# INFLUENCE OF PARASITIC INFESTATION ON SERUM PROTEIN AND SOME ENZYMES PROFILE IN SHEEP

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## *ABSTRACT*

*This study was designed to investigate the different infestation levels of Fasciola on some of the biochemical parameters specially on enzymes and protein using forty three adult male sheep, classified into four groups (control, light, moderate and heavy Fasciola infested) according to the intensity of infestation manifested by the fecal egg count. Liver enzymes AST, GGT, and GDH were increased and very diagnostic at all levels of infestation while ALT only increased in moderate infestation. The isoenzyme, fast liver ALP, liver and bone ALP isoenzymes, $LDH_4$ and $LDH_5$ were increased. This indicative to the liver damage caused by the migrating flukes. Total serum proteins were also decreased at all levels of infestation with a marked decrease in albumin and A/G ratio accompanied by marked increase in γ2 globulin. γ1 was diagnostic in moderate infestation, β1 in light, and heavy groups while b2 was indicative in moderate and heavy infestation.*

## *INTRODUCTION*

Fascioliasis, is primarily a zoonotic disease that produces liver infection with adult flukes. Fecal examination can be used for diagnosed of Fasciola infestation in sheep, but early infestation can not be diagnosed by fecal examination until liver damage associated with the immature migratory stage has occurred and adult flukes are producing ova **(Wyckoff and Bradley, 1985)**. The elevation of certain enzymes may be due to the escape from the disrupted hepatic parenchymal cells or altered membrane permeability e.g. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH) or cholestasis e.g. Alkaline phosphatase (ALP) and γ-glutamyl granspeptidase **(GGT) Coles (1986)**. Fasciolasis is known to produce a variety of biochemical effects in the host, including changes in plasma enzyme activities indicative

of liver injury **(Lopez, et al., 1993 and Perez et al., 2002).**

Isoenzymes of LDH or ALP are useful in diagnosis, because of their variation in properties activities from one tissue to another and determination of the type of isoenzyme presenting the serum in disease, as well as the change in total enzyme activity, can provide valuable additional information **Moss and Butterworth (1974)**. The importance of alkaline phosphates as a diagnostic tool in hepatobiliary disease and in bone disease associated with an increased osteoblastic activity. Different ALP isoenzymes can be introduced into the blood as a result of damage to a number of organs (liver, bone, kidney and intestine). Therefore, an increase in total serum ALP activity is not, itself, a direct reflection of damage to any one organ. For this reason, any attempt to determine the source of an increase in serum ALP activity requires an investigation of its tissue-specific isoenzymes. Because ALP isoenzymes retain their tissue specificity, when released into the circulation, they can be used to identify the tissue contributing the isoenzyme that is responsible for the increase in total serum ALP **(Dumas and Spano 1980 and Osamn et al., 1999)**. In liver diseases, an increase in LDH5 is found, especially acute hepatitis, but also in more chronic conditions such as cirrhosis and obstructive jaundice, even when the total serum LDH activity is within normal range **(Elliot and Wilkinson 1963)**.

GGT is useful clinically in diagnosis of diseases of the liver and biliary tract. Its degree of elevation is only moderate in hepatocellular liver disease, but is marked in obstructive liver disease, GGT helps to determine whether an elevated ALP is coming from bone or liver **Kaplan, et al. (1995) and Calleja et al. (2000)**. Serum Glutamate dehydrogenase (GDH) is a more sensitive indicator of liver necrosis than serum GOT activity **Sewell, (1967)**.

The present work aimed to study the effect of different stages and forms of Fasciola infestation on the pattern of Lactate dehydrogenase and Alkaline phosphatase isoenzymes, which may be used as a method of evaluation of the health status of sheep as well as a diagnosis of infestation. Also, studying the enzymatic changes of some liver enzymes. Protein and its fractionation also have been included.

## *MATERIAL AND METHODS*

A total of 43 male sheep native breed, collected from zagazig and El -Basateen abattoirs, about 20-40 kgm (Body weight) were used in this study. The animals were subjected to pre-slaughtering examination for detection of healthy animals. After slaughtering, blood, fecal and bile samples were collected individually from each animal. Postmortum examination is made on the liver for macroscopic detection of the parasite. Fecal and bile samples were collected from each animal directly from the rectum and the gall bladder in Polly-ethylene nylon sacs. The sam-

ples are transferred directly to the laboratory and examined for detection of fluke eggs according to Kelly (1984) and for determination of the laboratory and examined for according to the method of Coles (1986). The animals are classified into four main groups according to the egg count in both feces and bile:-

Serum samples were used for determination of ALT and AST activities according to the method described by Reitman and Frankel (1957). ALP assayed according to Kind and King (1954), GGT according to **Szasz et al. (1969)** GDH according to **Bergmeyer (1974)** and LDH according to the method recommended by the German clinical chemistry society (1970). ALP isoenzyme was estimated according to the methods described by **Lee and Kenny (1975)** and **Chapman, et. al. (1987)** and LDH isoenzyme by **Plummer (1978)**, Stroev and **Makarova (1989)**. Manchenko (1994). Total protein concentration by the method of **(Richardson 1977)** and protein electrophoresis by the methods of **Smith (1976), Gowenlock, et. al. (1987) and Hames and Rickwood (1990)**.

## *RESULTS*

The study resulted in significant increase in the activity of ALT in moderate infestation group. Significant increases in the activity of AST were observed not only at all degrees of infestation, but also increased according to the intensity of infestation. LDH, GGT, and GDH activities were significantly increased in all groups. ALP activity also increased in the moderate and heavy infestation (Table 1). ALP isoenzymes fraction showed that the fast liver fraction increased with all degrees of infestation; also liver and bone fractions are increased while intestinal fraction did not affected (Table 2). LDH isoenzymes results revealed in a non-significant increase in the activity of $LDH_1$, $LDH_3$ fractions, and $LDH_2$ fraction increased only in moderate and heavy infestation whereas $LDH_4$ and $LDH_5$ fractions were highly significantly increased (Table 3). Decreases in the total protein concentrations in serum of light, moderate, and heavy infested sheep, with decrease in albumin, A/G ratio and increases in globulin (Table 4).

## *DISCUSSION*

The results of ALT in moderate infestation group may be attributed to the hepatocellular damage caused by the migrating liver flukes as previously recorded by **Youssef (1983), Nasser and Abd-Rabo (1994) and Azza (1998)**. On the contrary, **Stromberg, et al. (1985) and Bashandy, et al. (1990)** reported non significant elevation in the enzyme level, because of the very low concentration of ALT in the liver of ruminants, which prevents any appreciable leakage of the

enzyme during disease process.

Significant increases in the activity of AST were observed not only at all degrees of infestation, but also increased according to the intensity of infestation. Similar findings was observed by **Radostitis, et al. (1994) and Ferre, et al. (1995)** in sheep, and also attributed the changes in AST values to the degree of damage of hepatic cells caused by migrating flukes and the penetration of this enzyme to the blood from cytoplasm and hepatocyte mitochondria .changes in the enzyme activity allow to determine the extent of damage occurring in the liver parenchyma, which in turn, depends on the intensity of infection .

GGT activity was significantly increased in all groups as recorded by **Jemli, et al. (1993), Ferre, et al. (1994) and Ferre, et al. (1996)** .

**Galtier, et al. (1986)** related the initial rise of GGT to the penetration of bile ducts by migrating flukes and would not be released into the circulation until bile duct damage occurs. This was also confirmed by **Sykes, et al. (1980), EI-Samani, et al. (1985) and Rowlands, et al. (1985)** they reported an elevated level of serum GGT and considered to be indicative of epithelial damage in bile ducts or cholestasis in sheep, the elevated values have been reported with fascioliasis, particularly in the chronic form when adult flukes are present in the bile ducts.

GDH showed a significant increase in all levels of infestation. **Haroun, et al. (1986)** recorded a significant increase in GDH activity in fasciola infested sheep. **Boyd (1962)** reported that serum GDH level was considered to be a high specific indicator for hepatocellular damage and high concentration of GDH was found in ovine liver. **Ferre, et. al. (1995)** related the increase in GDH level to the inflammatory state of the liver and to tissue destruction provoked by the parenchymal migration of juvenile flukes during the first stage of fascioliasis .

The results explained in table 2 declared a significant increase in ALP activity especially in the moderate and heavy infestation. These results are in accordance with **Degheidy, et al. (1990); Azza (1998); Cosme et al. (2001) and Massoud et al. (2001).** The significant increase was explained by **Kumar, et al. (1983)** which may be caused by the extra-hepatic billiary occlusion by mature flukes which resulted in ALP excretion through bile and its regurgitation into blood stream. In addition, **Abdou, et al. (1976)** attributed this increase to the irritation of liver cells by toxins or metabolic products of the worm and eggs.

ALP isoenzymes fraction showed that the fast liver fraction increased with all degrees of infestation; also liver and bone fractions are increased while intestinal fraction did not affected. **De-Broe (1985)** explained the origin of the fast liver ALP fraction in sera with cholestasis, it origi-

nates from the liver plasma membrane. Bile salts leach the enzyme off the sinusoidal and canalicular membranes. Upon reaching the serum, the membrane bound enzyme aggregates with lipids and lipoproteins. **Collin et al. (1987)** suggested that the measure of intestinal ALP fraction is one method of discriminating intra-hepatic from extra-hepatic jaundice, because intestinal ALP may be absent in extra-hepatic obstruction.

LDH activity in control sheep was 154.87±20.04 u/l which significantly increase in blood of sheep infested with fasciola regardless the degree of infestation light, moderate or severe (243.98±18.45, 236.10±20.43 and 390.06±20.41 u/l respectively. These findings were in agreement with that obtained by Bhattacharya, et. al. (1992) who concluded that LDH activity was significantly increased when young flukes migrating through the liver. On the other hand, the activity was reduced when the flukes enter the bile ducts. **Abdel- Razek, et. al. (1988)** attributed the occurrence of high LDH activity to the alternation in cell membrane permeability which may lead to leakage of the enzyme from the cytoplasm to plasma.

Total LDH activity can not consider as an accurate assay for liver status, and isoenzyme determinations are more costly. Results from table 3 revealed a non-significant increase in the activity of LDH1, LDH3 fractions, and LDH2 fraction increased only in moderate and heavy infestation whereas LDH4 and LDH5 fractions were highly significantly increased. These results are in accordance with **Stroev and Makarova (1989), Meyer, et al. (1992), Kaplan, et al. (1995) and Teitz (1996)** in human.

**Moss and Butterworth (1974)** remarked that LDH5 isoenzyme which occurs in high concentration in tissues such as liver and skeletal muscles that are able to undertake anaerobic metabolism. So when damage to these organs occur, the release of such isoenzyme of LDH into the blood stream is increased. Thus, comparing the serum isoenzyme pattern with that of the suspected tissue can identify the sources of an elevated LDH. **Schaffner and Schaffner (1987)** said that the activity of LDH isoenzymes were not as great as aminotransferases activity in hepatocellular injury. LDH is the predominant isoenzyme in hepatic anoxia and primary liver disease.

The present work manifested decreases in the total protein concentrations in serum of light, moderate and heavy infested sheep, as previously reported by **Degheidy, et al. (1990) Kumar and Sharma (1991) and Azza (1998). Furmaga and Gundlach (1967)** referred the decrease of total serum proteins caused by the liver flukes, which seriously affect the protein metabolism through mechanical and toxic injury of the liver cells and changes in the physiological function of the organ. On the contrary **Kadhim (1976) and Anderson, et al. (1978)** said that the total protein concentrations were increased, whereas **Agnes and Genchi (1977) and Radostitis, et al. (1994)** reported no changes in total protein concentrations. **Kadhim (1976)** discussed the

decrease in total serum proteins caused by the migrating juvenile flukes will depend on the size of inoculum and the duration of infection. This phenomenon will coincide with greatest destruction of cells brought about by the juvenile flukes just before they arrive in the bile duct.

The total albumin value was significantly decreased in all infested groups. **Kadhim, (1976) and Waweru et al. (1999)** thought that the hypoalbuminaemia may be due to two factors, one is the decreased synthesis and the other one is the higher catabolic rate due to the damage to the liver parenchyma caused by the migrating juvenile flukes. In addition, **Ghazy (1987)** stated that albumin is lost into the gastrolntestinal tract via the bile duct as a result of feeding habits of the flukes.

On dealing with the effect of fasciola on A/G ratio, the data revealed a significant decrease in A/G ratio at all level of infestation. These results are in consistence with that mentioned by **Bashandy, et al. (1990) and Azza (1998),** which discussed the obtained results for both albumin and globulins .

The non-significant increases in $\alpha_1$ & $\alpha_2$ fractions at all levels of infestation revealed. While $\beta_1$ was significantly decreased in light and heavy infestation, $\beta_2$ were significantly increased in medium and heavy infestation, while $\beta_3$ were not significantly changed, $\gamma_1$ was significantly increased in medium infestation while $\gamma_2$ was significantly increased at all levels of infestation (Table 4). The investigation of **El- Hawary, et al. (1971)** reported that the presence of parasite could be a stimulatory factor for an increase in the synthesis of gamma globulin which is the main globulin fraction. While **Mulligan (1972)** provided evidence that beta globulin fraction was significantly increased in liver cirrhosis and obstructive jaundice and attributed such elevation to the stimulation of reticulo- endothelial system. **Kadhim (1976)** reported that a1 globulin is not materially affected by the destruction of hepatic cells or by the presence of parasite.

The use of some hepatic marker enzymes GGT and GDH, which are found to be increased during the early migratory stage of Fasciola infestation. Isoenzyme application also is more useful in determining the origin of the elevated enzyme; also protein electrophoresis is useful indicator for the stage of liver damage. All of these tools can be used as a method of evaluation of the health status of the sheep as well as a diagnosis of infestation and the intensity of infestation.

| Groups | No. of sheep | Egg count/gm | Average |
|---|---|---|---|
| **Control** | 10 | ---- | --- |
| **Light infestation** | 11 | 100-200 | 150 |
| **Moderate infestation** | 12 | 200-400 | 300 |
| **Heavy infestation** | 10 | 400-800 | 600 |

**Table (1):** represent the mean activity S.E. of ALT, AST. GGT and GDH enzymes in control, light, moderate and heavy Fasciola infested sheep (U/l) .

| Group | ALT | AST | GGT | GDH |
|---|---|---|---|---|
| **Control** | 22.20± 2.52 | 130.20±23.98 | 28.56± 2.21 | 3.49± 1.35 |
| **Light infestation** | 26.27± 3.08 | 25.509±22.86* | 37.71±2.68* | 15.58±3.48* |
| **Moderate infestation** | 31.67±4.10* | 234.33±28.07* | 36.79±1.63* | 18.20±2.70* |
| **Heavy infestation** | 29.90± 3.52 | 243.20±33.20* | 42.36±1.28* | 17.16±3.53* |

* = Significance

**Table(2):** ALP isoenzymes in control, light, moderate and heavy Fasciola infested sheep (U/l).

| Group | Total ALP | Fast liver | Liver & bone | Intestinal |
|---|---|---|---|---|
| **Control** | 140.02±9.11 | 00.00±0.0 | 96.80±6.42 | 52.22±3.42 |
| **Light infestation** | 206.15±28.21 | 5.28±0.51* | 148.74±21.95 | 52.12±7.05 |
| **Moderate infestation** | 258.38±28.53* | 8.22±1.01* | 178.63±21.01* | 62.53±5.17 |
| **Heavy infestation** | 256.66±24.73* | 13.36±0.72* | 197.74±21.14* | 45.60±4.6 |

* = Significance

Table (3): Discuss LDH isoenzymes in control, light, moderate and heavy fasciola infested sheep (U/l).

| Group | Total LDH | $LDH_1$ | $LDH_2$ | $LDH_3$ | $LDH_4$ | $LDH_5$ |
|---|---|---|---|---|---|---|
| Control | 154.87 ±20.04 | 83.23 ±11.72 | 1.29 ±0.19 | 34.08 ± 5.23 | 6.93 ± 1.49 | 26.64 ±3.69 |
| Light infestation | 243.98 ±18.45* | 81.99 ±6.25 | 2.06 ±0.20 | 43.13 ±3.07 | 24.89 ± 3.83* | 91.88 ± 9.70* |
| Moderate infestation | 236.10 ± 20.43* | 86.50 ± 7.37 | 2.48 ± 0.32* | 47.40 ± 4.57 | 25.42 ± 2.83* | 74.29 ± 7.83* |
| Heavy infestation | 390.06 ± 20.41 | 89.79 ± 4.13 | 2.36 ± 0.42* | 42.85 ± 3.58 | 80.65 ± 5.56* | 174.12 ± 11.26* |

* = Significance

Table (4): Interpret the changes in total proteins, and its electrophoretic pattern control, light, moderate and heavy fasciola infested sheep (gm/l).

| Group | Control | Light infestation | Moderate infestation | Heavy infestation |
|---|---|---|---|---|
| Total proteins | 70.42± 3.07 | 58.15± 2.63* | 62.54± 1.57* | 53.94± 2.35* |
| Albumin | 30.69± 1.30 | 17.98± 0.93* | 17.87± 0.59* | 21.67± 0.36* |
| $\alpha_1$ Globulin | 1.42± 0.010 | 1.26± 0.08 | 1.465± 0.07 | 1.21± 0.08 |
| $\alpha_2$ Globulin | 2.65± 0.16 | 2.67± 0.15 | 2.69± 0.15 | 2.46± 0.15 |
| $\beta_1$ Globulin | 7.44± 0.37 | 6.24± 0.37* | 7.33± 0.19 | 5.98± 0.45* |
| $\beta_2$ Globulin | 0.81± 0.18 | 1.02± 0.10 | 1.48± 0.09* | 1.41± 0.25* |
| $\beta_3$ Globulin | 6.08± 0.41 | 6.65± 0.44 | 9.98± 0.45 | 5.59± 0.27 |
| $\gamma_1$ Globulin | 13.75± 0.53 | 14.84± 0.68 | 16.52±0.40* | 15.84± 0.94 |
| $\gamma_2$ Globulin | 7.40± 0.45 | 9.13± 0.58* | 9.43± 0.37* | 9.10± 0.45* |
| A/G Ratio | 0.78± 0.02 | 0.45± 0.01* | 0.40± 0.01* | 0.32± 0.02* |

* = Significance

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*الملخص العربى*

# تأثير الإصابة بالديدان على بروتينات الدم وبعض الإنزيمات فى الأغنام

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أجريت هذه الدراسة على ثلاثة وأربعون من ذكور الأغنام تم تقسيمها إلى أربعة مجموعات (مجموعة ضابطة ومجموعة بسيطة الإصابة ومجموعة شديدة الإصابة) على أساس عدد البيض فى البراز زيادة إنزيمات الكبد الأسبارتات أمينوتر انسفيراز وجاما جلوتاميل ترانساميناز وجلوتامات ديهيدروچيناز فى كل درجات الإصابة تعتبر من وسائل التشخيص بينما زاد إنزيم الألانين أمينوترانسفيراز فى المجموعة متوسطة الإصابة فقط أشباه الإنزيمات (جزء الكبد السريع وجزء الكبد والعظم لإنزيم الفوسفاتيز القلوى وكذلك الجزء الرابع والخامس من إنزيم اللاكتات ديهيدروچيناز) تدل على تأثر الكبد بالديدان المهاجرة داخل خلاياها. قلة مستوى البروتينيات الكلية فى جميع درجات الإصابة مع قلة مستوى الزلال ونسبة الألبيومين إلى الجلوبيولين.