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Chronic Gas Flaring Pollution-Induced Alterations in Kinetic Parameters of Lactate Dehydrogenase in *Gallus Domesticus* Native to Ebocha, Niger Delta, Nigeria

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Abstract

The effect of pollution due to chronic exposure to gas flaring on kinetic parameters of lactate dehydrogenase (LDH) of Gallus domesticus native to Ebocha, Egbema in the Niger Delta. Nigeria was investigated. Apparently healthy matured (6-9 months) male fowls (Gallus domesticus) native to Ebocha were used as test organisms and fowls from Okigwe with no history of petroleum hydrocarbon pollution served as control. Results obtained revealed that the mean LDH activity (ΔABS/min) of the fowls from Ebocha (288.546.54) was significantly ($p<0.05$) higher than that of fowls from Okigwe (256.48±7.35). The temperature optimum (37^{o}C) and pH optimum (8.0) were essentially the same for both locations. The Km and Vmax values were 0.607 and 448.84 respectively for fowls native to Ebocha, as against 0.842 and 416.78 respectively for those from Okigwe. The findings from this study indicated that only LDH activity, Km and Vmax of fowls from Ebocha were responsive to pollution due to gas flaring, while optimum temperature and pH values were virtually the same from the two locations.

Keywords: *Gallus domesticus*, gas flaring, lactate dehydrogenase, kinetic parameters.

1. Introduction

Nigeria is a country endowed with natural resources among which are crude oil and natural gas. Nigeria is the second world's highest flarer of Associate Gas (AG) with more than 120 gas flaring sites and the release of over 23 billion/m^3 of gas per annum (Olukoya, 2008). Due to poor infrastructure and unsustainable practices among oil companies, only 19% of the total associated gas is recovered (Evoh, 2002). According to World Bank estimates in 2011, the annual volume of natural gas

being flared and vented worldwide stood at about 140 billion cubic meters, this is enough to provide for the annual gas consumption of Central and South America and that of Germany and Italy (Orimogunje, Ayanlade, Akinkuolie & Odiong, 2010). Russia still tops the world's gas flaring countries, followed by Nigeria, Iran and Iraq. Inconsistent data and under-reporting of gas flaring by government and companies has complicated the global effort to track the progress on gas flaring reduction (Orimogunje, *et al*., 2010). Idodo-Umeh (2010) reported that the Nigerian crude oil is known to contain heavy metals such as Al, Zn, As, Ba, Fe, Pb, Co, Cu, Cr, Mn, Sb, Ni. It has been noted that surface and underground waters in gas-flaring environments tend to have more concentrations of heavy metals such as lead, barium, cadmium, selenium and copper than non-gas-flaring area (Nwankwo & Ogagaure, 2011; Egwurugwu, Nwafor, Nwankpa, Olorufemi & Okwara, 2013). In addition, high concentrations of heavy metals in soil and water exposed to gas flares in Niger Delta Region of Nigeria have been observed (Nwaogu & Onyeze, 2010; Nwankwo & Ogagaure 2011; Idodo-Umeh,2012). Various studies have investigated the impact of gas flaring on buildings (Nkwocha & Pat-Mbonu, 2010), micro-climate and vegetation (Efe, 2003), climate change (Emerole, 2008), human haematological parameters (Adiebo & Nwafor, 2010), air, soil and water quality (Ekanem, 2001; Nwaogu & Onyeze, 2010). Although these studies differ in their findings and conclusions, some revealed astonishing results. Despite these efforts, the impact of this pollution on enzyme in *Gallus domesticus* is lacking. Lactate dehydrogenase is one such important indicator of metabolic stress in the choice organism.

Lactate dehydrogenase (LDH) is an enzyme that catalyses the conversion of pyruvate to lactate and vice versa, using nicotinamide adenine dinucleotide (NAD^+) as cofactor. LDH is virtually present in a wide variety of organisms including plants and animals. Extracellular activity of this enzyme increases under condition of oxidative stress, since the cell integrity can be disrupted during lipid peroxidation process (Drent, Cobben, Herderson, wouters & Dieijen, 1996). Cellular enzymes in the extracellular space, although of no further metabolic function in this space, are still of benefit because they serve as indicators of disturbances of the cellular integrity induced by pathologic conditions. Lactate dehydrogenase is a cytoplasmic enzyme present in essentially all major organ systems (Drent *et al.,* 1996). The extracellular appearance of LDH is used to detect cell damage or cell death (Lott and

Nemensanszky, 1987). Lactate dehydrogenase is present in the form of its isozymes which are tetramers. Each tetramer consists of four sub-units or monomers each with a mass of 36 kDa giving the tetramer the mass of l44 kDa (Terence, Stavinos & Gary, 1999). Each monomer consists of a peptide chain of 334 amino acids with its own active centre (Terence et al.,1999). In the absence of lactate and NAD^+, the active centre is open and access to substrate binding site is allowed. In the presence of lactate-NAD^+ complex, the active centre is closed by peptide loop and no access is allowed to the binding site.

The lactate dehydrogenase isozymes are made up of two distinct subunits M and H having different amino acid sequences which make them different in terms of catalytic properties. The differences in sequence between the M and H subunits are mainly conservative, that is, both residues are of the same type e.g. glycine (G) and alanine (A), or arginine (R) and lysine (K). Non-conservative exchanges are less frequent e.g. Lysine (K) for glutamine (Q) or threonine (T) for glutamic acid (E). The H which is coded LDHA is more strongly negatively charged than M gene which is coded LDHB, due to higher number of acidic residues.

The isoenzymes all catalyse the same biochemical reaction but differ in their molecular structure, and are more or less organ specific (Moss and Henderson, 1986). Therefore, isoenzyme patterns can be used to localize the source of LDH release. The isoenzymes differ in reactivity to substrates, sensitivity to inhibitors, resistance to heat inactivation, cold ability, mobility in tertiary structure and charge. Therefore, isoenzymes can be separated electrophoretically (Drent *et al*., 1996). Activity of LDH is present in almost all cells of plants and animals and is found only in the cytoplasm (Moss and Henderson, 1986; Lott and Nemensanszky, 1987). Ebocha is a polluted environment, due to petroleum hydrocarbon pollution (Nwaogu and Onyeze, 2010), organisms tend to shift to anaerobic metabolism under free radical producing stress conditions. This shift is regarded as an adaptive phenomenon to maintain the capacity for ATP synthesis. During anaerobic respiration, pyruvate is reduced to lactate. This results in the production of high concentrations of lactate and an increase in the activity of lactate dehydrogenase (Jian, Xuchang & Lifong, 2012).

Gallus domesticus is a domesticated fowl, a subspecies of the red jungle fowl. As one of the most common and widespread domestic animals with a population of more than 24 billion in 2003 (Perrins *et al*, 2003), there are more chickens in the

world than any other species of birds. Humans keep chickens primarily as a source of food, consuming both their meat and their eggs.

Chickens may live for ten to fifteen years, depending on the breed.. This study was therefore undertaken to investigate the effect of pollution due to chronic exposure to gas flaring on kinetic parameters of LDH in *Gallus domesticus* native to Ebocha, Egbema in Niger Delta.

2. 0. Materials and Method

2.1. Reagent kits and Chemicals

Lactate dehydrogenase kit used was purchased from Randox Laboratories Ltd, Diamond Road, Crumlin, United Kingdom. The other chemicals used were of analytical grade.

2.2. Experimental Animals

The native fowls (*Gallus domesticus*) reared in Egbema were used as the experimental animals while fowls reared at Okigwe were obtained and used as the control fowls.

2.3. Preparation of Blood and Serum for Assay/Analysis

The native fowls were allowed to acclimatize in the laboratory for 24 hours. Incisions were then made into their thoracic regions and they were terminally bled by cardiac puncture using 5mL hypodermic syringes and needles. The blood samples were dispenced into sterile sample bottles. The blood samples were allowed to clot and were centrifuged at 3000 rpm for 10mins. The serum was separated using micropipette and used for the determination and assay of the various parameters.

2.4. Assay of Lactate Dehydrogenase Activity

LDH activity was assayed following standard procedures as described in the assay kits by the manufacturers from Randox laboratories Ltd, Diamond Road, Crumlin, United Kingdom.

2.5. Kinetic studies

The effect of pH, temperature and substrate concentration, Km and Vmax on lactate dehydrogenase activity was determined using the standard procedure.

2.6. Ethical Approval

The experiment was carried out in accordance with the approval of the Ethics Committee of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria, and all in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.7. Statistical Analysis

Each reading was taken in triplicate. Data were expressed as mean ±standard deviation and analysed for statistical significance using Student's t-test. Differences in means were considered significant at $p<0.05$.

3.0. Results and Discussion

Figure (1) shows LDH activity (ΔABS/min) of fowls from Okigwe and Ebocha. The figure indicates that the mean LDH activity was significantly ($p<0.05$) higher (288.54±6.54) in fowls from Ebocha than in those from Okigwe (256.48±7.35). The increase in LDH activity of fowls from Ebocha was as a result of pollution due to gas flaring in that environment.

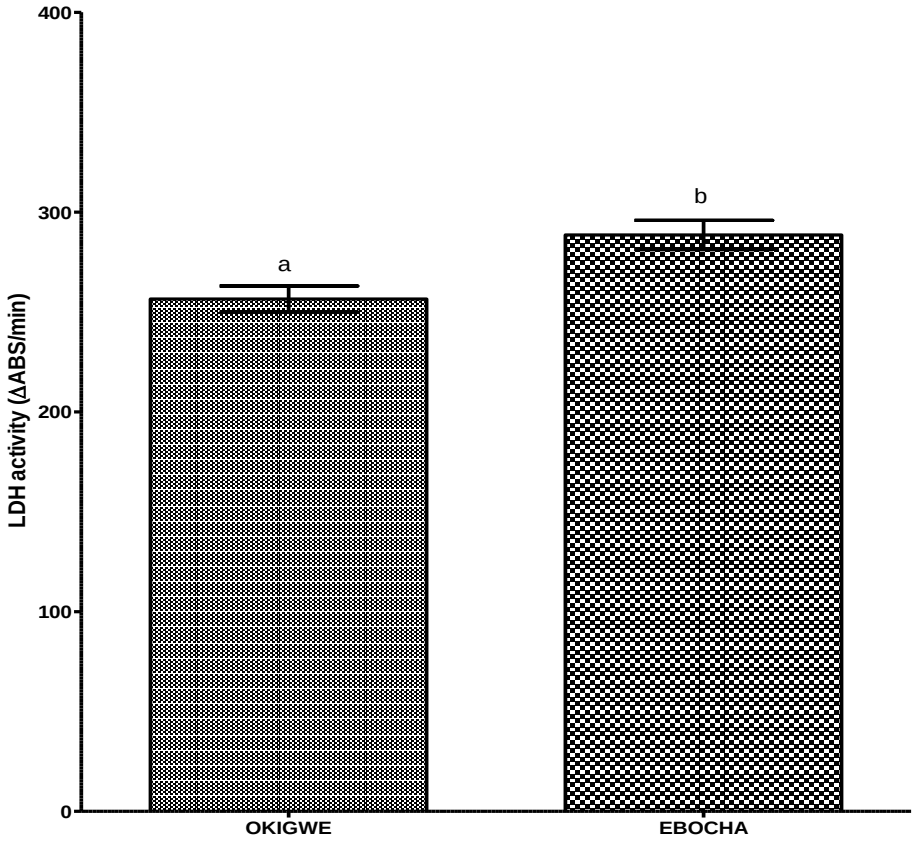

Fig. 1: LDH activities (ΔABS/min) of fowls from Okigwe and Ebocha.

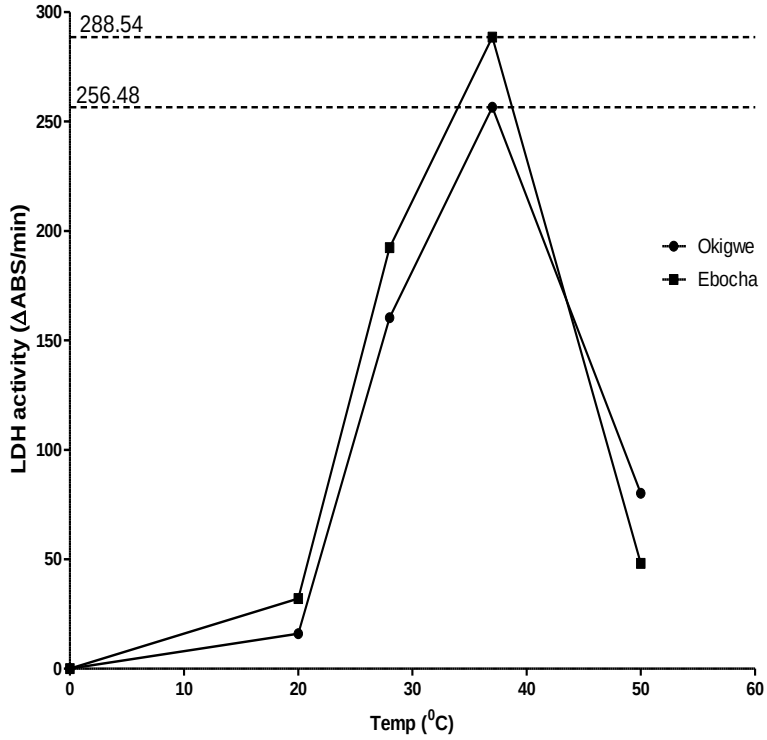

Fig. 2: Effect of temperature (^{0}C) on LDH activities (ΔABS/min) of fowls from Okigwe and Ebocha.

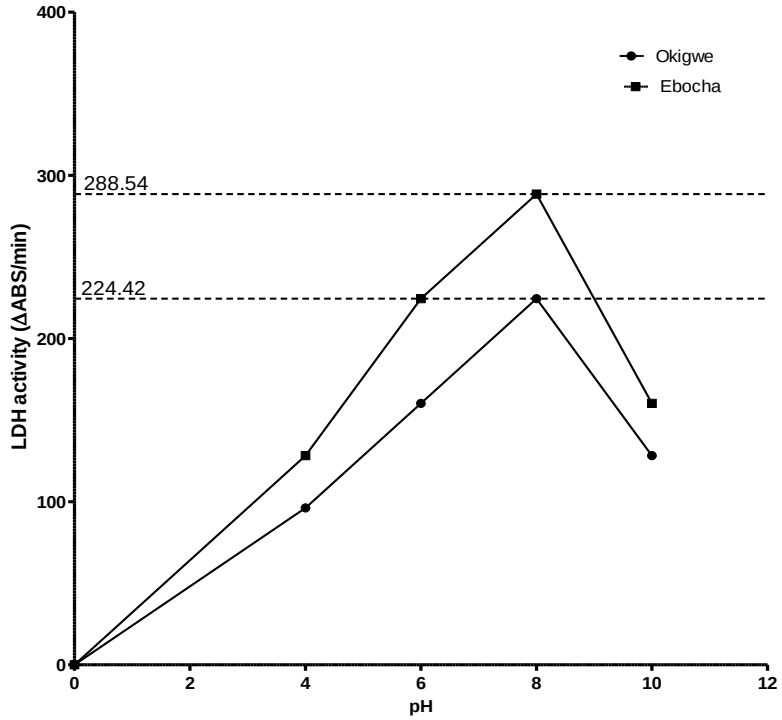

Fig. 3: Effect of pH on LDH activities (ΔABS/min) of fowls from Okigwe and Ebocha.

Lactate dehydrogenase (LDH) is an enzyme that catalyses the inter-conversion of pyruvic acid and lactic acid. This enzyme is found in both plants and animals (humans) and it responds to cellular damage or injury, which manifests as an increase in its activity.

Figure 1 indicates that there was a significant increase in LDH activity in fowls from Ebocha (288.54±6.45) when compared to that fowls from Okigwe (256.48±7.35). This result corroborates that reported by Halyna and Natalia, (2012) who worked on pollution-induced oxidative stress and biochemical parameter alterations in the blood of white stork nestlings *Ciconia ciconia* from regions with different degrees of contamination in Poland.

Figures 2 and 3 show the results of the effect of temperature and pH on lactate dehydrogenase activity of fowls from Okigwe and Ebocha respectively. Extremely high or low temperature values generally result in complete loss of the activity for most enzymes which may be due to ionization of amino acids in the active site of the enzyme lactate dehydrogenase.

The optimum pH or temperature of an enzyme is that pH or temperature at which the enzyme operates at maximum efficiency. These two parameters are factors in the stability of an enzyme. Enzymes work within pH and temperature ranges but have an optimal temperature and optimum pH in which their catalytic activity is at its peak. From this study, the optimum temperature and optimum pH of lactate dehydrogenase activity between Okigwe and Egbema were the same and 37^{o}C and 8.0 respectively.

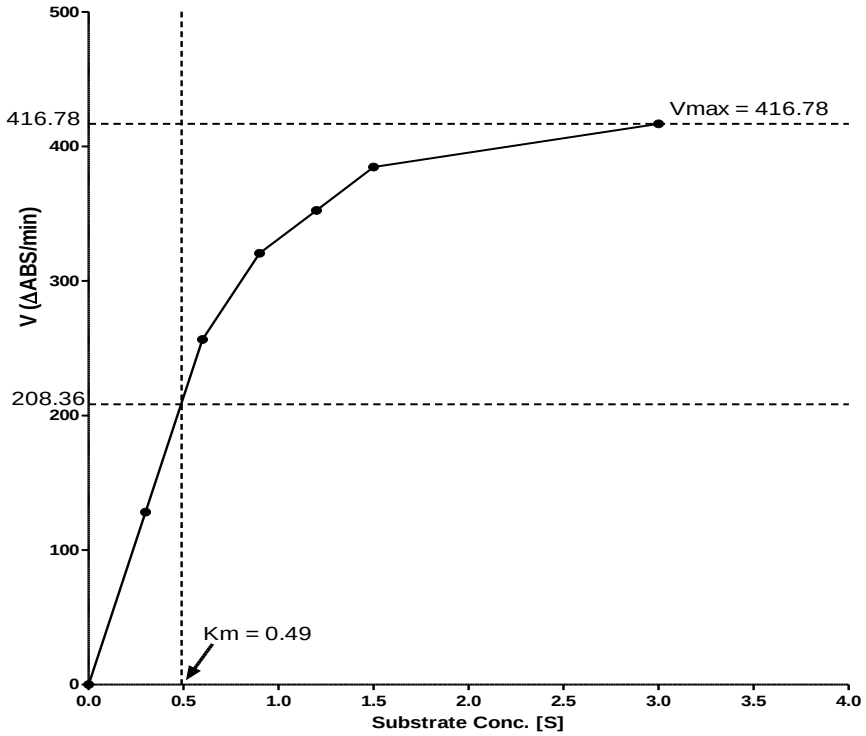

Fig. 4: Michaelis-Menten's plot of LDH activities (ΔABS/min) of fowls from Okigwe.

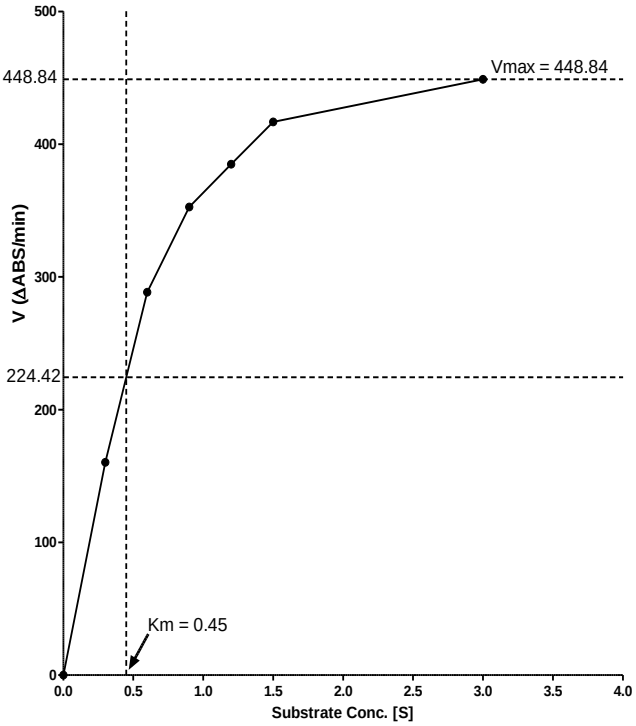

Fig. 5: Michaelis-Menten's plot for LDH activities (ΔABS/min) of fowls from Ebocha.

Figures 4 and 5 show the effect of substrate concentration on the lactate dehydrogenase activities of fowls from Okigwe and Ebocha respectively. For an enzyme catalysed reaction, there is usually a hyperbolic relationship between the rate of reaction and the concentration of substrate, this is because: at low concentration of substrate, there is a steep decrease in the rate of reaction with decreasing substrate concentration. The rate of reaction when the enzyme is saturated with a substrate is the maximum rate of reaction, Vmax. The relationship between the rate of catalysis for a substrate depends on the affinity of the enzyme for its substrate. This is usually expressed as the Km (Michaelis Constant) of the enzyme; an inverse measure of affinity.

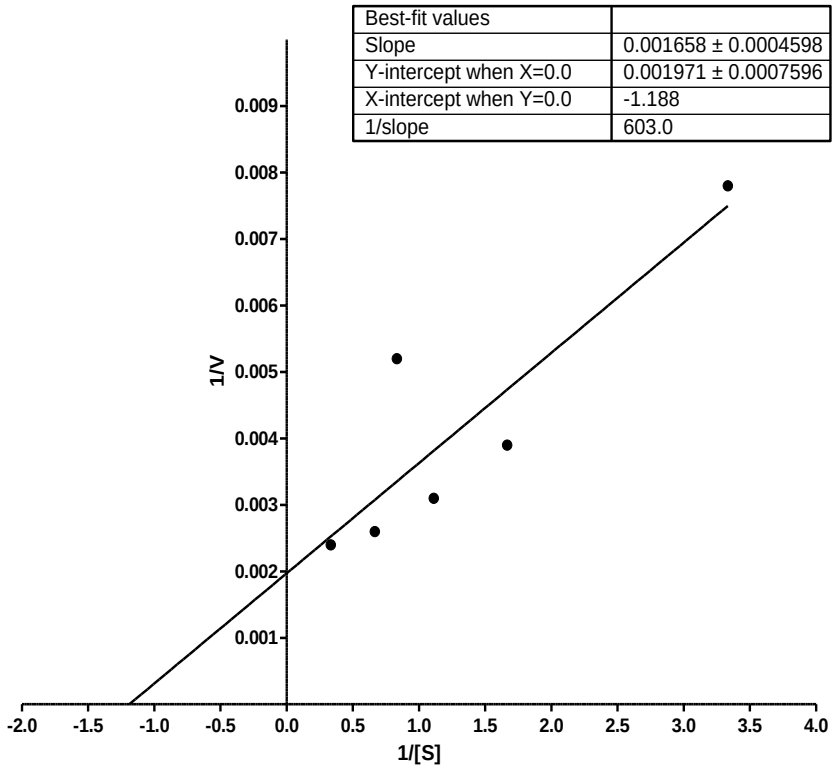

Fig. 6: Lineweaver-Burk's plot of LDH activity (ΔABS/min) of fowls from Okigwe.

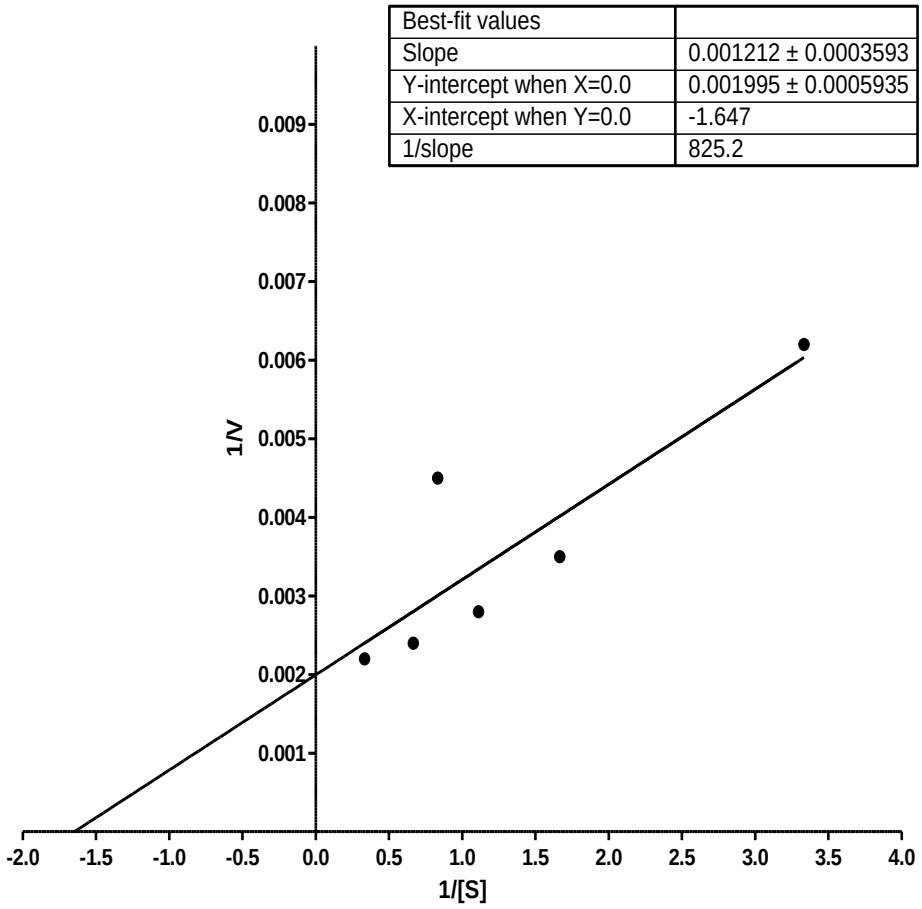

Fig. 7: Lineweaver-Burk's plot for LDH activities (ΔABS/min) of fowls from Ebocha.

Using Michaelis-Menten's plot and Lineweaver-Burk's plot (Figures 6 and 7). Fowls from Ebocha had higher Vmax and smaller Km than those from Okigwe. Alteration in the Km value may reflect structural modifications in enzyme at the active site or rearrangement of the subunit composition as lactate dehydrogenase, lactate dehydrogenase being an isoenzyme. This implies that pollution due to petroleum hydrocarbon (gas flaring) affected the enzyme activity by causing an increase in LDH activity. The result agrees with the results of Terence *et al*., (1999) who reported that physical training which is a form of stress induced alterations in lactate dehydrogenase kinetic parameters in rats.

4. 0. Conclusion

This study revealed that chronic exposure to pollution due to gas flaring increased the activities of lactate dehydrogenase, Vmax of fowls native to Ebocha, Niger Delta. The temperature and pH optima were not affected. These findings might imply that the pollution due to chronic exposure to gas flaring induced alterations in the kinetic parameters of LDH of *Gallus domesticus* native to Ebocha, Niger Delta.

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