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ORIGINAL ARTICLE

Alteration of Some Hepatic Enzyme Activities by Gastrointestinal Helminth Parasites in Domesticated Ostriches

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ABSTRACT
Using the conventional methods for parasitological examinations of the salt floatation and direct smear, ten ostriches infected with internal parasites were identified. Ten birds that were without internal parasites were used as the controls. The metabolizing enzymes glutathione peroxidase (GPX), DT -diaphorase (DTD) and succinate dehydrogenase (SDH) were assayed accordingly using liver samples from the control and infected birds. Malondialdehyde (MDA), a marker of lipid peroxdation, was also assayed. Results showed that cestode eggs occurred at twice the amount of nematode eggs in the faeces of infected ostriches. Infected birds showed significantly higher DTD activity when compared to non-infected birds (Student's t test, $p < 0.05$) whilst the activities of SDH and GPX were statistically not altered (Student's t test $p > 0.05$). MDA levels were significantly elevated in infected birds compared to controls (Student's t test, $p < 0.001$). These results suggest that infected birds are under oxidative stress due to the parasitic infection .
Key words: *Cestodes, nematodes, glutathione peroxidase, DT-diaphorase, Succinate dehydrogenase, malodialdehyde.*

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INTRODUCTION

References to ostriches are found in the ancient Egyptian, Greek and Roman literature implying that these birds may have existed millions of years ago in the Euro-Asiatic continent though these birds have become native to Southern Africa [1]. The birds are being commercially raised to meet the ostrich hide and feather industry needs as well as meat requirement [2, 3].

Commercial ostrich farming in Zimbabwe began in the mid 1980s and has since become a popular alternative to livestock and crop production on several farms [3, 4]. The primary purpose of commercial ostrich production is to maximize the number of birds for processing that will produce the highest yields of skin and meat of the best quality with a minimum cost of production [5]. Several challenges such as feed availability and diseases militate against this. The outbreak of the H5N2 strain of the Avian Influenza virus grossly affected production on Zimbabwe ostrich farms confining sales of products to the local market [6].

Ostriches are known to be susceptible to intestinal helminthic parasites especially Cestoda: *Houttuynia struthionis*; Nematoda, eg. *Codiostomum struthionis* and *Libyostongylus douglassii* [7, 8, 9]. *C. struthionis* is generally regarded as harmless unless it occurs in large numbers where it results in intestinal irritation and loss of appetite. The tapeworm, *H. struthionis*, is dangerous in young ostriches in which it could result in anorexia, diarrhea, poor growth and poor feathers [10, 11]. *Libyostrongylus* is a small blood-sucking trichostrongylid nematode found under the mucosal lining of the proventriculus of ostriches. *L. douglassii* appear to be common and widely distributed in ostriches and is the most pathogenic of all ostrich parasites [12, 13].

Whilst there have been several studies on antioxidant enzymes as a protective mechanism of parasites from reactive oxygen species arising from host immune response [14] studies on the effect of the parasites on the host's antioxidant enzyme activity are few [15, 16, 17]. Parasites cause inflammation when imbedding and penetrating the mucosa. Inflamation reactions are accompanied by generation of reactive oxygen species [15, 17, 18]. The oxidative damage is deleterious to the cell and may lead to senescence and cell death [19]. However, the cell is equipped with several antioxidant defense enzymes

which protect the cell from oxidative damage, as long as generation of reactive oxygen species does not overwhelm the activities of the antioxidant enzyme system. These enzymes include glutathione peroxidase and catalase which breakdown hydrogen peroxide to water [20], DT-diaphorase which is deemed to protect against lipid peroxidation by the hydroxyl radical (·OH) as well as against oxidation of proteins and DNA [21]. We set to use coprological techniques to demonstrate infection of ostricthes by cestodes and nematodes by the presence eggs in faeces. As the liver can is regarded as the major target for the detection of changes in metabolic enzymes [22] and parasites can cause shifts in metabolic or molecular function of the liver [23], we investigated the effect of parasitic infections on the activity of some selected hepatic enzymes.

MATERIALS AND METHODS

Sample collection and preparation

Parasitological Analysis

Faecal samples for helminthic parasitological examination were collected from the small intestines or rectum of the slaughtered eleven-month old female ostriches. Samples were placed in screw cap plastic bottles and transported to the laboratory in cooler boxes containing ice. Faecal egg counts were done using the salt floatation technique, as described by Soulsby [22] and Thienpont [23]. The method involved taking 2 g of faecal sample into a test tube, adding 21 ml of water and shaking the tube thoroughly to mix the contents. The mixture was filtered using muslin cloth to discard the large debris particles and the resulting filtrate was centrifuged at 3 000g for 3 minutes to settle the heavier parasite eggs. The supernatant was discarded and the tubes re-filled with saturated salt solution and re--centrifuged for 2 minutes at 3 000g so as to float the nematode eggs in the solution whose specific gravity was between 1.10 and 1.20 [24]. A glass rod was then used to transfer the floating eggs in the supernatant onto a microscope slide where they were viewed under both low and high power. A total of twenty birds from two farms consisting of 5 non-infected birds and 5 infected birds were identified from each farm and used in this study.

Biochemical Analysis

Liver samples for enzymatic assays were collected, alongside faecal samples, from the slaughtered birds. The samples were transported on ice in cooler boxes to the laboratory and kept at -80°C until they were required for assays. The liver samples were thawed, weighed and diced into small pieces using a scalpel and then homogenized in five volumes of 0. M potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at 9 000g for 10 minutes at 4^{o}C to sediment the heavier cell debris, nuclear and mitochondrial factions. The supernatants were stored in Eppendorf tubes in small aliquots of 500 - 1 000 µl at -80°C until required for enzymatic assays. The pellets were diluted with 10 volumes of buffer (pH 7.4) and similarly stored in eppendorf tubes until required for succinate dehydrogenase assay.

Diphosphotriphosphodiaphorase (DT-Diaphorase) assay

DT-Diaphorase activity was measured according to a previously described method [25] which was modified and adapted for a 96 well microplate reader in our laboratory. The reaction mixture, in a final volume of 200 µL, consisted of 50 mM Tris-HCl buffer, pH 7.5 (140 µl), 10% Triton X-100 (20 µl), 400 µM 2,6 Dichlorophenolindophenol and S-9 fraction at 1mg/ml protein (20 µl). A decrease in absorbance at 600 nm over 3 minutes at 30^{o}C was recorded. DT-diahorase activity was calculated using the extinction coefficient for DCPIP (ε = 21 $mM^{-1}cm^{-1}$) after correcting for the blank.

Glutathione peroxidase assay

Hydrogen peroxide was used as a substrate to determine selenium-dependent glutathione peroxidase (Se-GPX) activity and consumption of NADPH monitored at 340 nm over 3 minutes at 30^{o}C as described by Scholz et al., [26] after adapting the method for a 96 well microplate reader. In brief, the reaction mixture consisted of main reagent (95 µl), S-9 fraction at 1mg/ml protein in 50 mM Potassium Phosphate buffer, pH 7.4, (15 µl), 1.5 mM H_2O_2 (15 µl), and 50 mM Potassium Phosphate buffer, pH 7.4, (15 µl). Se-GPX activity was calculated using the coefficient extinction for NADPH (ε = 6.22 x 10^3 $M^{-1}cm^{-1}$) after correcting for a blank.

Lipid peroxidation assay

Lipid peroxidation was measured by determining MDA concentration in the liver according to the method of Draper and Hadley, [27]. Tissue (0.2 g) was homogenised in 5% aqueous TCA (5 ml) and 0.05% methanolic butylated hydroxytoluene (0.5 ml) and then heated in a capped tube in a boiling water bath for 30 mins. The cooled samples were centrifuged to remove particulate matter. A 1 ml aliquot of the supernatant was mixed with 1 ml saturated thiobarbituric acid and boiled for a further 30 mins. Absorbance of the cooled reaction mixture was read at 535 nm. Concentration of MDA was calculated using the coefficient of extinction, ε = 1.5 x 10^5 $M^{-1}cm^{-1}$ after correcting for reagent blank

Succinate dehydrogenase assay

A modified method of Ackrell et al., [28] was used to assay SDH. The test solution consisted of 3 ml potassium phosphate buffer (pH 7.4) which was added to 1 ml of 0.01% 2,6 DCPIP solution and 0.5 ml of the protein extract. To this mixture, 1 ml of the substrate, O.1 M sodium succinate was then added followed by addition of paraffin oil and the decrease in absorbance of 2,6,DCPIP was measured at 600 nm. A blank lacking the enzyme was simultaneously run.

Statistical Analysis

Results obtained were expressed as means ± SD. Statistical analysis was performed using GraphPad Instat Programme, Graph Pad Prism, San Diego, USA. Student's t test was used to determine differences between non-infected and infected birds.

RESULTS AND DISCUSSION

Based on egg counts (Table 1) and microscopic identification, cestodes were found to occur at over two fold prevalence when compared to nematodes on the two farms studied,. The thin shelled oval shaped nematode eggs were not larvated but could not be positively identified as they could either be of Strongyle origin or of *Codiostomum struthionis.* The cestodes, on the other hand, were positively identified as those from the tapeworm. Gastrointestinal parasites have been proven to cause marked production losses in farm animals throughout the world [29]. They need to be controlled but their control has become increasingly difficult because of their resistance to anthelminthic drugs [30].

Table 1. Parasite egg counts (eggs / gram) in rectal faeces collected from ostriches on two different farms. Values are means ± SD of 5 ostriches

Farm	Parasite egg	
	Nematode	Cestode
Farm A	57 ± 27	114 ± 60
Farm B	76 ± 38	114 ± 62

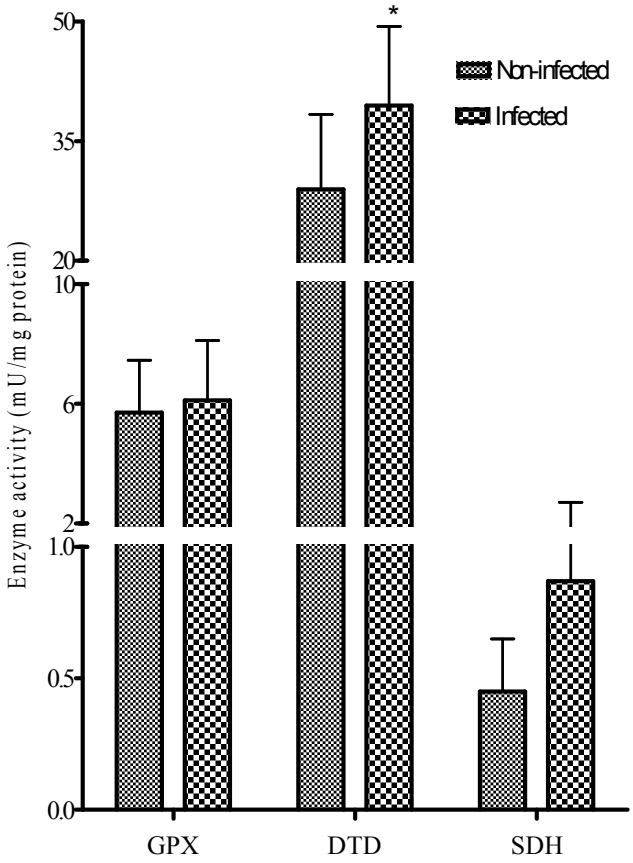

Figure 1. A comparison of enzyme activities of lovers of non-infected infected ostritches. Values are mean ± SD from 5 birds in each category.*p < 0.05 significantly different from non-infected birds , Student t test.

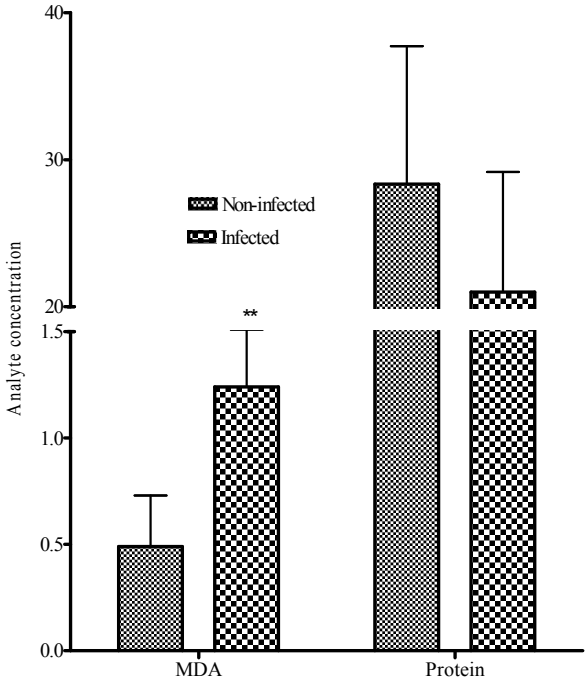

Figure 2. MDA concentration (µmole/g iver tissue) and protein concentration (mg/g liver tissue) in non-infected and infected ostriches. Values are means ± SD from 5 birds in each category. **p < 0.01 significantly from non infected birds, Student t test.

The effect of parasites on the activities of hepatic GPX, DTD, and SDH are shown in Fig 1. There was no significant difference in the activities of GPX between infected and non-infected ostriches ($p > 0.05$). On one hand, these results are in contrast to studies suggesting that parasitism results in significantly higher levels in hepatic GPX activity of infected animals [16, 31]. On the other hand, some studies have reported a decreased GPX activity in the liver of parasitized animals [32] and a decrease in erythrocyte GPX activity in mice infected *Plasmodium vinkei* [33]. Our results are however in agreement with studies done using fish. Neves et al., [34] found no difference in the activity of GPX in shrimp, (*Palaemonotes argentinus* Nobili 1901) infested by *Probopyrus ringueleti.* Bello et al., [17] also found that enzyme activities of CAT, an isofunctional enzyme, were similar in muscle of freshwater fish (*Rhamdia quelen*) infested by the parasite *Clinostomum detruncatum.* These results suggest that no simple relationship exists between GPX activity and oxidative stress in parasite- infected animals compared to non-infected animals.

Significantly higher DTD activity was manifested in infected ostritches compared to controls ($p < 0.05$). The higher DTD levels in infected birds indicates that parasitism leads to oxidative stress. DTD is an antioxidant enzyme that prevents initiation and propagation of lipid peroxidation by maintaining coenzyme Q in reduced antioxidant state [35, 21]. The higher activity of DTD could be an adaptive response in an effort to slow down lipid peroxidation.

That parasitism leads to oxidative stress in the ostrich is also shown by statistically higher levels of MDA in the liver of the infected ostrich ($p < 0.01$) when compared to non infected birds (Fig 2). The livers of infected ostriches had significantly higher MDA levels than those of non-infected ostriches ($p < 0.01$). Lipids are one of the main cellular components that are susceptible to damage by reactive oxygen species produced as a result of oxidative stress. It follows that peroxidation of lipids is the most obvious symptom of oxidative stress and is indicated by MDA levels. MDA is a product of lipid peroxidation. The higher the level in a tissue, the higher the level of peroxidation. The three-fold increase in MDA levels in infected ostriches implies that these ostriches are in a higher oxidative stress status compared to non-infected birds. Our results are in agreement with studies of Oliveira and Cecchini [36] who found elevated levels of MDA in livers of hamsters infected with *Leishmania* (L.) *chagasi*. Higher MDA levels were also found in cattle infected with Fasciola gigantic compared to non-infected cattle [16] whilst Kaya et al. [37] found higher MDA levels in serum of human patients with fasciolaiasis when compared to controls.

As parasitism has been reported to induce higher oxygen and energy consumption in some animals, e.g. in fish [38] we investigated the activity of SDH in hepatic mitochondria of infected and non-infected ostrich.

Higher but non statistically significant levels of SDH activity ((p = 0.08)) was observed in infected ostriches (Figure 1) suggesting a relatively higher turn over of oxygen within the citric acid cycle as the infected birds try to cope with infection. This would result in higher reactive oxygen species such as the hydroxyl radical responsible for lipid peroxidation [20].

All in all, the present study indicated that parasites significantly increased lipid peroxidation as shown by increased MDA levels in livers of infected ostriches. This was followed by an increase in the activity of an appropriate defense enzyme, DTD.

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REFERENCES

1. Batty, J. (1995). Ostrich Farming. Midhurst Publishing House. pp. 87-99.
2. Thomson, R.S. (1997). Raising Emus and Ostriches. US Department of Agriculture pp 306-310
3. Siwela, A.H., Shonai, A., Foggin, C.M. and Revol, B. 2000. Establishment of baseline values of copper, zinc and manganese in ostriches in Zimbabwe: A preliminary study. Zimbabwe *Vet. J*., **31**: 57-64
4. Shanaway, M.M. [online]. Recent developments in ostrich farming. http//www.fao.org. [Accessed 31 March 2006]
5. Perelman, B. (1998). Veterinary Aspects of preventative medicine in ostriches. *J. Med.*, **67**: 181-186.
6. Alexander, D.J. (2000). A review of Avian influenza in different bird species. *Vet. Microbiol*., **74**: 3-13.
7. Cooper, R.G. and Mahroze, K.M. (2004). Anatomy and Physiology of the gastrointestinal tract and growth curves of the Ostrich (*Struthionis camelus*). Animal Sci. J., **75**: 491-498.
8. Dzoma, B.M. and Dorrestein, G.M. (1998). Problems in Ostrich rearing. *Equator* **10**: 1-6.
9. Hooper, C., Cooke, M., Hill, F., Julian, A., McKenna, P., Thompson, J., and Williamson, C. (1999). Quarterly review of diagnostic cases- January-March 1999. Agriquality Laboratory Network. Surveillance **26**: 16-17.
10. Cooper, R.G. (1999). Ostrich production in Zimbabwe. Zimbabwe Sci. News **33***:*73-78.
11. Cooper, R.G. and Mahroze, K.M. 2004. Anatomy and Physiology of the gastrointestinal tract and growth curves of the Ostrich (*Struthionis camelus*). *Animal Sci. J*., **75**: 491-498.
12. McKenna, P.B. (2001). Register of new host-parasite records. Surveillance, **28**: 4-5,
13. McKenna, P.B. (2005). *Libyostrongylus* infections in ostriches- a brief review with particular reference to their detection in New Zealand. *New Zealand J. Vet.*, **53**: 267-270.
14. LoVerde, P.T., Carvalho-Queroz, T., and Cook, R. (2004). Vaccination with antioxidant enzyme confers protective immunity against challenge infection with Schistosoma mansoni. Mem Inst Oswaldo Cru., **99**: 37-43.
15. Egwunyenga, A.O., Isamah, G. and Nomorsi, O.P. (2004). Lipid peroxidation and ascorbic acid levels in Nigerian shildren with acute falciparum malaria. *African J. Biotechnol*., **3**: 560-563.
16. Choto, K. and Dube, S. (2012). Stress response to Fascioliasis by cattle. *Advances Biores.*, **3**: 100-108
17. Bello, A.R.R., Forks, E. Bello-Klein, Bello, A.A. Rabaldo, A, Binchini, A. 2000. Lipid peroxidation induced by *Clinostomum dentrucatum* in muscle of the freshwater fish *Rhandia quelen*. Dis. Aq. Org.,s **42**: 233-2361.
18. Siwela, A.H., Mambvura, C.I., Masanganise, K.E. and Dube, S. (2010). Oxidative stress associated with nematode infection in sheep.: a preliminary study. *Int. J. Biol. Chem. Sci*., **4**: 351-357.
19. Sies, H. (1991). Oxidative stress: from basic research to clinical application. Ann. J. Med. **91**: 315-385.
20. Kappus, H. (1987). Oxidative Stress in chemical toxicity. Arch Toxicol., **60**: 144-149.
21. Ernster, L. and Dallner, G. 1995. Biochemical physiological and medical aspects of ubiquinone function. *Biochem Biophys Acta*., **1271**: 195 – 201.
22. Soulsby, J.D. 1982. Helminthes, Arthropods and protozoa of Domesticated Animals. London.
23. Thienpont, D., Rochette, F. and Vanparijs, O.F.S. (1979). Diagnosing helminthiasis through coporogical examination. Janssen Research Foundation Beerse, Belgium.
24. Urquhat, G.M., Armour, J, Duncan, J.L, dunn, A.M and Jennings F.W. (1987). New Veterinary Parasitology. Longman Scientific and Technical, London, pp 71-76.
25. Lind, C., Cadenas, E., Hochstain, P. and Ernsten, L. (1990). D-T-Diaphorase: Purification, properties and function. *Methods Enzymol.*, **186**: 287-301.
26. Schloltz, R.W. Cook, L.S. and Todhunter, D.A. (1981). Distribution of selenium dependent and non-selenium dependent glutathione peroxidase activity on tissue of young cattle. *Am. J. Vet. Res*., **42**: 1724-1729.
27. Draper, H. and Hadley, M. (1990). Malondialdehyde determination as an index of lipid peroxidation. *Methods Enzymol*., **186B**: 421-431.
28. Ackrell, B.A.C, Kearney, E.B. and Singer, T.B. (1978). Mammalian succinated dehydrogenenase. In: Biomembranes. (Eds. Fleishcher, S and Parker, L) Academic Press, New York. pp. 466-483.
29. Sykes, A.R. (1994). Parasitism and Production in Farm animals. Animal Production **59**: 155-172.
30. Prichard, R. (1994). Antihelmenthic resistances. *Vet Parasitol*, **54**: 259-268.
31. Benzer, F. and Ozan, T.S. (2003). The status of lipid peroxidation, antoxidant enzymes, and nitric oxide in sheep infected with *Fasciola hepatica* . *Turkish J. Animal Sci.* **27**: 657-661.
32. Kolodziejczyk, L., Siemieniuk, E. and Skrydlewska, E. (2005). Antioxidant potential of rat liver in experimental infection with *Fasciola hepatica*. *Parasitol Res.* **96**: 367-372.

33. Stocker, R., Hunt, N.H., Buffiniton. G.D., Weidemanm, M.J. Lewis-Hughes, P.H and Clark, I.A. (1985). Oxidative Stress and protective mechanism in erythrocytes in relation to *Plasmodium vinckei* load. *P.N.A.S.*, **82**: 548-551.
34. Neves, C.A., Santos, E.A. and Bainy, A.C.D. (2000). Reduced superoxide dismutase activity in *Palaeomonetes argentinus* (Decapoda, Palemonidae infected by *Probopyrus ringueleti* (Isopoda, Bopyridae). *Dis. Aq. Org.*, **39**: 155-158.
35. Halliwell, B., and Gutteridge, J.M.C. (1986). Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. *Arch Biochem Biophys.*, **246**: 501-504.
36. Oliveira, F.J..D.A. and Cecchini, R. 2000. Oxidative stress of liver in hamsters infected with Leishmania (L) chagasi. *J. Parsitology* **86**, 1067-1072.
37. Kaya, Siitcii, R., Cetin, ES, Aridogan, B.C., Delibas, N and Mustafa, D. (2007). Lipid peroxidation level and antioxidant enzyme activities in blood of patients with acute and chronic fascioliasis. *Int. J. Infectious Dis.*, **11**: 251-255.
38. Lemly, D. and Eseh, M.J. (1984). Platelminta. In: (Ed. de Almeida, A..) *Elementos de ictioparasitologia.* Fundacao Eng Porto p 158.

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