Original Research Article

Effect of biodigester effluent on carbohydrate metabolizing enzymes and oxidative stress markers in the african catfish (*Clarias gariepinus*)

Abstract

Background: Although the use of biodigester effluent as fish feed have been previously reported, however, its effect on the metabolic activities of fish, which might inform its safety and administration guideline in fish farming is poorly understood.

Aim: The aim of the present study was to investigate the effects of pig dung biodigester effluent (PDBE) on key metabolic enzymes and antioxidant markers in *Clarias gariepinus*.

Methodology: Fifteen catfish (120±10 g) were purchased and grouped into three. Group I (control): exposed to freshwater; group two: exposed to raw PDBE; and group three: exposed to 1:10 dilution of PDBE. After exposure, serum lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) activities and total protein concentration were measured using commercially available kits while serum reduced glutathione concentration and glutathione peroxidase activity were measured using standard methods.

Results: The TP (g/dl) for control, group II and III were 6.00±0.12; 20.0±0.10 and 6.86±0.02 respectively, while their LDH activities (IU/L/min) were 115.00±1.57; 422.00±5.64 and 105.00±5.18 respectively. The activities of G-6-PDH (U/L/min) in control, group II and III were 8.00±0.40; 32.00±0.73; 7.00±0.40 respectively, while their glutathione concentrations (µg/ml) were 6.5±0.01; 0.5±0.01 and 9.83±0.12 respectively. The activities of GPx (µg/ml/min) in control, group II and III were 15±0.25; 64±1.99 and 10±1.33.

Conclusion: Raw PDBE caused a significant alteration in the metabolic parameters of catfish however these alterations were absent in a 1:10 dilution of the PDBE. This study's novel inclusion of both raw and diluted PDB effluent exposures offers valuable guidelines for effluent usage in aquaculture.

Keywords: Effluent, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, glutathione peroxidase, reduced glutathione

1 Introduction

Clarias gariepinus, also known as African sharp tooth catfish is a species of the air breathing catfish which grows very fast and multiply quickly [1]. It is indigenous to the inland freshwaters of Africa that is why it is called the African catfish [2]. Nigeria is the world's largest commercial producer of the African catfish. Given the continuous rise in the demand for catfish, catfish farming is currently a very lucrative business in Nigeria [3, 4]. Catfish production occurs in several communities in Bayelsa State [4]. Bayelsa is a State in Nigeria, which lies between River State and Delta State [5] and is part of the largest wetland in Africa, the Niger Delta [6]. Catfish ponds and sales are very common in Bayelsa State [4, 7]. Aside from *Clarias gariepinus*, other species of catfish found in Nigeria are; *Heterobranchus bidorsalis*, a hybrid of *Clarias gariepinus and Heterobranchus bidorsalis* known as *Heteroclarias, and Clarias nigro-digitatus* [8].

The high cost of fish feed is a major constraint to catfish production [4, 7]. A previous study found that fish feed accounted for 77.4% of the total cost of catfish production [9]. The conventional pelleted floating feeds for catfish consist of oil seed meal such as groundnut cake, Soybean cake, soybean flour [8], cotton seed meal and canola meal [10]. Sometimes

unconventional animal by-products that meet the nutrient requirements of catfish are also used as feed for catfish [10].

Active research on fish feed formulations with high quality and low cost is on-going [11, 12]. A previous research showed that fish residing close to wastewater effluent discharges tend to have more weight than those of control [13].

Biodigester effluent is a type of wastewater that is released from a biodigester during anaerobic digestion of biodegradable wastes [14]. A previous study showed that bio-digester effluents contained a good amounts of nitrogen, potassium, phosphates, calcium, magnesium and sodium but with *Escherichia. coli* presence which was removed upon treatment [15]. This suggests that wastewater effluent contains food and nutrients which are needed for fish growth [13]. Other previous studies have reported the use of biodigester effluent as a rich source of nutrient for plant growth [16, 17] and as fertilizer for fish growth [18, 19]. Although studies have showed that biodigester effluent can be used as fish feed, however, evaluation of its effect on key pollution biomarkers in catfish is limited in literature. Concerns have been raised over the safety of biodigester effluent as fish feed [20, 21]. Concern has also been raised over environmental pollution during its application [19].

Previous studies have not examined the metabolic impact of PDBE on *Clarias gariepinus*. Therefore, in order to determine the safety protocol and guideline underlying the use of biodigester effluent in catfish farming, the present study investigated the effect of pig dung biodigester effluent (PDBE) on the activities of lactate dehydrogenase, glucose-6-phosphate dehydrogenase, glutathione peroxidase, reduced glutathione and total protein concentration in African Catfish (*Clarias Gariepinus*).

2. Methodology

2.1 Study location

This research was conducted in the University of Africa Toru – Orua, Sagbama Local Government Area, Bayelsa State, Nigeria. The State is situated on the core of Niger Delta, Nigeria [22] on longitude 6.06990° E and latitude 4.77190° N [23]. Toru-Orua is a swampy environment that is well watered by the freshwater from Forcados River, surrounding creeks and lakes that flows across it [24].

2.2 Study design

Fifteen catfish (120 ± 10 g) were purchased from one of the commercial catfish traders in Toru – Orua community that rear and sell catfish as their major occupation [25]. They were acclimatized to laboratory conditions for four hours in freshwater from Toru – Orua River [26]. Effluent from pig dung biodigester was obtained from the Biogas Production and Research Centre in the University of Africa Toru – Orua, Bayelsa State. The catfish were grouped into three. Group I (control water): exposed to 20 litres of freshwater from Toru – Orua River; group II exposed to 20 litres of raw effluent from pig dung biodigester; group III: exposed to diluted effluent from pig dung biodigester. Freshwater was used for dilution. International guidelines for the use of fishes in research was followed for the care and handling of the experimental catfish [27]. Freshwater was used as control because freshwater is the natural habitat of catfish [2] while 1:10 dilution was used as previously described [20].

2.3 Acute toxicity test

The short-term adverse effects of exposing catfish to two different concentrations of pig dung effluent was evaluated for a period of 24 hours [28, 13]. At the end of the experiment, blood

sample was collected via heart puncture from the fish. The universal ethical recommendations for taking blood from catfish was followed [29]. Blood was drawn into plain sample bottles for serum preparation [30].

2.4 Determination of total protein (TP) concentration

This was carried out using the manufacturer`s protocol for Randox total protein kit based on the Biuret method, with bovine serum albumin as the protein standard [31].

Principle: Cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a blue coloured complex, which exhibits maximum absorbance between wavelengths of 530-570 nm [31].

Procedure: Following the Randox kit protocol, 1 ml of reagent R1 [containing Sodium hydroxide (100 mmol/l), sodium-potassium tartrate (16 mmol/l), potassium iodide (15 mmol/l) and copper II sulphate (6 mmol/l)] was added `to 0.02 ml of serum sample. The mixture was incubated at 25^{o}C and the absorbance was measured against reagent blank at a wavelength of 546 nm. The total protein concentration was expressed as mg/dl.

2.5. Estimation of serum lactate dehydrogenase activity

Lactate dehydrogenase (LDH) activity was carried out following the manufacturer's protocol for Randox LDH Kit using UV method [32].

Principle: $\text{Pyruvate} + \text{NADH} + H^{+} \xrightarrow{\text{LDH}} \text{L–Lactate} + NAD^{+}$

The activity of the LDH that leaked from tissues into the blood during tissue damage was measured in the present study. The reaction principle is based on the catalytic activity of serum LDH which leads to the reversible reduction of pyruvate to L-lactate mediated by the hydrogen donor, NADH. The decrease in NADH, was harnessed as a basis for the measurement of LDH

activity. NADH in solution produces a significant absorbance peak at 340 nm, while NAD+ has virtually no absorbance at this wavelength. This difference was the basis by which LDH activity was monitored. For the calculation of LDH activity, the rate of absorbance change per minute at 340 nm due to the reduction of NADH was multiplied by 9683 (a factor given in the manufacturer`s protocol for Randox LDH Kit) and the calculated activity was expressed as IU/L/min [32].

Protocol: Following the Randox kit protocol, 0.02 ml of serum sample was pipetted into a test tube followed by 1 ml of Randox reagent. The Randox reagent contained phosphate buffer (50 mmol/l, pH 7.5), pyruvate (substrate 0.6 mmol/l) and NADH (0.18 mmol/l). The serum sample and Randox reagent were thoroughly mixed together and absorbance was read at 340 nm at 30 sec, 1, 2 and 3 min intervals. The activity of LDH was calculated by multiplying the change in absorbance per min (ΔA 340 nm/min) with 9683 (a factor given in the manufacturer`s protocol for Randox LDH Kit). The calculated activity was expressed as IU/L/min.

2.6 Estimation of Glucose-6-Phosphate Dehydrogenase (G-6-PDH) Activity

This was carried out following the manufacturer's protocol for Randox G-6-PDH Kit based on UV method [33, 34].

Principle: The activity **of** G-6-PDH in the serum was determined by the measurement of the absorbance change at 340 nm due to the reduction of $NADP^+$**.** This enzymatic dehydrogenase reaction takes advantage of the ability of the reduced form (NADPH), to absorb light at a wavelength of 340 nm while the oxidized form ($NADP^+$) do not [33].

$$\text{Glucose-6-Phosphate} + NADP^+ \xrightarrow{\text{G-6-PDH}} \text{Gluconate-6-Phosphate} + NADPH + H^+$$

Protocol: Following the manufacturer`s protocol for Randox kit**,** 1 ml of reagent R1 containing triethanolamine buffer (31.7 mmol/l, pH 7.6) and EDTA (3.2mmol/l) was pipetted into a test tube, followed by 0.03 ml of reagent R2 containing NADP (0.34 mmol/l) reconstituted with 2 ml of redistilled water and 0.015 ml of serum sample. The mixture was mixed thoroughly and incubated for 5 min at 37^0C. Reagent 3 containing glucose-6-phosphate dehydrogenase substrate (0.34 mmol/l) was reconstituted with 2 ml of redistilled water and was added to the mixture. The whole content of mixture was mixed thoroughly and the absorbance was taken at 0 sec 1, 2 and 3 minutes.

Activity of G-6-PDH **(**U/L/min) = 33650 (factor) x change in absorbance at 340 nm/min.

2.7 Determination of Reduced Glutathione (GSH) Concentration

The method originally described by Ellman [35] was used in estimating the level of reduced glutathione (GSH).

Principle: The reduced form of glutathione comprises in most instances the bulk of cellular non-protein sulfhydryl groups. The principle of reaction is therefore based upon the oxidation of glutathione by the sulfhydryl reagent 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) resulting in the formation of a relatively stable yellow chromogenic derivative called 5'-thio-2-nitrobenzoic acid (TNB), measured at a wavelength of 412 nm. The sulfhydryl reagent is the Ellman`s reagent.

Procedure: Exactly 0.2 ml of serum was added to 1.8 ml of distilled water and 3 ml of protein precipitating solution (2 ml of 5% TCA). The mixture was mixed together and allowed to stand for approximately 5 min, after which it was centrifuged and filtered. Exactly 1ml of the supernatant was added to 3.0 ml of 0.2 M phosphate buffer (pH 8.0) and 0.5 ml of Ellman`s reagent (19.8 mg of 5', 5'-dithiobis-2-nitrobenzoic acid, DTNB in 100 ml of 0.1% sodium

nitrate) was added and the absorbance was read at 412 nm against a reagent blank. Reduced GSH concentration is proportional to the absorbance of the final mixture at 412 nm wavelength. GSH concentration was extrapolated from the standard curve for reduced GSH [35].

2.8 Evaluation of glutathione peroxidase activity

Glutathione peroxidase activity was determined according to the method of Rotruck *et al.*, [36].

Principle: Glutathione peroxidase catalyzes the oxidation of hydrogen peroxidase (H_2O_2) using reduced glutathione. The reduced glutathione is oxidized in the process. Glutathione peroxidase activity is arrested by TCA (Trichloroacetic acid) and the remaining glutathione in the reaction mixture is determined by the method originally described by Ellman [35]. In this protocol, thiols present in the remaining glutathione in the reaction mixture interact with 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to form a highly yellow coloured anion with maximum peak at 412nm. Glutathione peroxidase activity is proportional to the amount of glutathione that was consumed in the reaction.

Equation of reaction

Glutathione peroxidase

Reaction: $2\ GSH + H_2O_2 \longrightarrow GSSG + 2\ H_2O$

Abbreviations used: GSH = Reduced glutathione; GSSG = oxidized glutathione

Procedure: The reaction mixture contained 0.2 ml of 0.4 M Tris-HCl buffer, pH 7.0, 9.1 ml of 10 mM sodium azide, 0.2 ml of serum, 0.2 ml of 4 mM glutathione and 0.1 ml of 0.2 mM hydrogen peroxide. The reaction mixture was incubated at 37°C for 10 min and was arrested by the addition 0.4 ml 0f 10% TCA, followed by centrifugation at 3000 rpm for 5 min. The supernatant was assayed for glutathione content by using Ellman`s reagent. Briefly, to 1ml of the

supernatant was added 0.5 ml of Ellmans reagent (19.8 mg of 5', 5'-dithiobis-2-nitrobenzoic acid, DTNB in 100 ml of 0.1% sodium nitrate) and 3.0 ml of 0.2 M phosphate buffer (pH 8.0) and the absorbance was read at 412 nm against a reagent blank. Glutathione peroxidase activity was obtained by plotting a GSH standard curve and the concentration of the remaining GSH in the reaction mixture was obtained from the standard calibration curve by extrapolation from the curve.

Calculation: GSH consumed = initial GSH concentration – remaining GSH in the mixture.

2.9 Statistical analysis

IBM SPSS software version 24 was used for data analysis. One-way ANOVA followed by Duncan multiple range test was used to test for significant difference between groups and results were presented as mean ± standard error of mean of five replicates. *P*-value was set at 0.05 [37, 38].

3.0 Results

3.1 Effect of PDB effluent on serum total protein concentration in African catfish (*Clarias gariepinus*)

The serum total protein concentration of African catfish (*Clarias gariepinus*) exposed to PDB effluent is presented in Figure 1. Compared with control, a significant ($P < 0.05$) increase in total protein concentration was found in the serum of group II catfish exposed to raw PDB effluent (control: 6.00 ± 0.12 g/dl versus group II: 20.0 ± 0.10 g/dl), while no significant difference was found in group III fish (Figure 1) compared with control (control: 6.00 ± 0.12 g/dl versus group III: 6.86 ± 0.02 g/dl).

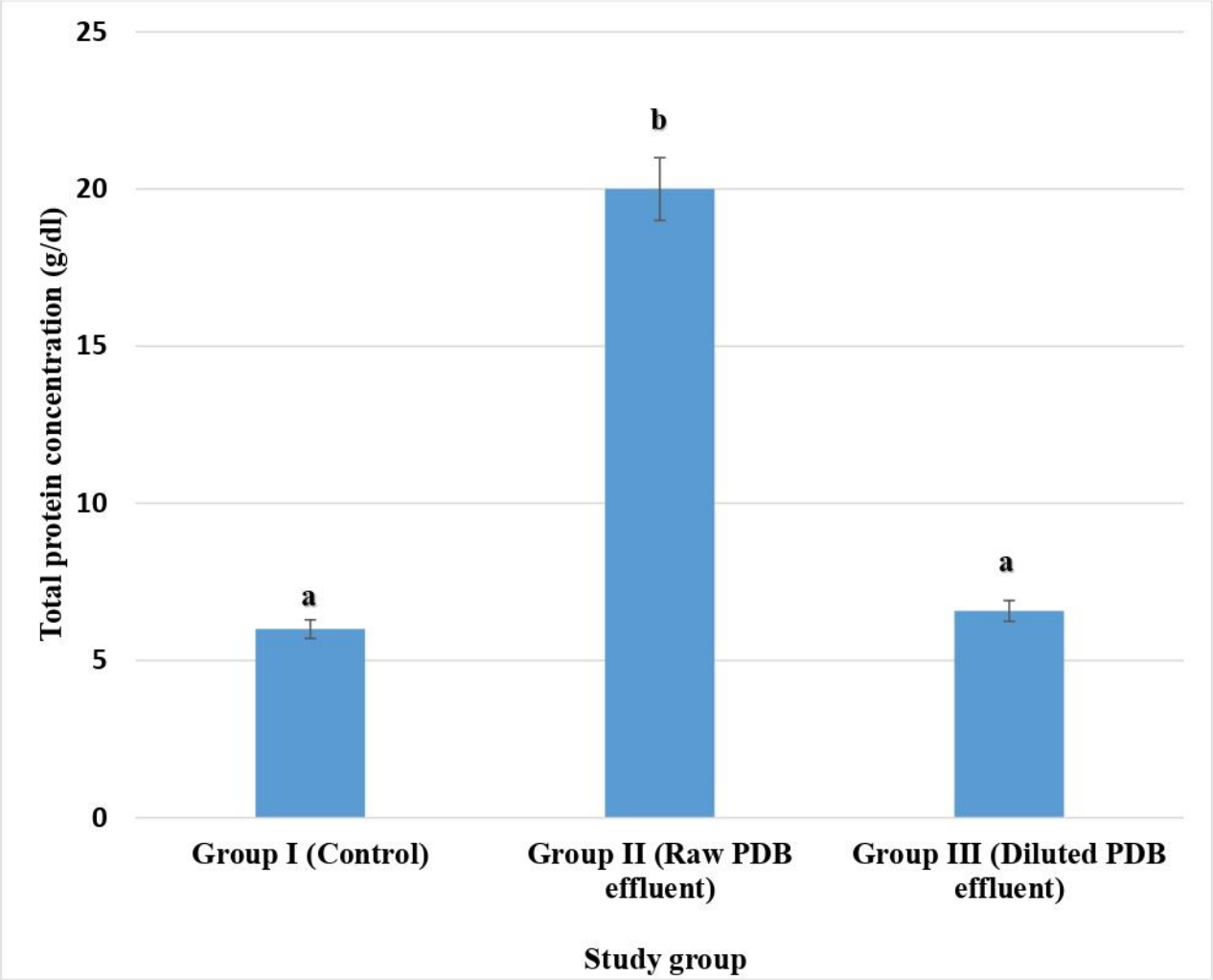

Figure 1. Concentration of serum total protein in the serum of African catfish (*Clarias gariepinus*) exposed to PDB effluent.

Result presented as mean ± standard error of mean (SEM); bars with different superscripts are significantly different ($p < 0.05$) while bars with the same superscript are not significantly different. PDB: pig dung biodigester. Group I fish (control): exposed to freshwater. Group II: exposed to raw PDB effluent and group III: exposed to diluted PDB effluent (1:10 dilution).

3.2 Effect of pig dung biodigester effluent on the activity of serum lactate dehydrogenase in African catfish (*Clarias gariepinus*)

Compared with control, a significant ($p < 0.001$) increase in the activity of LDH was found in group II (Figure 2) catfish exposed to raw PDB effluent (control: 115.00 ± 1.57 IU/L/min versus group II: 422.00 ± 5.64 IU/L/min). On the contrary, no significant difference in the activity of LDH was found in group III catfish (105.00 ± 5.18 IU/L/min) compared with control.

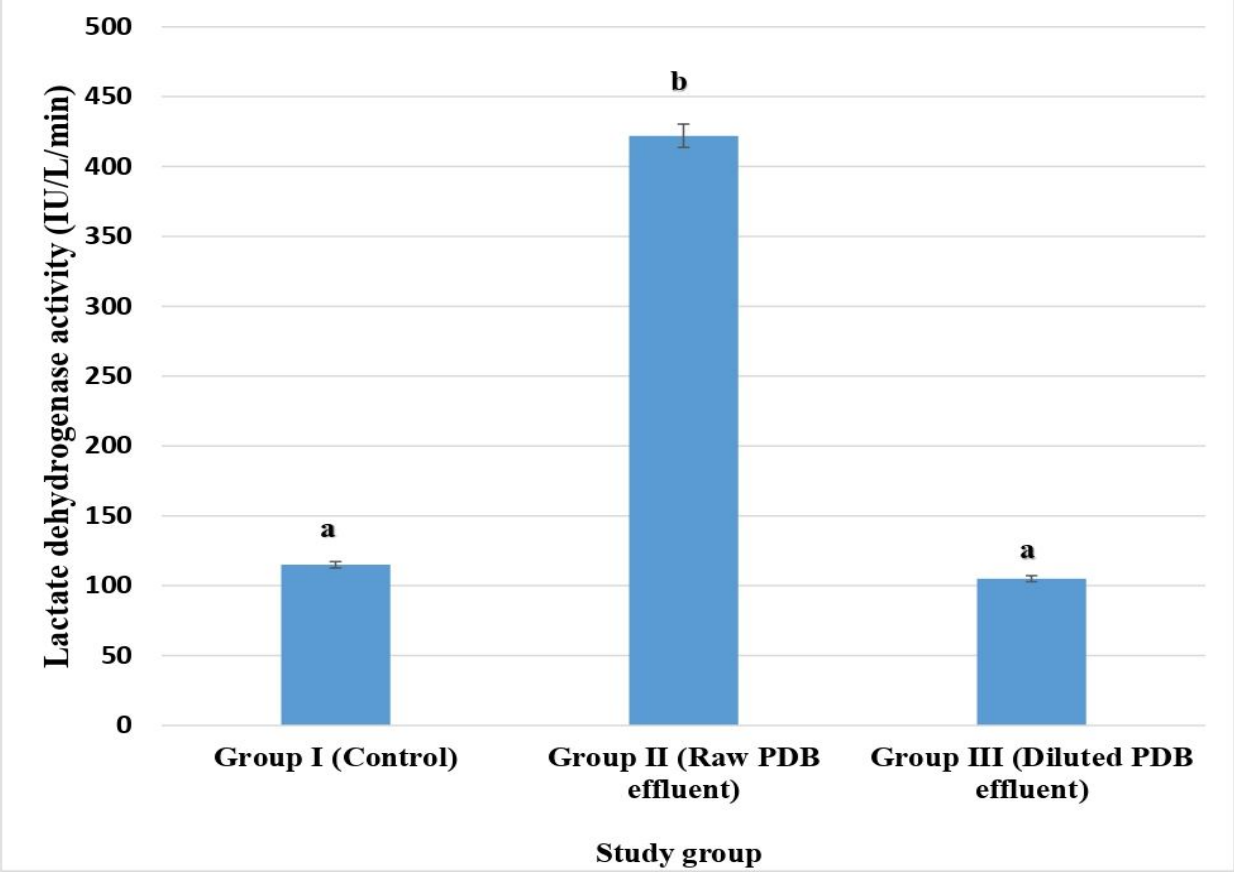

Figure 2 Effect of PDB effluent on serum lactate dehydrogenase (LDH) activity in African catfish (*Clarias gariepinus*).

Result presented as mean ± standard error of mean (SEM). Bars with different superscripts are significantly different $p < 0.05$) while bars with the same superscript are not significantly

different. PDB: pig dung biodigester. Group I (control): exposed to freshwater. Group II: exposed to raw PDB effluent and group III: exposed to diluted PDB effluent (1:10 dilution).

3.3 Effect of pig dung biodigester effluent on the activity of serum glucose-6-phosphate dehydrogenase in African catfish (*Clarias gariepinus*)

Results for the effect of PDB effluent on the enzyme activity of glucose-6-phosphate dehydrogenase (G-6-PDH) in the serum of catfish (*Clarias gariepinus*) is presented in Figure 3. As shown in Figure 3, compared with control a significant ($p < 0.001$) increase in the activity of G-6-PDH was found in the serum of group II (control: 8.00 ± 0.40 U/L/min versus group II: 32.00 ± 0.73 U/L/min; $P < 0.001$), while the activity of G-6-PDH in group III catfish exposed to diluted PDB effluent was found to be similar to that of control with no significant difference (control: 8.00 ± 0.40 U/L/min versus group III: 7.00 ± 0.40 U/L/min; $p > 0.05$).

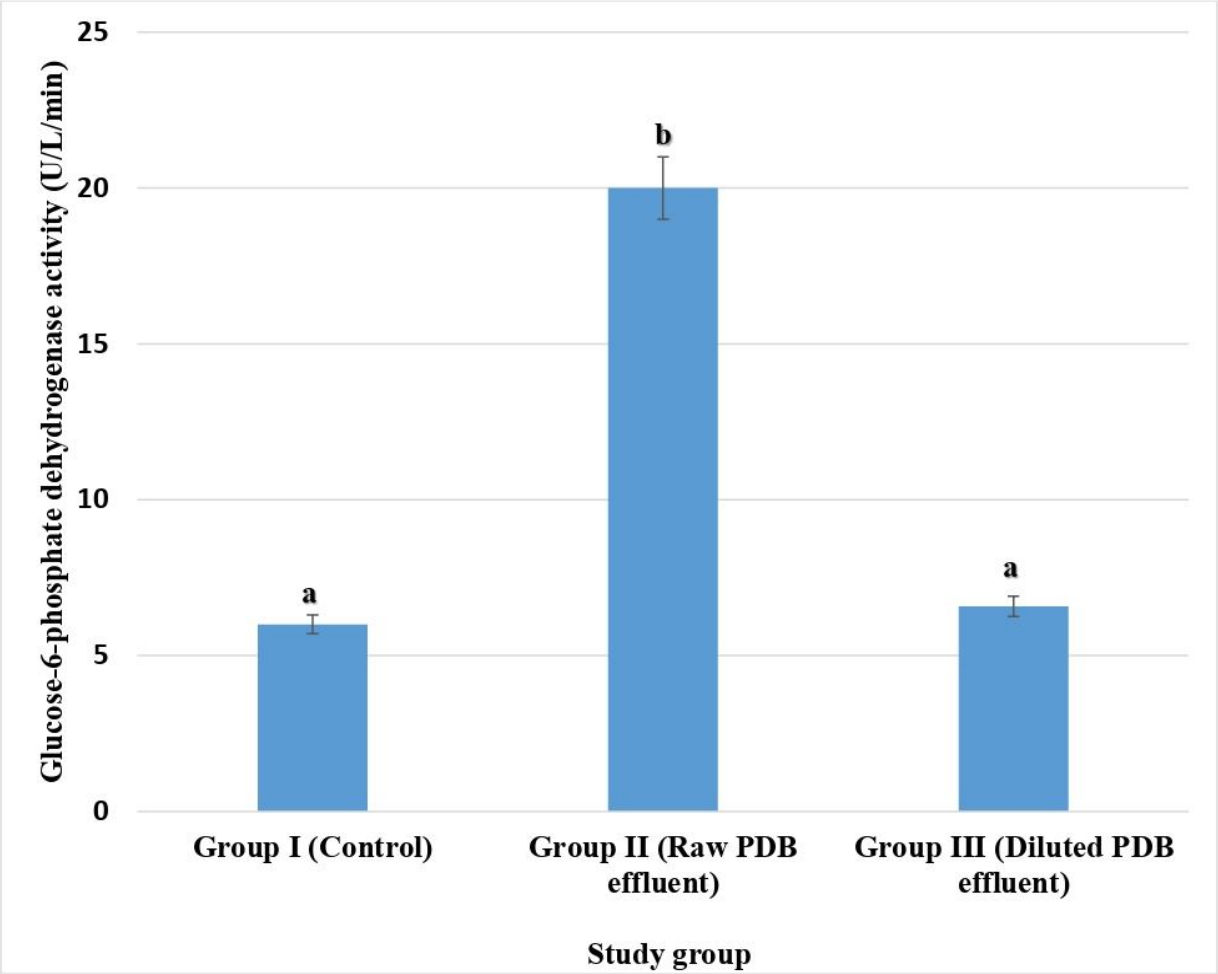

Figure 3 Effect of PDB effluent on serum glucose-6-phosphate dehydrogenase (G-6-PDH) activity in African catfish (*Clarias gariepinus*).

Result presented as mean ± standard error of mean (SEM). Bars with different superscripts are significantly different ($p < 0.05$) while bars with the same superscript are not significantly different. PDB: pig dung biodigester. Group 1 (control) exposed to freshwater. Group II: exposed to raw PDB effluent and group III: exposed to diluted PDB effluent (1:10 dilution).

3.4 Effect of pig dung biodigester effluent on reduced glutathione concentration in the serum of African catfish (*Clarias gariepinus*)

Results for the effect of PDB effluent on the concentration of reduced glutathione in the serum of catfish (*Clarias gariepinus*) is presented in Figure 4. As shown in Figure 4, compared with control a significant $p < 0.001$) decrease in the concentration of reduced glutathione was found in group II. However, significant ($p < 0.001$) elevation in the concentration of reduced glutathione was found in group III compared with control.

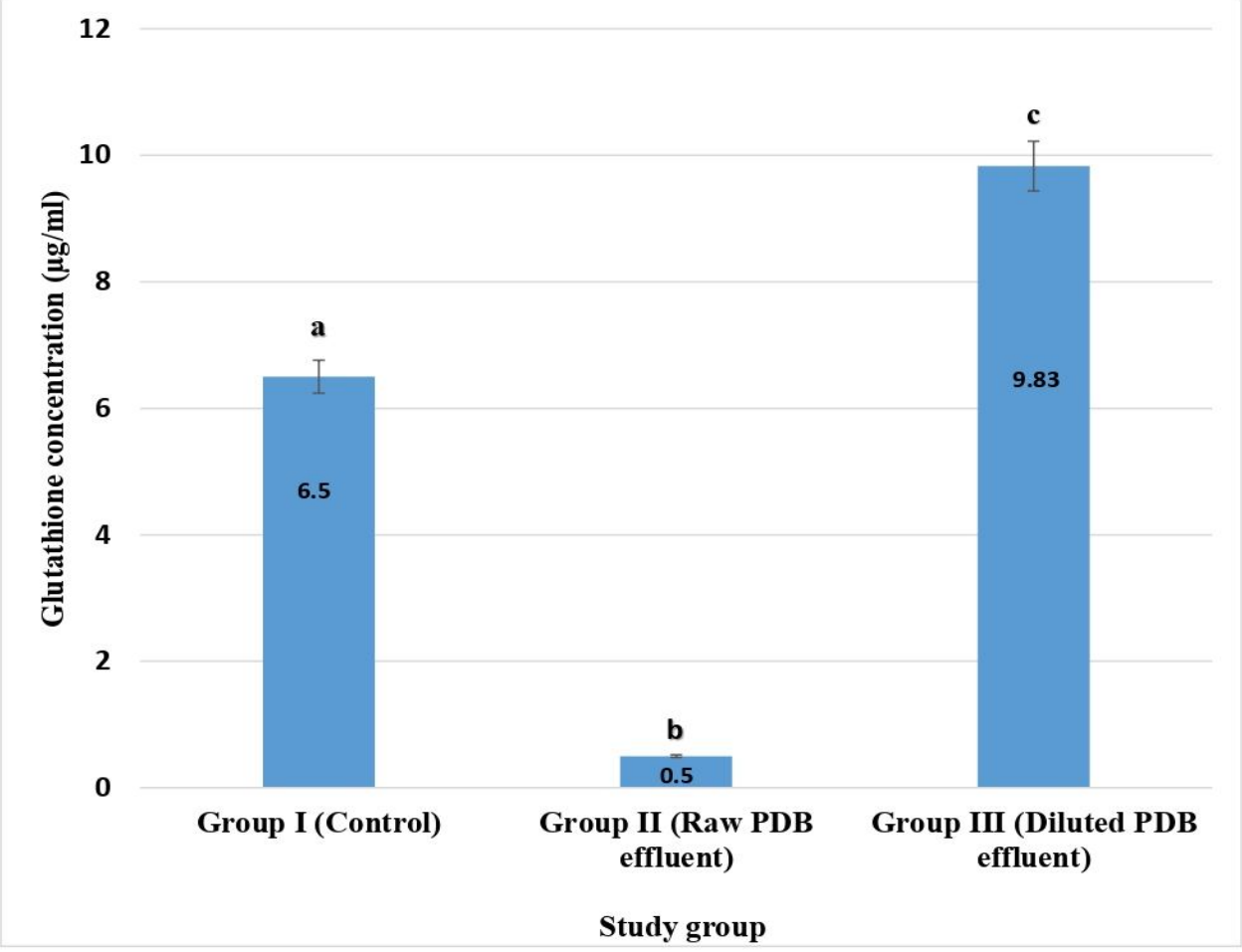

Figure 4 Effect of PDB effluent on reduced glutathione in the serum of African catfish (*Clarias gariepinus*).

Result presented as mean ± standard error of mean (SEM). Bars with different superscripts are significantly different ($p < 0.001$) PDB: pig dung biodigester. Group 1 (control): catfish exposed to freshwater. Group II: catfish exposed to raw PDB effluent, and group III: catfish exposed to diluted PDB effluent (1:10 dilution).

3.5 Effect of pig dung biodigester effluent on serum glutathione peroxidase activity in African catfish (*Clarias gariepinus*)

As shown in Figure 5, compared with control, the activity of serum glutathione peroxidase (GPx) was found to be significantly higher in group II catfish exposed to raw PDB effluent than in control. However, the enzyme activity of GPx decreased significantly ($p < 0.001$) lower than that of control following a 1:10 dilution of PDB effluent.

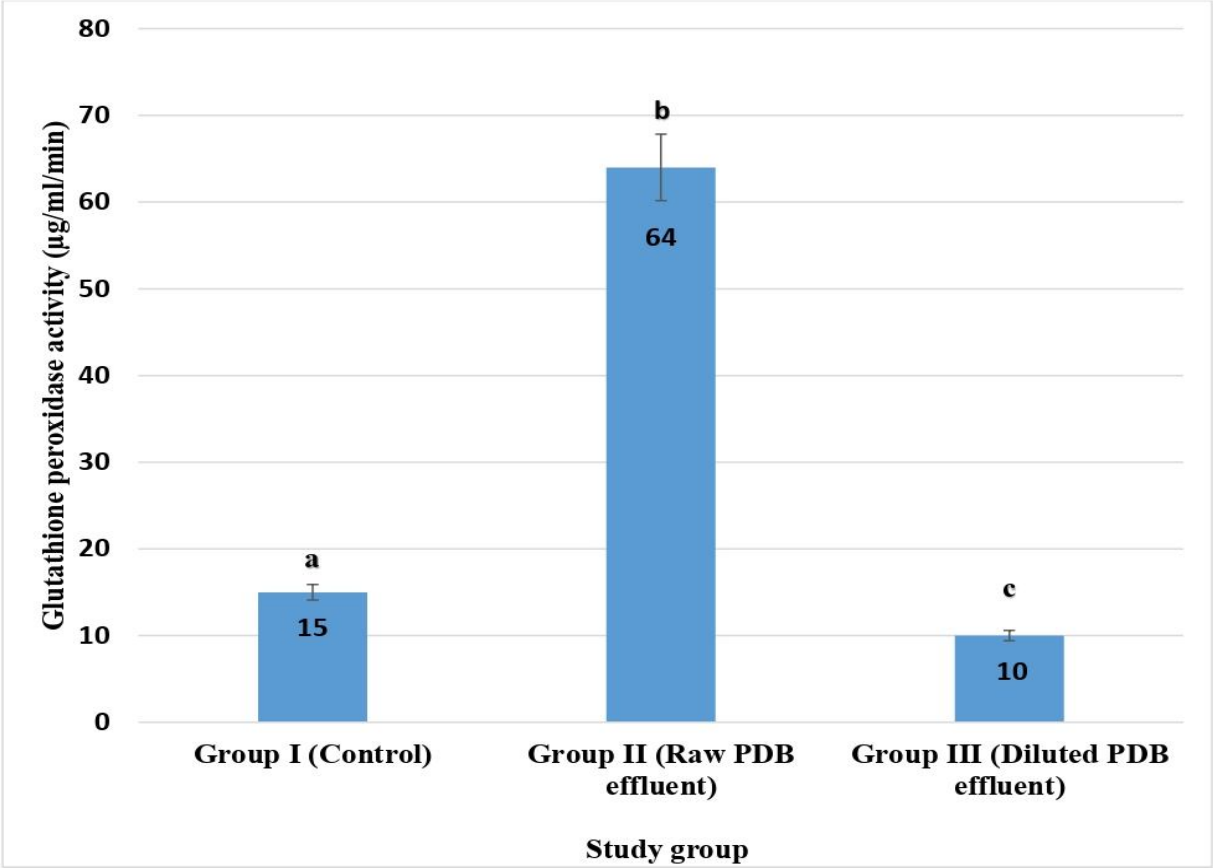

Figure 5 Effect of PDB effluent on the activity of glutathione peroxidase in the serum of African catfish (*Clarias gariepinus*).

Results presented as mean ± standard error of mean (SEM). Bars with different superscripts are significantly different ($p < 0.001$). PDB: pig dung biodigester. Group 1 (control): catfish exposed to freshwater sample. Group II: catfish exposed to raw PDB effluent, and group III: catfish exposed to diluted PDB effluent (1:10 dilution).

Discussion

Comparative evaluation of the biomarkers of pollution in catfish exposed to raw PDB effluent and catfish exposed to diluted PDB effluent was carried out in the present study. Previous studies

have not examined the metabolic impact of PDBE on *Clarias gariepinus*. This makes the present study unique. Given that the liver is one of the richest sources of LDH [32], the increase in serum LDH activity beyond normal serum level which was found in the present study in group two after exposure to pig dung biodigester effluent suggests that raw PDBE induced excessive damage and destruction to the liver cells of catfish. This may have led to increased permeability of liver cells and leakage of LDH as well as other parameters into the blood. However this was not found in group III catfish which were placed in diluted PDBE

Findings of the present study corroborates findings of a previous study where an increase in lactate dehydrogenase was found in the freshwater fish called *Channa punctatus* following exposure to increasing concentration of tannery waste water [39]. Increase in the activity of LDH was also found in the liver and muscle cells of tilapia fish (*Oreochromis mossambicus*) following exposure to fumaronitrile, a persistent organic pollutant of industrial waste water [40]. Significant changes in the activity of LDH was also previously found in the armored catfish, *Rhinelepis strigosain (Siluriformes, Loricariidae)* after exposure to hypoxia and temperature variation compared with the unexposed group [41].

A previous study found that exposure of fish to cadmium and lead also resulted in a significant decrease in LDH and G-6-PDH activities [42]. Furthermore, another previous study also showed a reduction in the activity of glucose-6-phosphate dehydrogenase in the erythrocytes of dogfish and 5 other fish species from Black Sea after exposure to stress [43]. These findings are consistent with the findings of the present for group 2. However the reverse was found for group III catfish placed in diluted PDBE in the present study.

Enzymatic and non-enzymatic antioxidants serve as important biological defense against environmental oxidative stress and have been reportedly used as biomarkers of pollution in

several animal studies [44]. Comparative evaluation of reduced glutathione levels between catfish exposed to raw PDB effluent and catfish exposed to diluted PDB effluent was also carried out in the present study. The present study found a decrease in glutathione concentration and an increase in the antioxidant enzyme activity of catfish exposed to raw PDB effluent. This is in agreement with the findings of previous studies where a decrease in glutathione level was found in the freshwater snail called *Bellamya purificata* after exposure to landfill leachate effluent and bisphenol A [45]. The same was observed in the fish, *Channa punctatus* after exposure to a thermal power plant effluent in a previous study [46]. Elevation of glutathione peroxidase activity has also been reported in a previous study where the effect of waste water on fish was evaluated [47]. The present study suggests that the induction of glutathione peroxidase activity was caused by pollutants present in PDBE. GSH and GPx may be considered as potential specific biomarkers for PDBE pollution.

The present study found that the administration of 1:10 dilution of PDB effluent to group III catfish restored the altered metabolic parameters in group II to that of control and better than that of control for GSH and GPx. This suggests that the concentration of PDBE should be taken into consideration when using it as feed source or supplement in fish farming. The present study is quite novel because while previous studies mainly focused on only the effect of raw wastewater on fish, the present study focused on the effect of both the raw waste water and the diluted wastewater in order to determine the proper guideline for the application of PDBE for fish farming.

Aside from providing a guideline for PDBE administration in fish farming, the present study has also showed that when the levels of pollutants exceed safe limits, a reduction in the overall health of fish can occur. These impacts can also adversely affect human health [48].

The comparative evaluation of LDH and G-6-PDH activities in *Clarias gariepinus* exposed to raw and diluted PDB effluent in this study demonstrates the enzymes' utility as biomarkers of pollutant-induced stress. The marked increase in LDH activity, especially in fish exposed to raw PDB effluent, likely reflects hepatic cellular damage due to pollutant stress, causing enzyme leakage into the serum [49]. These findings align with similar studies showing LDH elevation in freshwater species exposed to wastewater pollutants, highlighting potential hepatotoxic effects [39 -43]. The restoration of these biomarkers to near-control levels in fish exposed to diluted effluent underscores the protective potential of effluent dilution, suggesting practical applications for safe effluent integration into aquaculture.

This study's novel inclusion of both raw and diluted PDB effluent exposures offers valuable guidelines for effluent usage in aquaculture, emphasizing the importance of effluent concentration management. Given the potential for toxic bioaccumulation, it is crucial to control pollutant levels to safeguard fish health and, by extension, human health upon consumption. Therefore, regulating PDB effluent levels for use in aquaculture could be a viable strategy for balancing nutrient supply with environmental and food safety. I can acknowledge that while the findings are indicative, larger samples might offer more generalizable insights instead of the small sample size of fifteen catfish used in this present study.

Conclusion

It can be concluded from the present study that although fishes had reduced metabolic activity after been exposed to a high concentration of raw pig dung biodigester effluent, however, their metabolic activities were restored to normal when the effluent was diluted in a 1:10 dilution. Thus, the present results suggest that a 1:10 dilution of pig dung biodigester effluent might be a very effective practice for the detoxification of PDBE for catfish farming.

The findings of this study contribute valuable insights into the potential utilization of PDB effluent in aquaculture, specifically for enhancing fish metabolism and optimizing cost-effective feeding strategies. While high concentrations of raw PDBE adversely affected catfish metabolism, a 1:10 dilution of PDBE significantly improved metabolic health, suggesting that appropriate dilution mitigates toxicity while providing metabolic benefits.

Furthermore, biomarkers such as total protein, G-6-PDH, LDH, GSH, and GPx are shown to be reliable indicators for monitoring PDBE pollution in aquaculture settings. This research therefore provides a foundation for developing safe guidelines for PDBE use in fish farming and offers a practical recommendation for fish farm managers: a 1:10 dilution may be an effective practice for detoxifying PDBE, making it a viable resource for sustainable fish farming.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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