source for various animals, including horses, poultry, rabbits (Chat *et al*., 2005; Oluwasola *et al*., 2008), and it also contributes to increasing milk production and promoting fattening in livestock species (Euclides *et al*., 2008; Peres *et al*., 2012). To enhance its digestibility and nutritional value for aquaculture, biological additives can be used to treat and break down the grass fiber, improving its suitability for fish feed. For Grass Carp (*Ctenopharyngodon idella*), the only species in the genus *Ctenopharyngodon* (Cudmore and Mandrak, 2004), which are herbivorous freshwater fish, their diet mainly consists of aquatic vegetation, but they can consume small fishes, worms, and insects when plant food is scarce. In pond culture, however, they show a preference for pelleted food over

vegetation (Köprücü, 2012). Adult Grass Carp primarily feed on aquatic macrophytes, with 95% of their diet coming from such sources (Fedorenko and Fraser 1978), while feeds for juvenile Grass Carp typically consist of 33% digestible protein, 6% lipids, and 10.7 kJ/g of digestible energy (Köprücü, 2012). This study examines replacing yellow corn with Gunia Grass in Grass Carp diets, evaluating effects on growth, feed efficiency, survival, and hematological parameters. The research aims to establish a sustainable feeding strategy using locally available resources, benefiting farmers and supporting environmentally responsible aquaculture practices.

MATERIALS AND METHODS

1 Experimental diets

Thirteen iso-proteinaceous (25% CP) and iso-caloric (442.8 kcal/100 g) diets were prepared. The thirteen experimental diets were formulated as described in Table (3). The ingredients were ground and thoroughly mixed, and the oil was slowly added at the same time of mixing with warm water (45°C) until the diet began to clump. Diets were processed by a minced machine and dried for 48 hours in a drying oven at 70°C. The pellet size was 0.6 mm in diameter and 2 mm in length and stored dried at -20°C. Experimental fry were fed 3% of live body weight 6 days a week for 92 day. The preparation of guinea grass treated with probiotics involves several steps, including harvesting (1.6 m height), drying (solar dryer for 7 days), and treated with probiotics (ZAD® and Bacti Silage, both of them). Experimental diets were prepared according to the following categories:

Untreated Grass (D_2, D_3, D_4): These diets substitute yellow corn meal (YCM) with 10%, 20%, and 30% dried Gunia grass without any treatments.

ZAD®-Treated Grass (D_5, D_6, D_7): In these diets, grass is treated with ZAD®.

Bacti Silage-Treated Grass (D8, D_9, D_{10}): In these diets, replace YCM had been replaced with Gunia grass treated with Bacti Silage.

Combination Treatment (D_{11}, D_{12}, D_{13}): In the final three diets, grass is treated with both ZAD® and Bacti Silage. ZAD® (patent No. 22155) is the biotechnical product made from natural sources to elevated level of cellulose enzyme from anaerobic bacteria which convert the polysaccharide into monosaccharide by specific enzymes according to the procedure of Gado (1997). The used probiotic ZAD® is a mixture of anerobic Bacteria (*Ruminococcus sp.*) at concentration 28×1012 c.f.u/ml. This species are producer for cellulase, xylanase, alpha amylase and protease enzyme, Bacti Silage is a mixture of *Ruminococcus flavefaciens*, Lactic acid bacteria, *Lactobacillus plantarum* and *Pediococus pentosaceus* at concentrations (15×1012, 1.0×1013, 6.0×1012 and 4.0×1012 CFU).

2 Experimental fish

Apparently healthy 390 (*C. idella*) fry were obtained from a private commercial freshwater fish farm in Motobas, Kafr–El Sheikh Governorate, Egypt. Experimental fry (with an average initial body weight of 0.98 ± 0.02g / fry.) were kept indoors in circular cement tanks for two weeks as an acclimation period and fed on diet contained 30% crude protein (Prior to the start the experiment, (ninety fish were frozen at -20°C for initial whole-body chemical analysis.

3 Experimental facility

Fish were placed in Thirty-nine net hapas (thirteen treatments each with a three replicates) with dimensions of (120 cm L * 120 cm W * 100 cm H) each. Fish were kept before starting the experiments for 15 days as an acclimatization period in circular cement tanks until distributed into the experimental hapas. Each hapa was stocked with fifteen fry of (*C. idella*) that were weighed, and their weight was recorded biweekly.

Table (1): Chemical analysis (% on DM basis) of dietary ingredients used in the present study

Ingredient	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)	Crude fiber (%)	NFE (%)	GE (Kcal/100g)
Fish meal	92.32	65.68	18.14	16.18	0	0	512.51
Soybean meal	89.11	44.5	4.13	4.45	7.3	39.62	453.69
Wheat bran	88.82	14.5	4.17	6.32	10.73	64.28	386.17
Yellow corn	86.86	7.61	3.41	1.23	1.86	85.89	429.08
DGG	90.36	7.15	0.6	12.17	27.51	52.58	262.7
DGGZ	89.91	7.55	0.41	13.75	21.3	55.99	277.21
DGGBS	89.16	7.86	0.32	14.25	20.5	65.25	316.26

NFE: Nitrogen free extract (Calculated by differences).
GE: Gross Energy: - Gross energy was calculated using factors 5.65, 9.45 and 4.15 kcal/g of protein, lipid and carbohydrate, respectively (Gatlin III, 2010). DGG: dried gunia grass. GGZ: dried gunia grass with of (ZAD®). GGBS: dried gunia grass with of (Bacti Silage).

Proximate chemical analysis

Samples of the experimental diets and fish (initial and final samples) were chemically analyzed to determine dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), and ash contents according to the methods of AOAC (2000). Nitrogen free extract (NFE) was calculated by differences, by deducting the sum of percentages of CP, EE, CF and ash from 100. Gross energy (GE) contents of the experimental diets and fish samples were calculated by using factors of 5.65, 9.45 and 4.15 kcal/g of protein, lipid and carbohydrates, respectively (Gatlin III, 2010). Proximate analysis composition of the experimental diets was presented in Table (3).

Survival rate and Growth performance parameters:

Average weight gain (AWG), average daily gain (ADG), specific growth rate (SGR% day), feed/gain ratio, feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate were calculated according to the following equations:

Average weight gain (g/fish) (AWG)

$$WG = W_2 - W_1$$

Where: W_1 = the initial weight (mg)
W_2 = the final weight (g)
Average daily gain (mg/fish) (ADG):

$$ADG = \frac{W2 - W1}{T}$$

Where: W_1= the initial weight (mg)
W_2= the final weight (g)
T = Experimental period (d)

Specific growth rate (SGR):

$$SGR = \frac{Ln.\ Wf - Ln.\ Wi}{Period\ (days)} \times 100$$

Where Ln = Natural Logarithm (log)$^{-10}$
Wi= Mean initial weight (g)
Wf= Mean final weight (g)
Survival rate (%): SR % = (Total number of fish survived/Total number of fish stocked) × 100.

Feed and nutrient utilization:

Feed conversion ratio (FCR):

$$FCR = \frac{\text{Dry feed intake (g)}}{\text{Live weight gain (g)}}$$

Protein efficiency ratio (PER):

$$PER = \frac{\text{Live weight gain (g)}}{\text{Protein intake (g)}}$$

Protein productive value (PPV %):
PPV% = 100× (Protein gain/ Protein applied)
Where: Protein gain= ($P_t - P_O$)
P_O: Protein content in fish at start.
P_t: Protein content in fish at end.

Energy utilization (EU %):

EU% = 100× [($E_t - E_O$)/ energy intake (kcal)].
Where: E_t= Energy in fish (kcal) at end.
E_O= Energy in fish (kcal) at start.

Biometric parameters:

Viscerosomatic index (VSI):
VSI = 100 × (viscera weight / whole-body weight)
Intestosomatic index (ISI):
ISI =100 × (intestine weight / whole-body weight)

Hepatosomatic index (HIS):
HIS = 100 × (hepatosomatic weight / whole-body weight)
Condition Factor (CF):
CF = whole-body weight ×100 / length

Water Quality:

Water temperature and dissolved oxygen were measured daily using an oxygen meter (YSI Model 58, YSI Industries, and Yellow Spring Instruments, OH, USA). The pH- value was monitored twice weekly using an electronic pH meter (pH pen, Fisher Scientific, Cincinnati, OH, USA). Total ammonia, nitrite, and nitrate were measured weekly using spectrophotometer (Spectronic 601**,** Milton Roy Company, San Diego, CA, USA) according to APHA (2003). Total alkalinity was monitored twice weekly using the titration method of Golterman *et al*. (1978).

Blood sampling collection and preparation:

At the end of the experiment, five fish were randomly sampled from each hapa (15 per treatment) and sedated (clove powder, 200 mg L^{-1}) to collect blood samples from caudal vein using a 2 mL syringe. The blood was collected into tubes with and without heparin for routine blood testing and serum preparation, respectively. Serum was separated from the latter by allowing the samples to stand at room temperature for 2 h and then at 4 °C overnight, the tubes were then centrifuged at 3000 rpm/min for 10 min to separate the serum that was used for blood physiological and biochemical parameters testing.

Hematological assays:

The heparinized blood was diluted in PBS to quantify white blood cells (WBC) and red blood cells (RBC) using a hemocytometer slide (Sarder, *et al*., 2001). Hemoglobin was measured by blending blood samples with Drabkin's Reagent and reading the absorbance of mixtures at 540 nm wavelength on a spectrophotometer (Blaxhall and Daisley, 1973; Klontz *et al*., 1994**).** The hemoglobin contents were reported as grams per deciliter (g dl−1). Hematocrit values (HCT) were estimated using the method of (Cyriac *et al*., 1989). Microhematocrit tubes were filled with heparinized blood and centrifuged at 10,000 rpm for 5 min. The length of packed red cells (mm) × 100 divided by the total length of the blood sample (mm) in the tube is reported as HCT. The mean cell volume (MCV), the mean cell hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) were estimated based on the formula presented by Ref (Benfey and Sutterlin, 1984).The serum samples were used for aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), triglycerides (TG), and lactate dehydrogenase (LDH) determinations that were measured using a Hitachi 7020 fully automated biochemical analyser (Tokyo, Japan) according to the manufacturers protocols.

Determination of Oxidant/antioxidant indices:

Glutathione peroxidase (GPX) and malondialdehyde (MDA) were assayed in hepatic tissues (three fish/replicates). The liver samples were homogenized in buffer at pH 7.5 and centrifuged for 15 minutes at 10,000 rpm at 4◦C to get the supernatant. The supernatant was then centrifuged for 1 hour to recover the final supernatant, and the pellet was collected, washed, and stored in the buffer (pH 7.5). The hepatic levels of malondialdehyde (MDA, catalog No. MD 25 29), GPX (catalog No. GP 2524) were measured spectrophotometrically using commercial kits (Bio diagnostics company, Cairo, Egypt).

Digestive enzyme activities:

At the end of the experiment, two anaesthetized fish from each replicate were dissected, the liver was washed using sterile chilled saline, kept in an icebox, then stored at -80°C until homogenization. The liver samples were minced and homogenized (10% w/v) in ice-cold sucrose buffer (0.25 M) in a Wise Tis® HG-15D homogenizer (Daihan Scientific, India). The homogenate was centrifuged at 10000 rpm for 20 min at 4°C. The resulting supernatant was collected and stored at -20°C. Enzyme activity of total proteases, amylase and lipase was expressed as specific activity (units per milligram of soluble protein; one unit (U) of

activity was defined as µmol of product generated per minute). Soluble protein concentration was determined using method of Bradford (1976), with bovine serum albumin solution as standard. Amylase and lipase activities were determined using the formula:

$$\text{mU mg protein}^{-1} = \frac{(\Delta DO/\Delta t) \times Vt \times f}{Ex \times 10^{-3} \times 10^{-9} \times Ve \times d \times P}$$

Where (Δ DO/ Δ t) is the decrease or increase of optical density / minute, Vt is the total reaction volume, f is the correction factor for the dilution of the extract, Ex is the molar extinction coefficient, 10-3 is the conversion factor of liter to milliliter, 10-9 is the conversion factor from mol to nmol, Ve is the volume of added extract in ml, d is the length of the light beam through the microplate (0.79 for lipase activity and 0.675 for amylase activity) and P is the mg of protein per ml.

Histological technique:

After fish dissection, the liver and intestine were removed, thoroughly washed with a physiological saline (0.9% NaCl) solution and blotted on filter paper then buffered formalin 10%. The fixed specimens were processed using a conventional paraffin embedding technique. From the prepared paraffin blocks, 5 mm thick sections were obtained and stained with hematoxyline and eosin (H and E) for light microscopic examination according to the method described by Culling (1974). Measurements of villi length and width were taken using microscope with a micrometer rule as described by (Spadoni *et al*., 2005; Eyarefe *et al*., 2008). Four different villi were measured in each slide per parameter, recorded and an average values calculated. For villi length, width and area of absorption.

Economic evaluation:

Economic valuation of the experimental diets has been calculated by evaluation the feed cost in Egyptian pound (L.E) needed to produce 1 kg of live weight gain of each experimental fish group.

Feed cost/kg weight gain = FCR × cost of kg feed

Statistical analysis:

The obtained data were subjected to one-way analysis of variance (ANOVA) to test the effect of replacement of yellow corn (10, 20 and 30 %) dried gunia grass without or with of ZAD®, Bacti Silage and both of them respectively in Grass carp diets as the three factors. Least significant difference (LSD) was used as a post hoc test to compare between means at $P \leq 0.05$. Data were analyzed by analysis of variance (ANOVA) using the SAS procedure (Statistical Analysis System, version 9.1.3, 2007). All percentages and ratio were transformed to arcsine values prior to analysis (Zar, 1984).

Table (2): Chemical analysis of feed ingredients (g/ 100 g) (% on DM basis).

Item	Experimental diets												
	Control	Dried Gunia grass (DGG)			Gunia grass With Z (GGZ)			Gunia grass With B (GGBS)			Gunia grass With ZB (GGZB)		
	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8	D_9	D_{10}	D_{11}	D_{12}	D_{13}
Feed ingredients (g/ 100 g)													
Fish meal	15	15	15	15	15	15	15	15	15	15	15	15	15
Soybean meal	25	25	25	25	25	25	25	25	25	25	25	25	25
Wheat bran	12	12	12	12	12	12	12	12	12	12	12	12	12
Yellow corn	44	39.74	35.48	31.21	39.31	34.63	29.94	39.57	35.13	30.69	39.44	34.88	30.32
DGG	0	4.26	8.52	12.79	0	0	0	0	0	0	0	0	0
GGZ	0	0	0	0	4.69	9.37	14.05	0	0	0	2.35	4.69	7.03
GGBS	0	0	0	0	0	0	0	4.43	8.87	13.31	2.22	4.44	6.66
Sunflower oil	3	3	3	3	3	3	3	3	3	3	3	3	3
Vit. and Min. mix.	1	1	1	1	1	1	1	1	1	1	1	1	1
Total	100	100	100	100	100	100	100	100	100	100	100	100	100

DGG: dried gunia grass. GGZ: dried gunia grass with of (ZAD®). GGBS: dried gunia grass with of (Bacti Silage).

Table (3): Proximate analysis of Experimental diets (% on DM basis).

Item	Experimental diets												
	Control	Dried Gunia grass (DGG)			Gunia grass With Z (GGZ)			Gunia grass With B (GGBS)			Gunia grass With ZB (GGZB)		
	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8	D_9	D_{10}	D_{11}	D_{12}	D_{13}
Proximate analysis (%) on DM basis:													
DM	90.14	90.17	90.16	90.18	90.18	90.19	90.19	90.20	90.21	90.20	90.22	90.21	90.20
Ash	5.20	6.13	6.17	6.25	6.12	6.13	6.16	6.10	6.04	5.98	5.87	5.70	5.52
OM	84.94	84.04	83.99	83.93	84.06	84.06	84.03	94.10	84.17	84.22	84.35	84.51	84.68
CP	25.28	25.20	25.20	25.22	25.30	25.31	25.30	25.32	25.28	25.29	25.30	25.32	25.31
EE	6.54	7.27	7.29	7.28	7.29	7.30	7.30	7.31	7.30	7.29	7.33	7.31	6.87
CF	4.84	6.64	6.71	6.85	5.47	5.68	5.87	6.43	6.21	5.91	5.34	5.26	5.14
NFE	58.14	54.76	54.63	54.40	55.82	55.58	55.37	54.84	55.17	55.53	56.16	56.41	57.16
GE (kca/100)	445.92	438.34	446.29	437.05	443.49	442.64	441.72	441.09	440.77	442.23	445.28	446.24	445.14
P:E	56.69	57.49	56.47	57.71	57.05	57.18	57.28	57.40	57.35	57.19	56.82	56.70	56.86

DM: Dry Matter CP: Crude Protein EE: Ether Extract (a measure of crude fat) OM: Organic Matter (difference between DM and Ash values) Ash: Mineral Content (inorganic matter left after combustion) CF: Crude FiberNFE: Nitrogen-Free Extract (calculated carbohydrates) GE (kcal/100): Gross Energy (measured in kilocalories per 100 grams)
P:E: Protein-to-Energy Ratio

RESULTS

1. Choice infestation test3.1 Growth performance:

Table (4) explains minimal variation in initial weights across treatments, ranging from 0.97g to 1.05g, indicating no significant ($P \leq 0.05$) differences at the start. The highest final weight (20.31g) and weight gain (19.34g) were recorded in treatment D_{13}, which utilized 30% DGG combined with both ZAD® and Bacti Silage, demonstrating the synergistic effect of these additives. In contrast, treatments with only DGG (D_2, D_3, D_4) had significantly ($P \leq 0.05$) lower final weights (7.68g, 7.44g, 7.26g) and weight gains (6.67g, 6.41g, 6.29g) compared to the control (D_1) (8.85g and 7.88g, respectively). Both Average Daily Gain (ADG) and Specific Growth Rate (SGR %) followed similar trends, with D_{13} showing exhibiting the most efficient growth metrics. Diets consisting solely of

DGG (D_2-D_4) had significantly ($P \leq 0.05$) poorer ADG and SGR% compared to both the control and other enhanced treatments. Most treatments achieved a 100% survival rate, except for D_1 (control), D_4 (30% DGG), D_9 (20% DGG + Bacti Silage), and D_{10} (30% DGG + Bacti Silage), where survival rates ranged from 86.67% to 97.78%. The lower survival rate in the control suggests that additives like ZAD® and Bacti Silage improve fish resilience. The results indicate that replacing yellow corn with DGG alone does not support optimal growth in Grass Carp, as seen in D_2-D_4. However, combining DGG with ZAD® and/or Bacti Silage significantly ($P \leq 0.05$) improves growth metrics, especially at higher inclusion levels (20% and 30%). The combination of ZAD® and Bacti Silage (D_{11}-D_{13}) showed the greatest positive effect on growth, with D_{13} outperforming all treatments. These findings align with (Aslam and Zuberi, 2017), who observed high biomass and growth in Grass Carp fed Duckweed under intensive culture. Gado *et al*. (2017b) also noted that exogenous fibrolytic enzymes, such as those in ZAD® and Bacti Silage, enhance digestibility and energy availability, contributing to improved growth. In conclusion, while DGG alone may not suffice as a yellow corn replacement, the addition of ZAD® and Bacti Silage can overcome its nutritional limitations, enhancing growth and survival in Grass Carp. This has potential applications in aquaculture, especially in areas where yellow corn is less accessible or cost-effective.

Table (4): Effect growth performance

Experimental Treatments	Initial weight (g)	Growth performance			SGR[4] %	Survival (%)
		Final weight (g)	Gain (g)	ADG (g/fish/day)		
D_1	0.97 [a] ± 0.02	8.85 [h] ± 0.07	7.88 [h] ± 0.06	0.09 [e] ± 0.002	2.46 [e] ± 0.02	86.67 [d] ± 0.87
D_2	1.01 [a] ± 0.02	7.68 [i] ± 0.07	6.67 [i] ± 0.06	0.07 [f] ± 0.002	2.25 [g] ± 0.02	100 [a] ± 0.87
D_3	1.03 [a] ± 0.02	7.44 [j] ± 0.07	6.41 [j] ± 0.06	0.07 [f] ± 0.002	2.2 [g] ± 0.02	100 [a] ± 0.87
D_4	0.97 [a] ± 0.02	7.26 [j] ± 0.07	6.29 [j] ± 0.06	0.07 [f] ± 0.002	2.24 [g] ± 0.02	93.33 [c] ± 0.87
D_5	1.00 [a] ± 0.02	9.34 [g] ± 0.07	8.34 [g] ± 0.06	0.09 [e] ± 0.002	2.48 [e] ± 0.02	100 [a] ± 0.87
D_6	1.06 [ab] ± 0.02	9.88 [e] ± 0.07	8.82 [e] ± 0.06	0.1 [d] ± 0.002	2.49 [e] ± 0.02	100 [a] ± 0.87
D_7	1.00 [a] ± 0.02	10.29 [d] ± 0.07	9.29 [d] ± 0.06	0.1 [d] ± 0.002	2.59 [d] ± 0.02	100 [a] ± 0.87
D_8	1.05 [ab] ± 0.02	8.76 [h] ± 0.07	7.71 [h] ± 0.06	0.09 [e] ± 0.002	2.36 [f] ± 0.02	100 [a] ± 0.87
D_9	0.99 [a] ± 0.02	9.41 [g] ± 0.07	8.42 [g] ± 0.06	0.09 [e] ± 0.002	2.5 [e] ± 0.02	95.56 [bc] ± 0.87
D_{10}	1.03 [a] ± 0.02	9.67 [f] ± 0.07	8.64 [f] ± 0.06	0.1 [d] ± 0.002	2.49 [e] ± 0.02	97.78 [ab] ± 0.87
D_{11}	1.05 [a] ± 0.02	12.71 [c] ± 0.07	11.65 [c] ± 0.06	0.13 [c] ± 0.002	2.76 [c] ± 0.02	100 [a] ± 0.87
D_{12}	0.99 [a] ± 0.02	16.77 [b] ± 0.07	15.78 [b] ± 0.06	0.18 [b] ± 0.002	3.15 [b] ± 0.02	100 [a] ± 0.87
D_{13}	0.97 [c] ± 0.02	20.31 [a] ± 0.07	19.34 [a] ± 0.06	0.22 [a] ± 0.002	3.38 [a] ± 0.02	100 [a] ± 0.87

SGR4%: Specific Growth Rate (% increase in weight per day) ADG (g/fish/day): Average Daily Gain (grams per fish per day)

2. Feed and nutrient utilization

Table (5) demonstrates the effects of replacing yellow corn with varying levels of Dried Gunia Grass (DGG), DGG with ZAD®, DGG with Bacti Silage, and both additives on feed and nutrient utilization in Grass Carp. Treatments combining ZAD® and Bacti Silage (D_{11}-D_{13}) exhibited the best performance, with D_{13} showing the highest feed intake, lowest feed conversion ratio (FCR), and superior protein efficiency ratio (PER), protein productive value (PPV%), and energy utilization. In contrast, treatments with only DGG (D_2-D_4) showed the poorest results, with the highest FCR and lowest nutrient utilization. Moderate improvements were observed in treatments using either ZAD® or Bacti Silage alone (D_5-D_{10}), but they did not achieve the same level of enhancement as when both additives were combined. The lowest FCR was recorded in D_{13}, indicating the most efficient feed utilization at 30% DGG with both additives, while DGG without additives (D_4) had the highest FCR, reflecting poor feed efficiency. Both Protein Efficiency Ratio (PER) and Protein Productive Value (PPV %) significantly ($P \leq 0.05$) improved in treatments with ZAD® and Bacti Silage, particularly at 30% replacement (D_{13}). Similarly, energy utilization peaked in D_{13}, highlighting the optimal nutrient assimilation when both additives were used together. The data showed significant ($P \leq 0.05$) increases in PPV%, feed intake, and feed efficiency in treatments D_{11}, D_{12}, and D_{13}, likely due to the synergistic effects of ZAD® and Bacti Silage. These findings align with Gado *et al*.

(2006, 2011, Gado and Salem, 2013), who noted improved protein content, digestibility, and fiber degradation when ZAD® was used. ZAD®, a biotechnological product, enhances nutrient digestibility, body weight gain, and feed conversion by breaking down polysaccharides into monosaccharides. In contrast, treatments with only DGG (D_2-D_4) showed the highest FCR and lowest nutrient utilization, likely because DGG alone is less effective as feed ingredient. Superior results from combining ZAD® and Bacti Silage underscore the synergistic effect on feed efficiency and nutrient utilization in Grass Carp diets, particularly at higher inclusion levels (20% and 30%). Overall, the combination of ZAD® and Bacti Silage consistently improved feed intake, feed efficiency, protein utilization, and energy utilization, making it a superior alternative to untreated DGG or control diets.

Table (5): Effect of replacement of yellow corn with different levels of DGG, DGG with ZAD®, DGG with Bacti Silage and Both of them on Feed and nutrient Utilization of Grass Carp *(Ctenopharyngodon idella)*.

Experimental Treatments	Feed intake (g/fish)	FCR	Feed utilization		Energy utilization (%)
			PER	PPV (%)	
D_1	14.96^{de} ± 0.94	1.9^{bc} ± 0.12	2.08^{cd} ± 0.07	34.73 ^{d} ± 1.07	17.85^{ef} ± 0.55
D_2	13.37 ^{e} ± 1.05	2^{bc} ± 0.21	1.98 ^{d} ± 0.12	32.18 ^{e} ± 0.81	17.72^{ef} ± 0.15
D_3	13.4 ^{e} ± 0.45	2.09 ^{b} ± 0.11	1.90 ^{d} ± 0.06	29.18 ^{f} ± 0.61	16.79 ^{f} ± 0.53
D_4	15.34^{de} ± 0.69	2.44 ^{a} ± 0.13	1.63 ^{e} ± 0.07	25.18 ^{g} ± 0.61	14.55 ^{g} ± 0.93
D_5	15.63 ^{d} ± 0.64	1.88^{cd} ± 0.14	2.11^{cd} ± 0.1	34.8 ^{d} ± 0.7	19.03^{de} ± 1.19
D_6	15.24^{de} ± 0.7	1.73^{de} ± 0.12	2.29^{bc} ± 0.11	38.99 ^{c} ± 1.13	20.84^{bc} ± 0.32
D_7	16.41 ^{d} ± 0.48	1.77^{cd} ± 0.06	2.24 ^{c} ± 0.12	37.22 ^{c} ± 0.7	19.35^{de} ± 0.79
D_8	15.66 ^{d} ± 0.57	2.03^{bc} ± 0.04	1.95 ^{d} ± 0.11	32.37 ^{e} ± 1.32	16.85 ^{f} ± 0.81
D_9	16.88 ^{d} ± 0.62	2.01^{bc} ± 0.05	1.97 ^{d} ± 0.13	32.45 ^{e} ± 0.98	17.61^{ef} ± 0.9
D_{10}	16.72 ^{d} ± 0.29	1.93^{bc} ± 0.06	2.04^{cd} ± 0.09	33.48^{de} ± 1.02	18.29^{ef} ± 0.79
D_{11}	20.63 ^{c} ± 0.76	1.77^{cd} ± 0.09	2.23 ^{c} ± 0.12	38.33 ^{c} ± 0.72	19.86^{cd} ± 1.05
D_{12}	24.98 ^{b} ± 1	1.58^{de} ± 0.15	2.50^{ab} ± 0.26	42.56 ^{b} ± 1.44	21.49^{ab} ± 0.9
D_{13}	28.34 ^{a} ± 0.49	1.46 ^{e} ± 0.11	2.70 ^{a} ± 0.21	46.94 ^{a} ± 0.96	22.95 ^{a} ± 1.19

FCR: Feed Conversion Ratio PPV (%): Protein Productive Value (%)PER: Protein Efficiency Ratio

3. Body chemical composition

Table (6) presents the body chemical composition of Grass Carp when yellow corn is replaced with varying levels of Dried Gunia Grass (DGG), DGG with ZAD®, DGG with Bacti Silage, and combinations of both additives. Dry matter, ether extract, gross energy, and ash content remained statistically similar across all treatments, indicating a consistent nutrient profile. Significant differences ($P \leq 0.05$) were observed in crude protein content, with the highest level recorded in treatment D_{13} (25.67 a ± 0.58), which included DGG with both ZAD® and Bacti Silage at the 30% replacement level. This suggests that combining these additives enhances crude protein content, contributing to better growth performance compared to other treatments and the control. The crude fiber content was lowest in the control treatment (D_1), while treatments with both ZAD® and Bacti Silage (D_{11}-D_{13}) showed reduced crude fiber, especially at the 30% replacement level (D_{13}), suggesting improved fiber digestibility. Nitrogen-Free Extract (NFE) values differed significantly ($P \leq 0.05$) among the experimental treatments, reflecting variability in carbohydrate content. The control group (D_1) had the highest NFE value (59.03 a ± 1.72), while treatments involving DGG with additives generally showed lower NFE values. Notably, D_{13} (DGG with both ZAD® and Bacti Silage at 30% replacement) had an NFE value of 56.9 ab ± 0.61, significantly ($P \leq 0.05$) higher compared to several other treatments, highlighting the potential of these additives to improve carbohydrate availability. The protein-to-energy ratio (P) was slightly higher in treatments D_3, D_4, D_8, D_9, and D_{13}, indicating a balanced nutrient composition conducive to efficient growth.

Overall, combining ZAD® and Bacti Silage, particularly at higher replacement levels (D_{13}), appears to optimize the chemical composition of Grass Carp, potentially enhancing growth

performance and overall health. The improvement in ether extract with DGG treated with ZAD® and Bacti Silage, although not statistically significant (P≤0.05), yielded better results than the control group. These findings align with previous studies (Bassuny *et al*., 2005; Gado *et al*., 2006; Gado and Salem, 2013), which reported improved crude protein and fiber digestibility when using ZAD® and fungal additives with low-quality roughages. In the case of crude fiber, treatments D_{11}, D_{12}, and D_{13} showed a notable decline compared to other groups. This could be due to the action of cellulose enzymes secreted by bacteria or microorganisms in rumen liquor or by commercial cellulose enzymes. These results are consistent with Gado *et al*. (2007).

Table (6) :Body chemical composition

Experimental Treatments[1]	Dry matter (%)	On dry matter basis (%)				NFE	GE	Ratio P: E
		Crude protein	Ether extract	Ash	Crude Fiber			
D_1	90.20 ^{a} ± 1.82	24.30 ^{b} ± 0.67	6.57 ^{a} ± 0.74	5.24 ^{a} ± 0.61	4.85 ^{g} ± 0.55	59.03 ^{a} ± 1.72	442.62 ^{a} ± 4.15	54.90 ^{c} ± 0.74
D_2	90.19 ^{a} ± 1.71	24.26 ^{b} ± 0.72	7.31 ^{a} ± 0.36	6.17 ^{a} ± 0.77	6.66^{ab} ± 0.58	55.6^{bc} ± 1.07	435.2 ^{a} ± 3.7	55.74^{bc} ± 0.77
D_3	90.22 ^{a} ± 2.08	25.25^{ab} ± 0.33	7.34 ^{a} ± 0.33	6.19 ^{a} ± 0.4	6.74^{ab} ± 0.4	54.47^{bc} ± 1.51	436.46 ^{a} ± 5.93	57.85 ^{a} ± 0.54
D_4	90.17 ^{a} ± 1.97	25.26^{ab} ± 0.38	7.35 ^{a} ± 0.29	6.25 ^{a} ± 0.33	6.87 ^{a} ± 0.18	54.27 ^{c} ± 1.35	435.8 ^{a} ± 4.82	57.96 ^{a} ± 0.62
D_5	90.2 ^{a} ± 1.85	25.33^{ab} ± 0.57	7.34 ^{a} ± 0.57	6.16 ^{a} ± 0.18	5.51^{ef} ± 0.33	55.66^{bc} ± 1.47	441.79 ^{a} ± 3.37	57.33^{ab} ± 0.77
D_6	90.17 ^{a} ± 1.9	25.03^{ab} ± 0.54	7.33 ^{a} ± 0.29	6.16 ^{a} ± 0.16	5.71^{ef} ± 0.31	55.77^{bc} ± 0.55	440.44 ^{a} ± 2.99	56.82^{ab} ± 0.91
D_7	90.19 ^{a} ± 1.98	24.65^{ab} ± 0.48	7.30 ^{a} ± 0.5	6.22 ^{a} ± 0.22	5.91^{de} ± 0.15	55.92^{bc} ± 0.87	438.68 ^{a} ± 3.08	56.19^{bc} ± 0.79
D_8	90.18 ^{a} ± 1.15	25.37^{ab} ± 0.29	7.34 ^{a} ± 0.57	6.15 ^{a} ± 0.13	6.46^{bc} ± 0.33	54.69^{bc} ± 0.6	437.99 ^{a} ± 4.47	57.92 ^{a} ± 0.51
D_9	90.21 ^{a} ± 1.53	25.31^{ab} ± 0.26	7.32 ^{a} ± 0.51	6.07 ^{a} ± 0.16	6.25^{cd} ± 0.25	55.05^{bc} ± 1.11	438.94 ^{a} ± 4.87	57.65 ^{a} ± 0.63
D_{10}	90.19 ^{a} ± 1.6	25.35^{ab} ± 0.36	7.29 ^{a} ± 0.38	5.96 ^{a} ± 0.49	5.95^{de} ± 0.42	55.46^{bc} ± 0.46	440.59 ^{a} ± 2.88	57.53^{ab} ± 0.81
D_{11}	90.18 ^{a} ± 1.57	25.01^{ab} ± 0.5	7.37 ^{a} ± 0.75	5.86 ^{a} ± 0.49	5.35^{fg} ± 0.49	56.41^{bc} ± 0.83	443.38 ^{a} ± 3.68	56.41^{bc} ± 0.99
D_{12}	90.19 ^{a} ± 1.51	25.01^{ab} ± 0.36	7.35 ^{a} ± 0.25	5.68 ^{a} ± 0.33	5.29^{fg} ± 0.36	56.68^{bc} ± 0.47	444.24 ^{a} ± 3.83	56.29^{bc} ± 0.52
D_{13}	90.19 ^{a} ± 1.84	25.67 ^{a} ± 0.58	6.71 ^{a} ± 0.41	5.55 ^{a} ± 0.58	5.17^{fg} ± 0.3	56.9^{ab} ± 0.61	442.85 ^{a} ± 4.19	57.96 ^{a} ± 1.13

NFE: Nitrogen-Free Extract (represents the carbohydrate fraction of feed). GE: Gross Energy (total energy content of a feed or food item).

P:E: Protein-to-Energy Ratio (the balance of dietary protein relative to energy.

4 Biometric parameters

Table (7) evaluates the effects of replacing yellow corn with varying levels of Dried Gunia Grass (DGG), DGG treated with ZAD®, Bacti Silage, and their combination on the biometric parameters of Grass Carp. Viscerosomatic Index (VSI), which assesses the proportion of visceral organ weight relative to the total body weight of fish, was significantly higher (P≤0.05) in treatment D_{13} (14.36 ± 0.44), where 30% of yellow corn was replaced with DGG combined with both ZAD® and Bacti Silage. This result indicates enhanced internal organ development, likely reflecting improved health, metabolic activity, and nutrient absorption. Treatments D_2 to D_{10}, which involved various levels of DGG, DGG with ZAD®, or DGG with Bacti Silage, showed generally lower VSI values than D_{13}. The control diet (D_1) and treatments with only DGG (D_2 to D_4) or a single additive (D_5 to D_{10}) consistently had lower VSI values compared to D_{13}.

Intestosomatic Index (ISI), measuring the weight of the intestines relative to the total body weight, also showed significantly ($P \leq 0.05$) higher values ($P \leq 0.05$) in D_{13} (5.35 ± 0.33). This suggests enhanced intestinal development, likely due to the combination of ZAD® and Bacti Silage improving nutrient digestion and absorption. Treatments D_2 to D_{10}, involving different levels of DGG and additives, exhibited lower ISI values compared to D_{13}, while the control diet (D_1) also had a lower ISI. The Hepatosomatic Index (HSI), reflecting liver weight relative to body weight, was significantly higher ($P \leq 0.05$) in D_{13} (3.82 a ± 0.54). This may indicate improved liver function and metabolic activity in fish receiving both ZAD® and Bacti Silage. The Condition Factor (CF), a measure of overall fish health and growth, was also significantly ($P \leq 0.05$) higher in D_{13} (1.89 ± 0.18) compared to all other treatments, reflecting superior growth performance. Treatments D_2 to D_{10} showed consistently lower CF values, while the control diet (D_1) and treatments with only DGG or one additive also had lower CF values than D_{13}. The increased VSI in D_{13} suggests that the combined diet of DGG, ZAD®, and Bacti Silage at a 30% replacement level promoted higher VSI, reflecting increased metabolic activity and energy storage in the visceral organs. This improvement may be attributed to better nutrient digestibility and absorption, leading to enhanced visceral organ growth (Ali *et al*., 2010). The synergistic effects of ZAD® and Bacti Silage likely improved the fish's ability to utilize nutrients efficiently, contributing to better feed quality and digestion. ZAD® enhances nutrient availability, while Bacti Silage supports fermentation processes, which could explain the improved physiological function and increased organ mass. These findings align with previous studies (Storebakken *et al*., 2000). Regarding ISI, the combined additives in D_{13} significantly ($P \leq 0.05$) improved intestinal development, enhancing nutrient absorption and overall digestive efficiency. The increased ISI reflects better intestinal health, supporting improved growth performance and fish condition. Similar results were reported by Shiau and Hsu (2002). For CF, the combination of ZAD® and Bacti Silage in D_{13} led to significantly ($P \leq 0.05$) better body condition, indicating enhanced nutrient availability and feed utilization. This resulted in superior growth and health, as evidenced by the improved weight-to-length ratios in $_{D13}$. These results are consistent with findings by Bureau *et al*. (2008) and Lemos (2009).

Table (7): Biometric parameters

Experimental Treatments	VIS	ISI	HIS	CF
D_1	$11.42^{d} \pm 0.57$	$4.51^{bc} \pm 0.2$	$3.00^{ef} \pm 0.39$	$1.55^{b} \pm 0.14$
D_2	$10.20^{h} \pm 0.58$	$2.65^{d} \pm 0.37$	$2.55^{g} \pm 0.33$	$0.95^{ef} \pm 0.08$
D_3	$10.30^{gh} \pm 0.55$	$3.98^{c} \pm 0.62$	$2.80^{fg} \pm 0.35$	$1.14^{ef} \pm 0.14$
D_4	$11.20^{de} \pm 0.58$	$2.99^{d} \pm 0.58$	$3.10^{ef} \pm 0.2$	$1.16^{ef} \pm 0.15$
D_5	$10.90^{ef} \pm 0.58$	$4.20^{bc} \pm 0.49$	$2.90^{ef} \pm 0.4$	$1.30^{cd} \pm 0.17$
D_6	$11.50^{d} \pm 0.58$	$4.30^{bc} \pm 0.58$	$3.20^{de} \pm 0.35$	$1.20^{de} \pm 0.17$
D_7	$13.28^{b} \pm 0.74$	$5.05^{ab} \pm 0.42$	$3.66^{ab} \pm 0.28$	$1.35^{cd} \pm 0.17$
D_8	$10.58^{fg} \pm 0.65$	$3.92^{c} \pm 0.57$	$2.83^{fg} \pm 0.3$	$1.08^{ef} \pm 0.05$
D_9	$11.26^{d} \pm 0.63$	$3.94^{c} \pm 0.59$	$3.18^{de} \pm 0.22$	$0.87^{f} \pm 0.02$
D_{10}	$12.24^{c} \pm 0.71$	$4.81^{bc} \pm 0.53$	$3.23^{cd} \pm 0.2$	$1.08^{ef} \pm 0.03$
D_{11}	$11.50^{d} \pm 0.74$	$4.51^{bc} \pm 0.38$	$2.96^{ef} \pm 0.44$	$1.48^{bc} \pm 0.23$
D_{12}	$13.07^{b} \pm 0.76$	$4.73^{bc} \pm 0.48$	$3.41^{bc} \pm 0.36$	$1.59^{b} \pm 0.34$
D_{13}	$14.36^{a} \pm 0.44$	$5.35^{a} \pm 0.33$	$3.82^{a} \pm 0.54$	$1.89^{a} \pm 0.18$

VIS: Viscerosomatic index ISI: Intestosomatic index
HIS: Hepatosomatic index CF: Condition Factor

5. Water quality parameters

Table (8) presents the impact of replacing yellow corn with various levels of dried grass (DGG), DGG treated with ZAD® (GGZ), DGG treated with Bacti Silage (GGBS), and DGG treated with both GGZ and GGBS on water quality parameters in grass carp culture. Water temperature across all treatments (D_1-D_{13}) remained stable, with no significant ($P \leq 0.05$) differences. Similarly, dissolved oxygen (DO) levels ranged from 5.2-5.9

mg/L, showing no significant ($P \leq 0.05$) differences between treatments. Regarding pH values, slight variations were observed, ranging from 7.3-8.04. Lowest pH values were recorded in diets treated with Bacti Silage (D_8: 7.30, D_9: 7.60, D_{10}: 7.50), possibly due to acidifying effects of fermentation process. Total ammonia nitrogen (TAN) levels were lowest in treatments D_{12} (1.91 mg/L) and D_{13} (1.87 mg/L), which included the highest levels of Gunia grass treated with both ZAD® and Bacti Silage. In contrast, untreated DGG diets (D_2, D_3, D_4) showed higher TAN values. Nitrite levels remained low across all treatments (0.12-0.21 mg/L), with no significant ($P \leq 0.05$) differences, although slightly higher nitrite concentrations were observed in untreated DGG diets (D_3 and D_4). Nitrate concentrations also varied slightly but remained within safe limits (1.92 to 2.48 mg/L), with the highest nitrate levels recorded in untreated DGG diets (D_4). The inclusion of dried Gunia grass and its treated forms did not notably impact water temperature, a crucial factor for maintaining optimal fish metabolism and health. Although the fermentation process in Bacti Silage caused a slight reduction in pH, it remained within acceptable levels for grass carp. The stable dissolved oxygen levels suggest that neither DGG nor its treated forms negatively affected oxygen availability, essential for fish respiration. The lower TAN levels in treatments with ZAD® and Bacti Silage indicate improved nitrogen management, possibly through enhanced nitrification or ammonia utilization, while untreated DGG led to higher nitrogen waste accumulation. Although nitrite and nitrate concentrations remained safe, the slightly elevated levels in untreated DGG diets highlight the importance of treatment in promoting efficient nitrogen conversion. The use of ZAD® and Bacti Silage-treated Gunia grass (especially in D_{11}, D_{12}, and D_{13}) appeared to promote better water quality by reducing nitrogenous waste, which is vital for maintaining a healthy culture environment. Controlling TAN and nitrate levels is particularly important in aquaculture systems to prevent toxic build-up and support fish growth and health. These results suggest that treatments with ZAD® and Bacti Silage may enhance nitrogen assimilation or denitrification processes, thus reducing nitrate accumulation in the water and improving environmental sustainability. Temperature is a critical environmental factor affecting fish metabolism, growth, and overall health. Grass carp (*Ctenopharyngodon idella*) (Norman, 2010; Ahmad and Harun, 2015), being ectothermic, rely on stable water temperatures for optimal physiological processes, with an ideal range between 25°C and 30°C (Ahmad and Harun, 2015; Graham, 2010). The recorded temperatures in this study fall within range (26.5°C to 27.3°C), ensuring normal metabolic activity and growth (Bostock *et al*., 2014). Importantly, the dietary replacement of yellow corn with DGG and its treated forms did not affect water temperature, an indication that the feeds did not introduce thermal stress to the fish. This stability is crucial as temperature fluctuations can compromise fish immunity and growth, leading to higher susceptibility to disease. The slightly lower pH values observed in the treatments with Bacti Silage (D_8, D_9, D_{10}) could be attributed to the fermentation process involved in its preparation. Fermentation is known to produce organic acids such as lactic acid, which can lower the pH of feed and subsequently water quality (Ricke, 2003). pH also plays a role in ammonia toxicity; higher pH levels increase the proportion of toxic unionized ammonia (NH_3). In this study, lower TAN levels in treatments with DGG treated with ZAD® and Bacti Silage may be attributed to the acidifying effect favoring the less toxic ammonium form (NH_4^+), thereby reducing stress on the fish (Colt and Armstrong, 1981). The recorded DO values (5.2 to 5.9 mg/L) were well above critical thresholds for grass carp (5.0 mg/L), ensuring recommended oxygen levels were sufficient for growth and metabolism (Boyd, 1990; Timmons *et al*., 2002). The stable DO levels across treatments suggest that even untreated DGG did not cause excessive oxygen demand, a risk associated with high fiber and organic matter in plant-based feeds. The treated DGG forms, especially with Bacti Silage, likely enhanced microbial balance in the system, reducing the biological oxygen demand (BOD) and supporting nitrification processes, where dissolved oxygen becomes available in the water, thus promoting the activity of nitrifying bacteria, leading to efficient conversion of toxic ammonia to less harmful nitrate (Boyd, 2015). Inclusion of alternative plant-based feed ingredients like DGG can sometimes impact DO levels by increasing organic matter load. However, the consistent DO levels in this study indicate that treated and untreated

DGG did not excessively raise organic matter, likely due to efficient nutrient digestion. Treated DGG diets, particularly those involving ZAD® and Bacti Silage, promoted better nutrient utilization, limiting the organic load in the pond and reducing oxygen demand. Maintaining proper DO levels is crucial for nitrification, the process by which ammonia (NH_3) is converted into nitrite (NO_2^-) and then into nitrate (NO_3^-). This process requires oxygen and is performed by nitrifying bacteria. In this study, the relatively low total ammonia nitrogen (TAN) and nitrite levels observed in treatments containing ZAD® and Bacti Silage suggest that nitrification was functioning efficiently, supported by the adequate oxygen supply. High DO levels are necessary for the optimal performance of nitrifying bacteria, which help prevent accumulation of toxic ammonia and nitrite (Colt and Armstrong, 1981). Inclusion of alternative feed ingredients, such as plant-based materials like DGG, can sometimes influence DO levels by affecting the nutrient load in the water. Untreated plant materials often contain higher levels of fiber and indigestible compounds, which can increase organic matter in the pond, thereby raising the oxygen demand during decomposition (Tacon and Metian, 2008). In this study, however, the consistent DO levels across all treatments indicate that even untreated DGG did not lead to excessive organic load or decomposition. The treatments involving fermentation (Bacti Silage) and enzyme additives (ZAD®) might have contributed to better digestion and nutrient utilization by the fish, thereby reducing the amount of undigested material entering the water. Improved feed efficiency can help limit the organic matter load in the pond, which in turn reduces the microbial oxygen demand and helps maintain higher DO levels (Boyd and Tucker, 1998). These findings highlight the environmental benefits of incorporating treated Gunia grass, especially with ZAD® and Bacti Silage, into aquaculture feeds. By improving water quality and promoting nitrogen management, these treatments can enhance the sustainability of plant-based feed alternatives in aquaculture.

Table (8): Water quality parameters

Experimental Treatments	Temperature (°C)	pH	DO (mg/L)	TAN (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)
D_1	26.91 ^{a} ± 0.64	7.9^b ± 0.64	5.47 ^{a} ± 0.68	2.2^c ± 0.17	0.18^a ± 0.01	2.28^d ± 0.09
D_2	27.3 ^{a} ± 0.64	7.94^b ± 0.66	5.45 ^{a} ± 0.66	2.27^b ± 0.14	0.19^a ± 0.01	2.37^c ± 0.15
D_3	27.1 ^{a} ± 0.35	7.93^b ± 0.71	5.55 ^{a} ± 0.31	2.3^b ± 0.2	0.2^a ± 0.01	2.41^b ± 0.21
D_4	27.1 ^{a} ± 0.47	8.03^a ± 0.08	5.44 ^{a} ± 0.08	2.4^a ± 0.14	0.21^a ± 0.01	2.48^a ± 0.14
D_5	26.9 ^{a} ± 0.49	7.8^b ± 0.36	5.2 ^{a} ± 0.27	2.03^d ± 0.06	0.17^a ± 0.01	2.35^c ± 0.2
D_6	27.1 ^{a} ± 0.12	8^a ± 0.4	5.55 ^{a} ± 0.43	2.22^c ± 0.13	0.17^a ± 0.01	2.4^b ± 0.14
D_7	26.9 ^{a} ± 0.23	8.04^a ± 0.31	5.53 ^{a} ± 0.13	2.31^b ± 0.18	0.18^a ± 0.3	2.36^c ± 0.18
D_8	26.99 ^{a} ± 0.4	7.3^b ± 0.17	5.5 ^{a} ± 0.17	2.25^b ± 0.14	0.12^a ± 0.01	2^d ± 0.15
D_9	26.91 ^{a} ± 0.53	7.6^b ± 0.23	5.7 ^{a} ± 0.42	2.4^a ± 0.14	0.15^a ± 0.01	2.06^d ± 0.07
D_{10}	26.9 ^{a} ± 0.35	7.5^b ± 0.29	5.8 ^{a} ± 0.71	2.33^b ± 0.19	0.14^a ± 0.01	1.99^d ± 0.06
D_{11}	27.1 ^{a} ± 0.35	8.03^a ± 0.25	5.9 ^{a} ± 0.17	2.02^d ± 0.07	0.12^a ± 0.01	1.92^d ± 0.07
D_{12}	27.05 ^{a} ± 0.32	7.8^b ± 0.29	5.3 ^{a} ± 0.17	1.91^d ± 0.06	0.13^a ± 0.01	1.95^d ± 0.06
D_{13}	26.5 ^{a} ± 0.06	8^a ± 0.29	5.44 ^{a} ± 0.25	1.87^d ± 0.06	0.12^a ± 0.01	1.94^d ± 0.05

pH: Acidity/Alkalinity DO (mg/L): Dissolved Oxygen TAN (mg/L): Total Ammonia Nitrogen (sum of NH_3 and NH_4^+ in water).
NO_2-N (mg/L): Nitrite-Nitrogen (amount of nitrogen in the form of nitrite). NO_3-N (mg/L): Nitrate-Nitrogen (amount of nitrogen in the form of nitrate).

6. Blood hematological parameters

Table (9) presents data on the hematological responses of grass carp (*Ctenopharyngodon idella*) to diets in which yellow corn was replaced with varying levels of dried Gunia grass (DGG), dried Gunia grass treated with ZAD® (GGZ), Bacti Silage (GGBS), or both. The highest haemoglobin levels were observed in fish fed diets D_9, D_{10}, D_{12}, and D_{13}, which included higher percentages (20-30%) of Gunia grass treated with Bacti Silage or both ZAD® and Bacti Silage. Fish on diet D_{13} (30% DGG treated with both additives) showed the highest haemoglobin (8.77 g/100 ml). Also, this treatment D_{13} and D_{10} produced the RBC counts compared with other treatments especially Diets D_2 and D_5 which had the lowest RBC count. Regarding

haematocrit values, it decreased with DGG addition or with increasing level in fish fed diets D_2, D_3 and D_4, while with the addition of GGZ and/or GGBS at all levels (10-30%) it increased significantly ($P \leq 0.05$), especially in the highest treatment groups (D_{12} and D_{13}) followed by control treatment. Similarly, mean corpuscular volume (M.C.V µm³/cell) value in control treatment was higher than treatments D_2-D_4 that included different levels of DGG alone, or treatments D_5-D_7 that included different levels of DGG plus GGZ. On the other hand, the highest mean corpuscular volume (M.C.V µm³/cell) value was observed in fish fed diet D_{13}.

Fish fed with higher levels (20-30%) of treated Gunia grass with ZAD® + Bacti Silage GGZB (D_{12} and D_{13}) showed the highest Mean Corpuscular Hemoglobin (M.C.H pg/cell) and Mean Corpuscular Hemoglobin Concentration (M.C.H.C %), while, lower MCH and MCHC values were observed in diets D_2, D_3 and D_4. The same trend was evident with White Blood Cells the WBC counts which were generally higher in fish fed diets with treated Gunia grass with ZAD® or Bacti Silage, particularly when treated with ZAD® + Bacti Silage GGZB in (D_{12} and D_{13}). Furthermore, Diet D_1 (control) had the highest WBC count (33.43). Increment of haemoglobin levels with D_9, D_{10}, D_{12}, and D_{13} indicating that these treatments might enhance oxygen-carrying capacity more effectively than other treatments or the control diet (D_1). Also, this increment noticed with Red Blood Cells content with higher replacement levels (30%) which indicating improving erythropoiesis with higher levels (30%) of DGG treated with Bacti Silage and both additives. While, Lower haemoglobin levels and RBC counts in diets D_2, D_3, and D_4 suggest that untreated diets with GGZ and/or GGBS might not be as effective in maintaining or improving blood haemoglobin levels and may be due to negatively affect erythrocyte production. Additives like ZAD® and Bacti Silage might improve the overall nutrient profile of the feed by enhancing the digestibility of DGG. This could lead to better absorption of micronutrients crucial for hemoglobin synthesis, such as iron and vitamin B_{12} (Wang *et al*., 2019). The improved hemoglobin levels suggest that treated diets enhance the overall health and metabolic function of the fish, potentially leading to better oxygen transport and overall growth performance (Wang *et al*., 2011; Tacon and Metian, 2008). The increase in RBC counts with higher levels of treated DGG (D_{10} and D_{13}) suggests that both Bacti Silage and ZAD® enhance the feed's nutritional quality. These additives likely improve the availability of essential nutrients needed for erythropoiesis, such as vitamins, minerals, and growth factors. The beneficial effects of Bacti Silage and ZAD® could be due to their roles in improving gut health and nutrient absorption (Lall and Dumas, 2022). The superiority at the highest replacement level (30% DGG), indicating that a higher concentration of treated DGG provides more significant benefits for RBC production. This suggests a dose-dependent effect where the nutritional enhancements from the additives become more pronounced at higher inclusion rates (Tacon *et al.*, 2006). Also, DGG inclusion with ZAD and /or Bacti Silage enhanced Haematocrit (%) this suggests that higher levels of treated DGG with GGZ and/or GGBS enhance the overall blood cell volume and may improve the fish's ability to transport oxygen and consequently producing larger larger RBC size when higher percentages of Gunia grass treated with both ZAD® and Bacti Silage which reflect a response to increased erythropoietic demand or changes in RBC morphology. This positive response led to enhance MCH, MCHC and White Blood Cells values which indicate improved immune function or a response to dietary treatment, possibly offering better disease resistance, while superiority of control treatment might suggest that some of the treatments initially stress the fish or alter their immune response compared to the control (Lall and Kaushik, 2021). Also, the higher MCH and MCHC values observed in diets D_{12} and D_{13} indicate that the combination of DGG with ZAD® and Bacti Silage improves hemoglobin content per RBC and its concentration. This suggests that treated DGG diets enhance the nutritional profile, especially minerals, leading to more efficient hemoglobin synthesis and greater oxygen-carrying capacity. Studies have demonstrated that improved feed quality and nutrient availability can enhance MCH and MCHC (Lall and Kaushik, 2021; Tacon *et al.*, 2006). The lower hematocrit values observed in untreated DGG diets (D_2, D_3, D_4) indicate a possible deficiency in essential nutrients or the presence of inhibitory factors affecting RBC production or overall blood volume. This could be due to reduced nutrient bioavailability or suboptimal nutritional profiles of the untreated DGG (Lall and Dumas, 2022). The

improvement in hematocrit values with diets containing ZAD® and Bacti Silage suggests that these additives contribute to better overall nutrition and metabolic function. Additives such as ZAD® and Bacti Silage may enhance the digestibility and bioavailability of crucial nutrients like iron, vitamins, and minerals necessary for maintaining healthy hematocrit levels (Wang *et al*., 2019). Generally, the inclusion of dried Gunia grass, particularly when treated with ZAD® and Bacti Silage, generally improved blood hematological parameters in grass carp, enhancing RBC counts, haemoglobin content, and overall blood health. Treatments involving additives (ZAD® and Bacti Silage) seem to mitigate any negative effects of untreated Gunia grass, enhancing blood parameters to levels comparable to or exceeding those of the control diet. The results suggest that appropriate processing of Gunia grass is crucial to maximizing its benefits as a feed component in grass carp diets.

Table (9): Blood hematological parameters

Experimental Treatments	Haemoglobin (g/100 ml)	R.B.Cs (×10/mm3)	Haematocrit (%)	M.C.V (µm3/cell)	M.C.H (pg/cell)	M.C.H.C (%)	WBCS (×10^3/mm3)
D_1	6.54 ^{c} ± 0.21	2.01^{ab} ± 0.08	17.60 ^{c} ± 0.49	95.63 ^{e} ± 1.07	33.33 ^{f} ± 1.29	34.33 ^{h} ± 1.29	33.43 ^{a} ± 1.04
D_2	6.33 ^{c} ± 0.35	1.16 ^{c} ± 0.08	12.47 ^{i} ± 0.64	88.23 ^{i} ± 1.43	25.47 ^{i} ± 1.19	26.40 ^{k} ± 1.24	23.63 ^{h} ± 1.07
D_3	5.57 ^{d} ± 0.32	1.21 ^{c} ± 0.09	11.47 ^{j} ± 0.61	86.03 ^{j} ± 1.37	27.67 ^{h} ±	28.67 ^{j} ±	24.57 ^{g} ±
D_4	5.43 ^{d} ± 0.23	1.32 ^{c} ± 0.19	10.76 ^{k} ± 0.44	83.43 ^{k} ± 1.44	28.43 ^{g} ±	29.43 ^{i} ±	25.57 ^{f} ± 1.08
D_5	7.31 ^{b} ± 0.21	1.80 ^{b} ± 0.11	12.87 ^{h} ± 0.61	92.53 ^{h} ± 1.13	33.73 ^{f} ±	34.63 ^{h} ±	26.81 ^{e} ±
D_6	8.08^{ab} ± 0.14	1.85^{ab} ± 0.18	13.90 ^{g} ± 0.61	93.73 ^{g} ± 1.19	34.83 ^{e} ±	35.90 ^{g} ±	26.83 ^{e} ±
D_7	8.31 ^{a} ± 0.21	2.05^{ab} ± 0.13	14.94 ^{f} ± 0.58	94.83 ^{f} ± 1.18	35.67 ^{d} ±	36.67 ^{f} ±	28.20 ^{d} ±
D_8	8.34 ^{a} ± 0.27	1.80 ^{b} ± 0.11	15.98 ^{e} ± 0.58	95.77 ^{e} ± 1.24	37.13 ^{c} ±	37.83 ^{e} ±	27.67^{de} ± 1.2
D_9	8.41 ^{a} ± 0.41	1.89^{ab} ± 0.12	16.75 ^{d} ± 0.63	96.67 ^{d} ± 1.18	37.43 ^{c} ±	38.63 ^{d} ±	28.00 ^{d} ±
D_{10}	8.72 ^{a} ± 0.01	2.09 ^{a} ± 0.13	17.73 ^{c} ± 0.58	97.10 ^{d} ± 1.44	37.67 ^{c} ±	38.73^{cd} ±	29.10 ^{c} ±
D_{11}	8.00^{ab} ± 0.49	1.87^{ab} ± 0.2	18.79 ^{b} ± 0.64	99.00 ^{c} ± 1.24	38.40 ^{b} ±	39.40^{bc} ±	29.80^{bc} ±
D_{12}	8.72 ^{a} ± 0.29	2.07^{ab} ± 0.13	19.79 ^{a} ± 0.44	100.83 ^{b} ±	38.67 ^{b} ±	39.79 ^{b} ±	30.47 ^{b} ±
D_{13}	8.77 ^{a} ± 0.47	2.10 ^{a} ± 0.21	19.89 ^{a} ± 0.61	102.87 ^{a} ±	39.43 ^{a} ±	40.57 ^{a} ±	30.67 ^{b} ±

R.B.Cs (×10/mm³): Red Blood Cells (count per cubic millimeter). Haematocrit (%): Hematocrit (percentage of red blood cell volume in blood).
M.C.V (µm³/cell): Mean Corpuscular Volume (average volume of red blood cells).
M.C.H (pg/cell): Mean Corpuscular Hemoglobin (average amount of hemoglobin per red blood cell).
M.C.H.C (%): Mean Corpuscular Hemoglobin Concentration (average concentration of hemoglobin in red blood cells).
WBCs (×10³/mm³): White Blood Cells (count per cubic millimeter).

7. Serum biochemical parameters

Data in Table (10) shows that, the highest levels of Aspartate Aminotransferase (AST) were observed in D_2 (184.2 U/I), while the lowest were in D_{13} (74.7 U/I). Generally, AST levels decrease with increased inclusion of dried gunia grass and additives. Similarly, the highest Alkaline Phosphatase (ALP) levels were recorded in D_2 (92.1 U/I), and the lowest in D_{13} (44 U/I), with ALP also showing a decreasing trend with higher levels of dried gunia grass and additives. For Total Protein (g/dl), the highest values were found in D_{13} (6.43 g/dl) and the lowest in D_2 (2.81 g/dl), indicating an increase in total protein with the inclusion of dried gunia grass and additives. Albumin levels were highest in D_{13} (2.20 g/dl) and lowest in D_2 (1.45 g/dl), showing a similar increasing trend with the inclusion of dried gunia grass and additives. Similarly, Globulin levels were highest in D_{13} (4.23 g/dl) and lowest in D_3 (1.36 g/dl), also increasing with higher levels of dried gunia grass and additives. On the other hand, Cholesterol levels were highest in D_2 (122.8 mg/dl) and lowest in D_{13} (66.52 mg/dl), decreasing with increased inclusion of dried gunia grass and additives. Triglycerides were highest in D_{13} (129.96 mg/dl) and lowest in D_6 (81.87 mg/dl), showing an increase with the inclusion of dried gunia grass and additives. High-Density Lipoprotein Cholesterol (HDL-C) was highest in D_{13} (124.85 mg/dl) and lowest in D_6 (97.22 mg/dl), with HDL-C levels increasing with higher levels of dried gunia grass and additives. In contrast, Low-Density

Lipoprotein Cholesterol (LDL-C) was highest in D_2 (127.92 mg/dl) and lowest in D_{13} (92.1 mg/dl), decreasing with higher levels of dried gunia grass and additives. An increase in enzyme activity associated with hepatic function like alanine transaminase (ALT) and aspartate transaminase (AST) are used as biological marker for indicating liver health status (Li *et al*., 2011; Jahanbin *et al*., 2012). As for enzyme activity (AST and ALP), the decrease in AST and ALP with higher inclusion levels of dried gunia grass and additives suggests a potential improvement in liver function or reduced liver stress. Lower enzyme activities often indicate better health and less liver damage. Wan *et al.*, (2016) found that AST and ALT decreased with increasing the inclusion of macroalgae (*Palmaria palmata*) in Atlantic salmon (*Salmo salar*) diets. Furthermore, AST and ALT reduced significantly ($P \leq 0.05$) in grass carp with addition or increasing Moringa leaf meal in diet from 1% to 5% (Faheem *et al*., 2022). The inclusion of dried plant leaves in the diet of grass carp can influence their protein profile, including total protein, albumin, and globulin levels. Various studies have examined the effects of using plant-based protein sources, like dried leaves or plant extracts, in fish diets. Including dried plant leaves in fish diets not only serves as a cost-effective alternative but also influences the protein metabolism and health of the fish, improving their serum protein levels (Köprücü and Sertel, 2012). The increase in Total Protein, Albumin, and Globulin (Protein Profile) with higher inclusion levels of dried gunia grass and additives suggests enhanced protein synthesis or improved protein utilization. This could be a result of better nutritional quality or digestibility of the diets. As for Inclusion of dried plant leaves in the diet of grass carp has shown effects on their blood lipid profile. For example, studies have explored the impact of plant extracts like the alcoholic extract of lotus leaves on juvenile grass carp, demonstrating significant lipid-lowering effects in the hepatopancreas and muscle. These extracts were found to reduce triglyceride levels and lipid accumulation by modulating the expression of genes involved in lipid metabolism, such as those related to fatty acid uptake, lipid synthesis, and catabolism (Yao *et al*., 2020). Lipid Profile, the decrease in Cholesterol and LDL-C with increasing additives level, combined with an increase in triglycerides and HDL-C, indicates changes in lipid metabolism. Lower cholesterol and LDL-C are generally favorable, while increased HDL-C is associated with improved lipid profiles. The increase in triglycerides might suggest higher fat content or altered fat metabolism with higher inclusion levels of dried gunia grass and additives. In a study where grass carp were fed diets including plant-based proteins using *Mesosphaerum suaveolens* leaf extract, found that changes were observed in their serum biochemistry, including total protein, albumin, and globulin levels. These changes can reflect the fish's nutritional status and overall health (Sattanathan *et al*., 2024). The same study, authors found that it affected several blood parameters, including total triglycerides and cholesterol. Fish fed diets supplemented with the extract showed altered levels of serum components and changes in lipid metabolism markers, which suggests that such plant extracts can influence the overall blood lipid profile of the fish (Sattanathan *et al*., 2024). These findings highlight that dried plant leaves, through various extracts, can modulate the blood lipid profile in grass carp, potentially offering benefits for fish health and aquaculture practices.

Overall, the observed changes in biochemical parameters suggest that the inclusion of dried gunia grass and its additives (ZAD®, Bacti Silage) positively influences the health and metabolic status of grass carp. The diets with higher levels of dried gunia grass and its additives appear to support better protein synthesis, improved liver function, and favorable lipid profiles. As a dietary impact, among the treatments, D_{13} (30% dried gunia grass with ZAD® + Bacti Silage) shows the most favorable results for several parameters, indicating that this combination might provide the best nutritional benefits for grass carp.

Table (10): Serum biochemical parameters

Experimental Treatments	AST (U/I)	ALP (U/I)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL – C (mg/dl)	LDL – C (mg/dl)
D_1	163.73 ^{d} ± 1.86	87.68 ^{b} ± 1.45	3.74 ^{f} ± 0.13	1.82^{de} ± 0.16	1.92 ^{e} ± 0.05	92.69 ^{d} ± 1.64	110.46^{cd} ± 1.76	104.81^{cd} ± 2.05	107.83^{de} ± 2.16
D_2	184.2 ^{a} ± 0.98	92.1 ^{a} ± 1.15	2.81 ^{i} ± 0.12	1.45 ^{g} ± 0.06	1.36 ^{e} ± 0.14	122.8 ^{a} ± 1.33	86.98 ^{h} ± 1.43	90.05^{fg} ± 1.01	127.92 ^{a} ± 1.34
D_3	179.08 ^{b} ± 1.49	90.05^{ab} ± 1.56	2.92^{hi} ± 0.05	1.56^{fg} ± 0.03	1.036 ^{de} ± 0.04	112.57 ^{b} ± 1.95	81.87 ^{i} ± 1.42	86.98 ^{g} ± 1.51	117.68 ^{b} ± 2.04
D_4	173.97 ^{c} ± 1.37	88.01 ^{b} ± 0.87	3.27 ^{g} ± 0.15	1.67^{fg} ± 0.16	1.6 ^{de} ± 0.12	100.29 ^{c} ± 1.61	92.1 ^{g} ± 1.2	102.33 ^{d} ± 1.63	112.57 ^{c} ± 1.77
D_5	133.03 ^{e} ± 1.46	66.52 ^{c} ± 1.15	3.17^{gh} ± 0.05	1.69^{ef} ± 0.03	1.48 ^{de} ± 0.04	112.57 ^{b} ± 1.95	102.33 ^{e} ± 1.77	105.4^{cd} ± 1.83	112.57 ^{c} ± 1.95
D_6	127.92 ^{f} ± 1.34	61.4 ^{d} ± 1.06	3.89 ^{f} ± 0.07	1.77^{ef} ± 0.03	2.12 ^{de} ± 0.04	92.1 ^{d} ± 1.6	107.45 ^{d} ± 1.86	97.22 ^{e} ± 1.68	107.45^{de} ± 1.86
D_7	102.33 ^{h} ± 1.77	59.35 ^{d} ± 1.03	5.32 ^{c} ± 0.09	1.88^{de} ± 0.03	3.44 ^{cd} ± 0.05	88.01 ^{e} ± 1.52	110.52^{cd} ± 1.91	105.4^{cd} ± 1.83	101.31^{fg} ± 1.75
D_8	113.71 ^{g} ± 1.97	65.13 ^{c} ± 1.13	4 ^{f} ± 0.07	1.78^{ef} ± 0.03	2.22 ^{de} ± 0.05	93.04 ^{d} ± 1.61	93.04^{fg} ± 1.61	93.04^{ef} ± 1.61	108.54^{cd} ± 1.88
D_9	109.5 ^{g} ± 1.9	60.38 ^{d} ± 1.05	4.3 ^{e} ± 0.07	1.87^{de} ± 0.03	2.43 ^{bc} ± 0.05	84.94 ^{e} ± 1.47	96.19^{fg} ± 1.67	94.15^{ef} ± 1.63	103.36^{ef} ± 1.79
D_{10}	98.24 ^{h} ± 1.7	51.17 ^{f} ± 0.89	5.97 ^{b} ± 0.1	2.04^{bc} ± 0.04	3.93 ^{ab} ± 0.05	78.8 ^{f} ± 1.36	97.22 ^{f} ± 1.68	90.05^{fg} ± 1.56	100.29^{fg} ± 1.74
D_{11}	92.1 ^{i} ± 1.6	56.28 ^{e} ± 0.97	4.34 ^{e} ± 0.08	1.94^{cd} ± 0.03	2.4 ^{cd} ± 0.05	83.91 ^{e} ± 1.45	114.61 ^{c} ± 1.99	108.47^{bc} ± 1.88	101.31^{fg} ± 1.75
D_{12}	89.03 ^{i} ± 1.54	53.21 ^{f} ± 0.92	5.01 ^{d} ± 0.09	2.09^{ab} ± 0.04	2.92 ^{ab} ± 0.05	73.68 ^{g} ± 1.28	119.73 ^{b} ± 2.07	112.57 ^{b} ± 1.95	97.22 ^{g} ± 1.68
D_{13}	74.7 ^{j} ± 1.29	44 ^{g} ± 0.76	6.43 ^{a} ± 0.11	2.2 ^{a} ± 0.04	4.23 ^{a} ± 0.05	66.52 ^{h} ± 1.15	129.96 ^{a} ± 2.25	124.85 ^{a} ± 2.16	92.1 ^{h} ± 1.6

AST (U/L): Aspartate Aminotransferase (measured in units per liter). ALP (U/L): Alkaline Phosphatase (measured in units per liter).
HDL-C (mg/dl): High-Density Lipoprotein Cholesterol (measured in milligrams per deciliter).
LDL-C (mg/dl): Low-Density Lipoprotein Cholesterol (measured in milligrams per deciliter).

8. Digestive enzymes activities, Liver Enzyme Activity and antioxidant parameters

Table (11) illustrates the effect of replacing yellow corn with different levels of Dried Gunia Grass (DGG), DGG with ZAD®, DGG with Bacti Silage, and their combination on digestive enzymes, liver enzyme activity, and antioxidant parameters in Grass Carp. Alanine Aminotransferase (ALT) activity increased significantly (P≤0.05) with increasing levels of DGG, while decreased in treatments (D_5-D_{13}) when combined with ZAD® or GGBS or both of them .On the other hand, Aspartate Aminotransferase (AST) activity decreased with increasing inclusion levels of DGG, GGZ, GGBS, or combination of the later additives. Malondialdehyde (MDA), a marker of oxidative stress, was significantly (P≤0.05) lower in D_{13} (8.63 h ± 0.58). On the other hand, Glutathione Peroxidase (Gpx) activity increased significantly (P≤0.05) in treatments with combined ZAD® and Bacti Silage (D_{11}-D_{13}), especially in D_{13} (8.47 ± 0.64) .Significant (P≤0.05) improvements in protease, lipase, and amylase activities were observed in D_{13}, with the highest activities recorded (Protease: 10.62 ±0.23, Lipase: 4.77 ±0.15, Amylase: 4.73±0.2). Since the liver plays a central role in metabolism and is sensitive to dietary changes, plasma levels of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) are key indicators of liver health (Yao *et al.*, 2022). Serum ALT and AST activities serve as sensitive markers for hepatocellular damage or inflammation (Chen *et al.* 2004; Faheem *et al.* 2019). In the present study, significant (P≤0.05) increases in AST and ALT were observed in Grass Carp fed with ZAD® and Bacti Silage, possibly due to hepatocytic damage induced by lactic acid and Ruminococcus anaerobic bacteria, leading to enzyme release into the bloodstream.

Additionally, significant (P≤0.05) increases in amylase and protease activities were noted, likely because diets containing enzyme supplements improved body weight gain and feed efficiency compared with the un-supplemented diets, consistent with findings by El-Sanhoury and Ahmed (2017). increasing Alanine Aminotransferase (ALT) activity with DGG suggesting increased liver stress, while decreasing with ZAD® or GGBS or both of them, suggesting reduced liver stress On the other hand, Aspartate Aminotransferase (AST) activity decreasing with DGG, GGZ, GGBS indicating that liver and other tissues improved with the addition of these additives, and consequently showing improved antioxidant defense, enhancing digestion and nutrient utilization especially with the combined additive treatment at the highest replacement level which resulted in a positive impact on fish health and growth.

Table (11): Digestive enzymes activities, Liver Enzyme Activity and antioxidant parameters

Experimental Treatments	ALT (U/mg)	AST (U/mg)	MDA (nmol/ml)	Gpx (nmol min-1 mg -1)	Protease (U mg−1)	Lipase (U mg−1)	Amylase (U mg−1)
D_1	50.73 ^{c} ± 1.55	90.57 ^{e} ± 1.09	10.33 ^{c} ± 0.44	5.07^{hi} ± 0.29	7.5 ^{h} ± 0.29	2.6 ^{i} ± 0.29	2.03 ^{j} ± 0.14
D_2	55.5 ^{b} ± 1.82	94.53 ^{a} ± 1.18	10.5 ^{c} ± 0.4	4.7 ^{i} ± 0.21	7.27 ^{h} ± 0.15	2.37 ^{j} ± 0.29	1.77 ^{k} ± 0.15
D_3	60.4 ^{a} ± 1.16	93.7 ^{b} ± 1.27	10.63 ^{c} ± 0.49	4.13 ^{j} ± 0.23	6.77 ^{i} ± 0.15	2.08 ^{k} ± 0.22	1.5 ^{l} ± 0.17
D_4	62.9 ^{a} ± 1.53	92.67 ^{c} ± 1.2	11.5 ^{a} ± 0.58	3.77 ^{j} ± 0.15	6.13 ^{j} ± 0.19	1.87 ^{l} ± 0.23	1.33 ^{m} ± 0.2
D_5	48.07^{cd} ± 1.36	91.67 ^{d} ± 1.2	11.03 ^{b} ± 0.26	5.57^{gh} ± 0.26	8 ^{g} ± 0.26	2.93 ^{h} ± 0.3	2.43 ^{i} ± 0.23
D_6	47.87^{cd} ± 1.45	90.5 ^{e} ± 1.15	11.57 ^{a} ± 0.23	5.97^{fg} ± 0.32	8.4 ^{f} ± 0.29	3.1 ^{h} ± 0.26	2.67 ^{h} ± 0.2
D_7	46.74^{de} ± 1.05	89.6 ^{f} ± 1.35	9.93 ^{d} ± 0.5	6.3^{ef} ± 0.32	8.6 ^{f} ± 0.23	3.4 ^{g} ± 0.21	2.95 ^{g} ± 0.22
D_8	45.83^{de} ± 1.45	89.03 ^{g} ± 1.11	9.7^{de} ± 0.44	6.7^{de} ± 0.32	8.97 ^{e} ± 0.26	3.62 ^{f} ± 0.2	3.22 ^{f} ± 0.19
D_9	42.31^{ef} ± 0.27	87.67 ^{h} ± 1.13	9.57 ^{e} ± 0.43	7.13^{cd} ± 0.33	9.3 ^{d} ± 0.26	3.87 ^{e} ± 0.17	3.4 ^{e} ± 0.16
D_{10}	42^{ef} ± 1.76	86.70 ^{i} ± 1.1	9.42^{ef} ± 0.43	7.17^{cd} ± 0.6	9.68 ^{c} ± 0.29	4.07 ^{d} ± 0.15	3.73 ^{d} ± 0.22
D_{11}	41 ^{f} ± 1.93	85.6 ^{j} ± 1.23	9.17^{fg} ± 0.47	7.67^{bc} ± 0.6	10 ^{b} ± 0.29	4.27 ^{c} ± 0.15	4.03 ^{c} ± 0.17
D_{12}	40.23 ^{f} ± 1.66	84.87 ^{k} ± 1.21	8.92^{gh} ± 0.48	8.1^{ab} ± 0.64	10.28 ^{b} ± 0.29	4.53 ^{b} ± 0.15	4.33 ^{b} ± 0.19
D_{13}	39.5 ^{f} ± 1.29	82.67 ^{l} ± 1.28	8.63 ^{h} ± 0.58	8.47 ^{a} ± 0.64	10.62 ^{a} ± 0.23	4.77 ^{a} ± 0.15	4.73 ^{a} ± 0.2

ALT (U/mg): Alanine Aminotransferase (measured in units per milligram). AST (U/mg): Aspartate Aminotransferase (measured in units per milligram).
MDA (nmol/ml): Malondialdehyde (measured in nanomoles per milliliter).
Gpx (nmol min^{-1} mg^{-1}): Glutathione Peroxidase (measured in nanomoles of substrate converted/ mg of protein).

CONCLUSION

The findings of this study suggest that for achieving sustainable and profitable aquaculture practices, incorporating feed additives such as ZAD® and Bacti Silage with alternative plant ingredients like Gunia grass is advantageous. This combination improves the nutritional quality of the feed, which in turn enhances fish growth performance and boosts economic returns. Overall, the data highlight the critical need to optimize feed formulations, focusing not only on reducing ingredient costs but also on maximizing the

biological efficiency and economic benefits in aquaculture systems.

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