Research Paper

Volume : 2 | Issue : 11 | November 2013 • ISSN No 2277 - 8179

Biochemical alterations due to Bovine Fascioliasis

Zoology

KEYWORDS : Fascioliasis, serum enzymes, creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase

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ABSTRACT

The biochemical alterations due to infection of bovine fascioliasis in cattle slaughtered within southeast Nigeria was investigated. Various biochemical parameters such as serum enzyme activities, serum protein levels, serum lipid contents and serum chemical compounds were determined using standard procedures. Statistically significant elevations were observed in the mean values of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea and creatinine of Fasciola-infected cattle when compared to those of the control group ($p<0.05$) while significant decrease was observed in the mean values of total proteins, albumin, glucose, cholesterol, triglycerides, high density lipoproteins and low density lipoproteins of Fasciola-infected cattle when compared with the control group ($p<0.05$). Total proteins, albumin, glucose, triglycerides and LDL parameters had moderate significant negative correlation with worm load ($r = -0.555, -0.457, -0.581, -0.550, -0.425$), while urea and creatinine had high and moderate significant positive relationships with worm load ($r = 0.806$ and 0.448 respectively)

Introduction

Bovine fascioliasis is a systemic parasitic disease of cattle caused by trematodes of the genus *Fasciola.* It is one of the most important hepatic diseases causing great economic losses in ruminants. Apart from its high veterinary importance, fascioliasis is also a known zoonosis infecting a good number of human populations in every continent of the world. The genus consists of two main species that are of medical and veterinary importance namely, *Fasciola gigantica* and *Fasciola hepatica,* commonly called liver flukes

Since the liver is the main metabolic organ of the body, infection of the hepatocytes is an essential feature of certain parasitic infections. In fascioliasis, the metabolic processes of the liver (Doaa *et al.,* 2007) and kidney are gradually reduced. Hepatic and renal functions can be assessed using biochemical tests such as blood glucose, creatinine, urea, serum proteins, and serum lipids, while the activity of certain serum enzymes such as AST, ALT and ALP, which increase following hepatic injury, can be measured (Craig *et al*., 1992). These enzymes have a predominantly intracellular action and thus, under normal conditions, the serum enzyme activity is very low or absent; any increase in their activity would be evidence of damage in the tissues in which they are lodged (Grunwaldt *et al*., 2005). AST is present in many tissues, particularly liver, striated and cardiac muscle, making it a good marker of soft tissue damage (Otto *et al*., 2000). AST and ALT are released early in cell disruption and clear slowly from the circulation. ALP levels are used as a test of hepatic excretory function in many animals (Gossett & French, 1984). It is persistently elevated in both acute and chronic liver diseases and reflects damage to the biliary system.

The hepatocytes play important part in controlling the levels of blood glucose, lipids and cholesterol. Triglycerides, cholesterol, phospholipids and enzymes essential for formation of cholesterol esters are synthesized in the hepatocytes while glucose is stored by the hepatocytes in the form of glycogen, which is the major source of glucose for other cells in the body during metabolism. Lipid profiles have been used for the diagnosis of metabolic diseases and as such, are helpful when interpreted in conjugation with history, clinical signs and laboratory tests (Nazifi *et al*., 2002).

Few studies have documented the biochemical alterations due to naturally acquired bovine fascioliasis. We therefore investigated the biochemical alterations in cattle due to bovine fascioliasis using cattle with naturally acquired fascioliasis and without other diseases using direct organ and tissue analysis and diagnostic procedure that are very reliable and readily affordable in endemic, resource-limited rural communities.

Materials and Methods

Collection of Blood Samples

Jugular blood samples were collected from 20 non-infected cattle and 57 cattle with naturally acquired bovine fascioliasis and no other disease, out of 659 cattle screened within Nsukka tropical ecosystem in southeast Nigeria. The selected slaughtered cattle were confirmed free from other possible diseases through visual inspection of the organs, intestine and tissues by qualified Veterinary officers. The blood samples were collected in clean dry serum separating tubes and left to clot. The samples were later centrifuged at 2000 rpm for 30 minutes for serum separation. The separated clear serum samples were aliquoted and stored at – 20°C to be used for biochemical determinations.

Biochemical Studies

Various biochemical parameters were adequately assessed using the prepared serum samples from both the infected and non-infected cattle. Serum enzyme activities, serum protein levels, serum lipids contents and serum chemical compounds were all determined using standard procedures.

Serum Enzyme Activities Determination

Liver enzymatic activities of AST and ALT were carried out calorimetrically according to Reitman and Frankel (1971). ALP activity was measured according to Haussament (1977).

Serum Protein Levels Determination

Serum total proteins was determined colorimetrically according to Doumas (1975) and serum albumin level was determined colorimetrically according to Doumas *et al*. (1971). Serum globulin levels were calculated as differences between total protein and albumin according to Varley (1979).

Serum Chemical Compounds Determination

Serum glucose was determined by using the On Call Plus Blood glucose meter and the On Call Plus Blood glucose test strips (Acon Laboratories inc. USA). Sodium fluoride was used as anti-

coagulant in collecting the blood sample. Serum creatinine urea were determined according to Patton and Crouch (1977).

Serum Lipid Contents Determination
The stored serum was assayed for triglycerides, cholesterol, high and low density lipoproteins. The determination of serum levels of cholesterol was carried out using colorimetric enzymatic end point method. Serum triglycerides were analyzed using colorimetric method after enzymatic hydrolysis with lipases. High density lipoprotein was determined using precipitant method. The determination was carried out using standard commercial test kits (RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom). Low density lipoprotein, measured in mmol/l, was calculated from the values of cholesterol and high density lipoprotein using the formula described by Friedewald *et al.* (1972).

Ethical Approval: The ethical requirements of the University of Nigeria involving research with livestock was fully complied with.

Analysis of Data
The data obtained was analyzed using SPSS version 16. The students t-test was used to analyze the significant differences between the biochemical parameters of the *Fasciola* infected and non-infected cattle. Values of $p < 0.05$ were considered significant and results were expressed as means ± SD. Correlation between worm load and biochemical values were tested using regression analysis.

Results
The Liver Enzymes Status: In the results as shown in Table 1, the activities of AST, ALT and ALP were observed to be higher in the infected cattle than in the control.

Liver Enzymes and Worm Burden: The result showed considerable increase in enzyme activities with increase in worm burden, although the positive correlation was significant only for ALT ($r = 0.557$). AST and ALP had no significant positive correlation with worm burden ($r = 0.294$ and $r = 0.360$) -(Fig. I).

The Serum Proteins: The levels of serum proteins that were assessed (Table 2), showed that the mean values of total protein and albumin of the infected cattle were much lower than that of the control and the differences between the means were highly significant ($P< 0.05$). There was no significant difference in the globulin level of the infected cattle and the control.

Serum Proteins and Worm Burden: The relationships between worm burden and the serum protein contents are illustrated in Fig. 2. The result showed a decrease in the serum proteins of infected cattle, the higher the worm burden, the lower the serum protein content. Regression analysis showed that total protein and albumin had significant negative correlation with worm burden ($r = -0.555$ and $r = -0.457$ respectively). Although globulin also had a negative correlation with worm burden, it was statistically insignificant ($r = -0.433$) (Fig. 2).

The Serum Chemical Compounds: The serum chemical compounds of the infected and non-infected cattle is shown in Table 3. The mean values of serum glucose level were lower in the infected cattle than in the control group while urea and creatinine levels were higher in the infected cattle than in the control group. Statistical analysis showed that high significant differences existed between the means of the serum chemical compounds of the infected and the control groups ($p< 0.05$).

Serum Chemical Compounds and Worm Burden: The result of the study of the effects of worm burden on the serum chemical compounds levels are presented in Fig. 3. The result showed considerable higher values for glucose in the lower worm counts than in the higher worm counts. On the contrary, it showed lower values for urea and creatinine in the lower worm counts than in the higher worm counts. This signified that the increase in worm burden caused a decrease in the serum glucose level and increase in the urea and creatinine levels. Multiple regression analysis revealed significant negative correlation between worm burden and glucose ($r = -0.581$) and significant positive correlation between worm burden and urea ($r = 0.806$). Creatinine had also significant positive correlation with worm burden, though at a lower rate than urea ($r = 0.448$) (Fig. 3).

The Serum Lipids Assay: The result obtained from the serum lipids assay is shown in Table 4. The mean values of cholesterol, triglycerides, high density lipoproteins and low density lipoproteins levels were much lower in the infected cattle than in the control. The statistical analysis indicated high significant differences between the mean values of the serum lipids of the infected and non-infected cattle ($p< 0.05$).

Serum Lipids and Worm Burden: In the study of the relationship between the serum lipids levels and *Fasciola* worm burden (Figs. 5, 6), it was revealed that intensity of infection had some effect on the lipids levels. It was observed that the serum lipids contents decreased progressively as worm burden increased. This showed that the damaging effects of *Fasciola* on the liver cells affects the production and usage of lipids in the infected body and the damage or degrading effect increases as the worm burden increases. Correlation analysis showed negative correlation between the serum lipid parameters and worm burden although the negative correlation was significant only for triglycerides and LDL, with the correlation coefficients as $r = -0.497$ (Fig. 5) and $r = -0.453$ respectively (Fig. 6). Cholesterol and HDL had no significant correlation with worm burden, $r = -0.270$ (Fig. 5) and $r = -0.359$ (Fig. 6).

Discussion
In this study, the biochemical parameters of non-infected cattle were found to be within the reference ranges for normal sheep (Kramer, 2000). Fascioliasis was found to produce hypoproteinaemia and hypoalbuminaemia. Total serum protein and albumin were significantly lower ($p<0.05$) in infected groups than the control and continued to decrease with increase in worm burden. This agrees with the report of Anderson et al. (1977) who found hypoalbuminaemia in infected cattle. Similarly, Vengust et al. (2003) observed decrease in serum total protein and albumin in fallow deer infected with F. hepatica. According to Thomas (1982), the hypoalbuminaemia may be due to the reduced albumin synthesis caused by liver damage. This produces cholangitis, biliary obstruction, destruction and fibrosis of hepatic tissue and anaemia (Blood et al., 1989). Matanović et al. (2007) mentioned that hypoproteinaemia is due to severe infection of the liver, which produced destruction of liver parenchyma resulting in drastic alteration in protein values.

In the present study, globulin levels were significantly higher ($p<0.05$) in infected groups than the control and increased with increase in worm burden. This result agrees with the report by Amer *et al.* (2002) and Matanović *et al.* (2007). The detected hyperglobulinaemia could be as a result of immune response to infection (Matanović *et al.,* 2007) and due to the increase in α and β globulin production (Duncan *et al.,* 1994).

In the present study, serum urea and creatinine levels were elevated due to *Fasciola* infection in cattle. The infected group showed significantly higher ($p<0.05$) serum urea and creatinine than the control group. This increase in serum urea may be due to the failure of detoxification of ammonia and other nitrogenous substances by cirrhotic liver. Results also showed that glucose level was significantly lower ($p< 0.05$) in the infected cattle than the control group. The hypoglycaemia may be due to the disturbance in gluconeo-genesis, which resulted from hepatic disorder (Soulsby, 1982); elevation of the ketone bodies from gastroenteritis could result in depression in blood glucose (Duncan *et al*., 1994), coupled with the depression of the hepatic glucogenic pathways and decrease in voluntary feed intake by the infected cattle (Phiri *et al.,* 2007).

During the study, cattle with higher worm burden showed significantly lower ($p<0.05$) glucose levels than those with less worm load. This agrees with Phiri *et al.* (2007) who stated that the more *F. gigantica* metacercariae in sheep, the greater the de-

crease in serum glucose levels. The decrease with worm burden could possibly be as a result of increased liver parechymal damages inflicted by higher worm load.

Liver enzymes assay in the present study indicated that serum AST, ALT and ALP levels were significantly elevated ($p< 0.05$) in the infected group. Ferre *et al.* (1995) observed the same elevation in AST of infected cattle which was indicated as a reflection of liver parenchymal damage denoting the migratory phase of infection. Takemoto *et al.* (1977) reported significant higher activities of AST, ALT and ALP in *Fasciolia* infected monkeys than in the control and suggested that the increase in AST and ALT was due to the damaging activities of the migrating immature flukes while the increase in ALP activities indicated penetration of the flukes into the bile ducts. The elevation in serum enzymatic activities may also be attributed to the degenerative changes and cirrhosis of the liver cells and enlargement of gall bladder. Moreover, the cellular changes from parasitism increase the permeability of the hepatic cells and in turn result in the release of the enzymes into the serum. In a similar report, El-Aziz *et al.* (2002) detected significant elevations in the activities of serum AST, ALT and ALP in *Fasciola* infected sheep and Ahmed *et al.* (2006) reported an increase of AST levels 2 weeks post-infection, synchronizing with the migratory phase of juvenile flukes in the liver parenchyma and a significant elevation in serum ALP activity 6 weeks post-infection, which they claimed have reflected changes in liver and bone function. Since ALP is known to be excreted via the bile duct, its elevation may have synchronized with the arrival of the flukes to the bile duct. In all, liver damage is the most important cause of increase in serum enzymes activities in infected animals (Singh *et al.,* 2004). So it is clear that the elevations in serum AST, ALT and ALP activities were sensitive indicators of hepatic cell damage and hepatic dysfunction in fascioliasis (Vengust *et al.,* 2003; Ahmed *et al.,* 2006) and that the hepatic damage was hepatobiliary.

Moreover, in the present study, serum ALT levels were observed to be significantly higher in cattle with higher worm load. AST and ALP have no significant positive correlation with worm burden. This is contrary to the report of *Anderson et al.* (1977) who detected an elevation in AST enzyme activities with increase in infecting doses of *Fasciola* worms in cattle. Doaa *et al.* (2007) observed similar increase in ALT with increase in worm burden. Singh *et al.* 2004 reported that liver damage is the most important cause of the increase in serum ALT activity in an infected sheep. Thus, it is reasonable to assume that ALT activities may reflect the intensity of *Fasciola* infection because the liver tissue is extensively destroyed by the immature liver flukes during their migration to the biliary passages.

Furthermore, this study showed that cholesterol, triglyceride, HDL and LDL have significant decrease in *Fasciola* infected cattle. These findings are in conformity with those in the reports of Ellah *et al.* (2010) and Kozat & Denizhan (2010) in their study of fascioliasis in sheep. **Mbuh and Mbwaye (2005) also found** similar results in *Fasciola gigantica* infestation in goats. The liver has a central role in various aspects of lipid metabolism. It produces bile, which is essential for efficient intestinal fat absorption. Additionally, the liver is not only the major producer of plasma lipoproteins, it is also the major site of clearance for these molecules (Canbay *et al*., 2007). The liver thus has a central metabolic function in various aspects of lipid and lipoprotein metabolism such as uptake, oxidation, and metabolic conversion of free fatty acids, synthesis of cholesterol and phospholipids**,** and formation and secretion of specific lipoproteins (Bauchart *et al*., 1996). In fascioliasis, the migrating flukes cause the death of the hepatocytes and the consequent severe pathology, which could result in the disturbance of lipids and lipoproteins in their serum levels (Latimer *et al*., 2003). The decrease in serum cholesterol ($p<0.01$) and triglyceride ($p<0.01$) levels could have occurred due to decreased synthesis by the liver initiated by the pathologic changes consequent to *Fasciola* infection. The impaired synthesis of cholesterol in the liver could also be the result of insufficient hepatocellular respiration due to hypoxia caused by anaemia in the *Fasciola* infected cattle in the present study. It was reported that the majority of serum cholesterol in ruminants is transported as HDL (Latimer *et al*., 2003). As such, the decrease in HDL and LDL levels, as was observed in this study, could retard cholesterol transport in the body and contribute to its lowered serum level. From these findings, it is reasonable to infer that *Fasciola* infections of cattle cause significant decrease in the serum levels of cholesterol, triglyceride, high density lipoprotein and low density lipoprotein.

In this study too, it was observed that the intensity of worm burden negatively correlates with the levels of serum lipids although the negative correlation was significant only for triglycerides and LDL. This implies that increase in worm burden can result in some decrease in the serum lipids levels, which can equally be due to the greater liver damages caused by the increased worm load.

Summary and Conclusion

In conclusion, the biochemical analyses indicated elevation in the liver enzymes activities, which were seen to be indicators of liver damage. Also hypoalbuminaemia, hypoproteinaemia and hyperglobulinaemia observed in the serum protein levels alteration including hypoglycaemia, increased urea and creatinine levels in the *Fasciola* infected cattle were all diagnostic proofs of liver dysfunction due to the necrotic changes in the hepatocytes caused by the migrating immature flukes. In addition, significant decrease noticed in the levels of serum cholesterol, triglycerides, high density lipoproteins and low density, which occurred due to decreased synthesis by the liver, were initiated by the pathologic changes consequent to *Fasciola* infection and the impaired synthesis of cholesterol could lead to hormonal and nuerological disorders in the infected individual, thereby causing infertility and sterility. These findings in animals could equally be observed in human beings and as such, these biochemical parameters could be deployed as proper diagnostic tools for both human and animal fascioliasis.

Table 1: The Mean Values of Liver Enzymes Activites of *Fasciola* Infected Cattle and the Control (±SD)

Liver Enzymes	Infected	Range	Control	Range
AST (U/L)	101.00 ± 3.268	65 – 120	35.70 ± 3.782	14 – 68
ALT (U/L)	83.99 ± 1.908	56 – 92.4	34.45 ± 2.537	21 – 56
ALP (U/L)	176.40 ± 4.384	103 – 192	111.05 ± 4.289	84 – 153

Table 2: The Mean Values of Serum Proteins of *Fasciola* Infected Cattle and the

Control (±SD)

Serum Proteins	Infected	Range	Control	Range
Total Protein(g/dl)	5.64 ± 0.263	4.0 – 7.9	7.10 ± 0.257	5.0 – 8.4
Albumin (g/dl)	1.52 ± 0.113	1.0 – 2.5	3.01 ± 0.144	2.0 – 4.1
Globulin (g/dl)	4.12 ± 0.218	3.0 – 5.9	4.9 ± 0.192	2.9 – 5.4

Table 3: The Mean Values of Serum Chemical Compounds of *Fasciola* Infected Cattle and the Control (±SD)

Serum Chemical Compounds	Infected	Range	Control	Range
Glucose (mg/dl)	51.12 ± 3.104	30 – 76	85.15 ± 3.326	65 - 120
Creatinine (mg/dl)	2.07 ± 0.296	0.7 - 4.50	0.73 ± 0.031	0.5 - 0.93
Urea (mg/dl)	35.73 ± 1.427	28 - 54.70	17.50 ± 1.029	15 – 26

Table 4: The Mean Values of Serum Lipids of *Fasciola* Infected Cattle and the Control (±SD) Serum Lipids and Worm Load

Serum Lipids	Infected	Range	Control	Range

Cholesterol (mg/dl)	72.60 ± 3.675	50 – 126	133.20±4.130	96 - 161
Triglycerides (mg/dl)	25.40 ± 1.027	19 – 32	42.40 ± 2.191	30 – 58
High Density Lipo-proteins (HDL(mg/dl)	39.85 ± 1.929	22 – 52 .1	63.34 ± 1.600	52 – 73.5
Low Density Lipo-proteins (LDL(mg/dl)	41.91 ± 1.357	33.8 – 56	78.49 ± 4.458	48.5 – 119

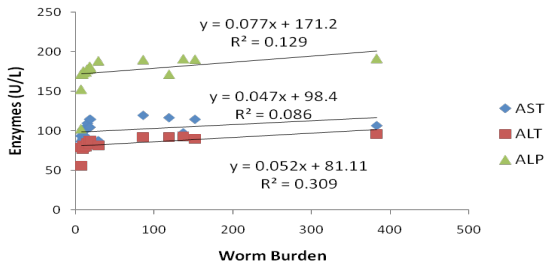

Fig. 1: The Linear Plot of the Liver Enzymes Activities against Worm Burden

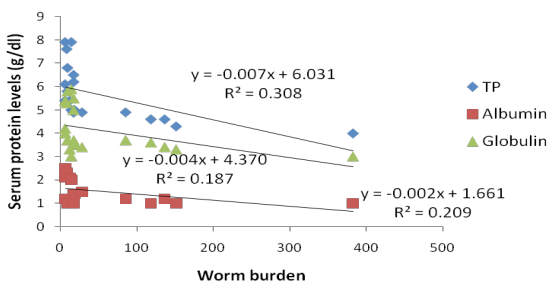

Fig 2: The Linear Plot of Serum Proteins Levels against Worm Burden

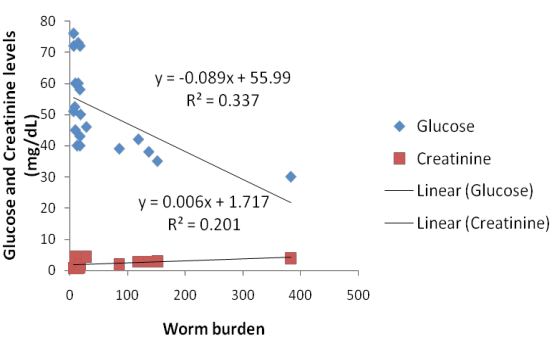

Fig. 3: The Linear plot of Serum Chemical Counpounds against Worm Burden

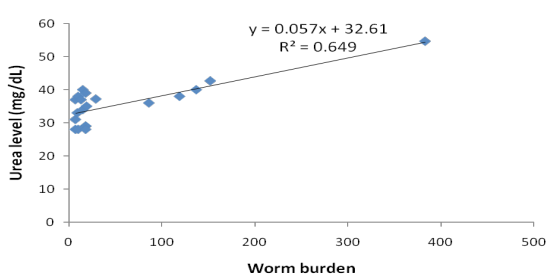

Fig. 4: The Linear plot of Serum Urea Levels against Worm Burden

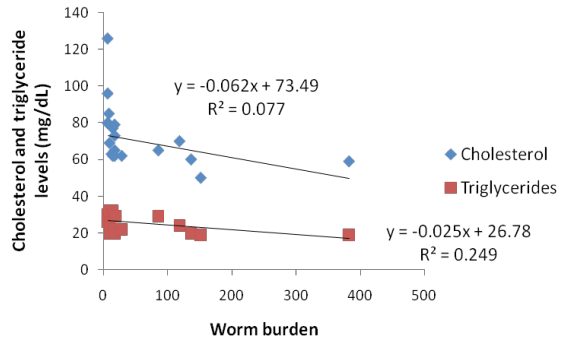

Fig. 5: The Linear Regression of Serum Lipids (Cholesterol and Triglycerides) against Worm Burden.

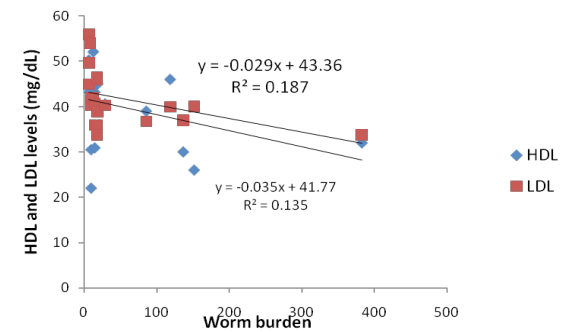

Fig. 6: The Linear Regression of Serum Lipids (HDL and LDL) against Worm Burden

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