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Biochemical parameters assessment in goats naturally infected with *Haemonchus contortus*

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Abstract

Haemonchus contortus is considered as main gastrointestinal parasite causing anaemia and hypoproteinemia in ruminants. The study was conducted to study the changes in biochemical parameters and its relationship with faecal egg counts in goats predominantly infected with *Haemonchus contortus*. A total number of 100 goats of either sex were divided into five groups (Group I, II, III, IV and V) on the basis of egg per gram (EPG). Results revealed a highly significant ($P<0.01$) inverse correlation of EPG with glucose, total serum protein, albumin and albumin/globulin (A/G) ratio. Whereas, highly significant ($P<0.01$) positive correlation of EPG with globulin and cholesterol were observed. Mean values of glucose, total protein, albumin and A/G were decreased in groups with high worms load whereas; globulin and cholesterol levels were increased. The mean values of all these parameters were showed highly significant ($P<0.01$) difference between all groups. Highly significant ($P<0.01$) difference in mean values of globulin were observed between the groups except group-I and group-II; and group-IV and group-V. The correlation of EPG with duo of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was positive and highly significant ($P<0.01$). The mean values of ALT and AST were elevated in groups with high worms burden and highly significant ($P<0.01$) difference was observed between the groups.

Keywords: Egg per gram, *Haemonchus contortus*, anaemia, hypoproteinemia, biochemical parameters

Introduction

India possesses 135.17 million goats and the total goat contributes around 26.40% of total livestock population (Livestock census, 2012) ^[10]. Goats have high potential to boost economy of developing countries like India and can be a good source of income for small and large-scale farmers. However, goats are very much prone to various parasitic diseases that are responsible for their poor health conditions, lowering of overall production and ultimately causing heavy losses due to reduced production, morbidity and mortality in animals (Singh *et al.*, 2015) ^[19].

Haemonchus contortus is one of the most prevalent and important gastrointestinal parasite in goats and sheep (Khalafalla *et al.*, 2011) ^[9]. The sub-clinical infections with lower worms load are generally not recognized. These are responsible for economic losses through lower fertility, lower weight gains, reduction in milk and meat production and increased treatment costs in animals (Fikru *et al.*, 2006) ^[6]. Epidemiological knowledge of the parasites present in an area is a prerequisite for the rational designing of the effective preventive and control measures against these dreadful gastrointestinal parasitic diseases (Rajarajan *et al.*, 2017) ^[16].

The aim of the present study was to study the alterations in biochemical parameters and its relationship with faecal egg counts in goats predominantly infected with *Haemonchus contortus*.

Materials and Methods**Selection of animals**

Goats of either sex slaughtered at slaughter house located at Cantonment Board, Mhow were screened and the goats predominantly infected with *Haemonchus contortus*, were taken in our study. Screening of goats was done until 100 numbers of goats were found predominantly positive for *Haemonchus contortus*.

Ethical approval

The present investigation was carried out with the approval of Institutional Animal Ethical Committee, College of Veterinary Science and Animal Husbandry, Mhow, Madhya Pradesh.

Collection of blood

Five ml of blood sample was collected aseptically from goats during slaughter in the blood collecting vial without anticoagulant for separation of serum from the blood. These tubes were kept in ice box for easy clotting of blood and were centrifuged at 3000 rpm for 10 minutes. The separated serum samples were stored at -20°C until biochemical analysis.

Methodology

Biochemical parameters viz. glucose (mg/dl), serum total protein (g/dl), albumin (g/dl), albumin / globulin (A/G) ratio, Cholesterol (mg/dl), alanine aminotransferase (ALT) (IU/L) and aspartate aminotransferase (AST) (IU/L) and were estimated using commercially available kits in a semi-automated biochemical analyzer (Erba Mannheim EM-200, Make- Transasia).

Collection of faecal sample

Approximately 5 g of faecal sample was collected directly from rectum of each animal in labeled polythene bag.

Examination of faecal sample

A. Qualitative examination

Floation method

Modified Sheather's Sugar floatation technique as described by Soulsby (1982) [22] was used for the detection of nematode eggs.

A sample of 2-3 gram faeces was taken in a mortar and mixed with 15-20 ml water to make a homogenous suspension and strained through a single mesh ordinary nylon tea strainer to remove coarse material. The suspension was then obtained and transferred to a 15 ml centrifuge tube. The mixture was centrifuged for 5 min. at speed of 1500-2000 rpm. The supernatant was carefully discarded and sediment was mixed with saturated solution of sugar in centrifuge tube and centrifuged for 3-5 min at 1500-2000 rpm. The eggs concentrated at the surface were removed by touching the surface with the end of a square cut glass rod and transferred on a slide. Then the slide was covered with cover slip and examined under microscope.

B. Quantitative examination

Mc-Master's counting technique

Mc-Master's counting technique was used for determining egg per gram of faeces. A suspension of faeces was prepared by homogenizing 3 gram faeces with 42 ml of water with the help of pestle and mortar. Suspension was divided in three equal parts (14 ml each) in 3 centrifuge tubes and centrifuged at 2000 rpm for 2 min. Supernatant was removed and sodium salt solution was added before straining. Then pipette 0.15 ml mixture (salt and fecal material) was taken on Mc-Master's slide, covered with cover slip and viewed under microscope. EPG of sample was calculated by multiplying 100 with number of eggs counted (Sloss *et al.*, 1994) [20].

Copro-culture for recovery of strongyle larvae

The faecal samples positive for strongyle eggs were pooled and subjected to copro-culture in separate glass tumbler

(capacity 300 ml) to harvest the infective stage larvae (L_3) (Roberts and Sullivan, 1949) [17].

About 75-100 grams of strongyle positive faecal mass was mixed with activated charcoal (3:1 ratio) and sufficient water was added to obtain a pasty consistency, and placed in a glass tumbler (capacity 300 ml). Mouth of the tumbler was covered with aluminum foil after cleaning its inner margin and was incubated at 25 to 28°C for 7 days. The culture tumbler was checked daily for optimum wetness in the sample. On 8th day, lukewarm water was filled in the tumbler till a convex surface was formed at the brim. A glass Petridish was placed on to the mouth of the tumbler with precaution that no air bubbles should be trapped within. The whole preparation was then so inverted that the tumbler stood in the Petridish and kept under artificial light in little slanting position. After 4-6 hrs, water in the Petridish was withdrawn and centrifuged at 1000 rpm for 2 min. Supernatant was discarded and hot formaldehyde (10%) was added to the sediment containing larvae so as to preserve them stretched.

Results and Discussion

A total number of 100 goats predominantly infected with *Haemonchus contortus* were divided into five groups (Group I, II, III, IV and V) on the basis of egg per gram (EPG) and subjected to the statistical analysis employing Pearson correlation analysis between EPG and biochemical parameters; and analysis of variance (ANOVA) employing completely randomized design (CRD) (Snedecor and Cochran, 1994) [21].

Grouping of goats

Faecal samples of goats were collected from slaughter house situated at Cantonment Board, Mhow and were checked for the presence of *Haemonchus contortus* infection. A total of 100 goats predominantly infected with *H. contortus* were selected for this study.

Animals were divided into five groups on the basis of egg per gram (EPG) as indicated in Table 01.

Table 1: Grouping of goats on the basis of egg per gram (EPG)

Groups	Egg per gram	Number of goats
Group-I	100-300	11
Group-II	400-600	20
Group-III	700-1000	50
Group-IV	1100-1200	10
Group-V	1300 and above	09

Biochemical parameters

The mean values of the serum glucose in group-I, group-II, group-III, group-IV and group-V were 64.30 ± 0.69 , 58.00 ± 0.40 , 51.80 ± 0.38 , 44.30 ± 0.88 and 36.50 ± 1.00 mg/dl, respectively (Table 02). There was a decline in glucose level in groups having high worms load and the difference was statistically highly significant ($P < 0.01$) between the all groups. Correlation between EPG and glucose was -0.989 which was inversely correlated and was highly significant ($P < 0.01$) (Table 03). These results may be correlated with reduced food intake and poor absorption of the dietary constituents due to gastrointestinal disturbances caused by parasitic infections (Hayat *et al.*, 1999) [8]. Murad *et al.* (2018) [12] reported mild changes in glucose level but not considered significant throughout the experiment.

In the present study, mean values of total serum protein in group-I, group-II, group-III, group-IV and group-V were

7.40±0.03, 7.20±0.02, 6.90±0.03, 6.70±0.03 and 6.20±0.03 g/dl, respectively (Table 02). The lower values were observed in groups of infected animal with high worms load and the differences were highly significant ($P<0.01$) between all groups. Decline in the total serum proteins may be attributed to haemodilution, which is a compensatory mechanism for the abomasal haemorrhage caused by the parasite. In this study hypoproteinemia and hypoalbuminemia were observed in goat infected by *H. contortus*, because these parasites may stimulate the proliferation of intestinal epithelial cells and replacement of abomasal acid-producing cells by immature cells. This leads to the loss of large quantities of serum protein into the gut. Furthermore, due to abomasal haemorrhage haemodilution occurs which can cause relative hypoproteinemia and hypoalbuminemia (Angulo-Cubillán *et al.*, 2007) [2].

The mean values of albumin in group-I, group-II, group-III, group-IV and group-V were 4.10±0.03, 3.80±0.02, 3.20±0.03, 2.60±0.03 and 2.10±0.07 g/dl, respectively (Table 02). The lower mean values were reported in groups of infected animal with high worms burden and revealed a highly significant ($P<0.01$) difference between the all groups. Decline of albumin level in *H. contortus* infected animals may affect the intravascular osmotic pressure (Nicholson *et al.*, 2000) [13]. Hypoproteinemia with decreased levels of total serum protein and serum albumin is an important consequence of haemonchosis which is responsible for protein losing enteropathy (Soulsby, 1982) [22].

The mean values of globulin in group-I, group-II, group-III, group-IV and group-V were 3.30±0.06, 3.40±0.00, 3.70±0.03, 4.10±0.03 and 4.10±0.03 g/dl, respectively (Table 02). These values were increased in groups having high worms level and statistically highly significant ($P<0.01$) difference was observed between the groups except group-I and group-II; and group-IV and group-V. Similarly high level of globulin was reported in infected animals by Diogenes *et al.* (2010) [5] for *H. contortus* infection in goats. The increased level of globulin could be correlated with the increased production of acute phase protein due to tissue injury and inflammation caused by the parasite (Petersen *et al.*, 2004; Lomborg *et al.*,

2008) [14, 11]. Ahmad *et al.* (1990) [1] reported significant increase in gamma globulin, a fraction of globulin in *H. contortus* infected animals.

The mean values of albumin/globulin (A/G) ratio in group-I, group-II, group-III, group-IV and group-V were 1.20±0.03, 1.10±0.00, 0.90±0.01, 0.60±0.00 and 0.50±0.03, respectively (Table 02). These values were declined in groups having high worms level and a highly significant ($P<0.01$) difference was reported between all groups. Alteration in albumin and globulin levels in infected sera caused the reduction of A/G ratio as also reported by Hosseini *et al.* (2012) [7] and Qamar and Maqbool (2012) [15]. Similar reports of decline in serum protein and albumin, and increase in serum globulin level were observed by Bordoloi *et al.* (2012) [3].

The correlation of EPG with total serum protein and albumin was -0.939 and -0.983, respectively (Table 03). These correlations were negative and highly significant ($P<0.01$). The correlation between EPG and globulin was 0.890 (Table 03). Which was highly significant ($P<0.01$) and positive. Highly significant ($P<0.01$) inverse correlation was observed between EPG and Albumin/Globulin ratio and it was -0.974 (Table 03).

The mean values of cholesterol in group-I, group-II, group-III, group-IV and group-V were 134.90±1.09, 145.90±0.69, 156.10±0.51, 166.20±1.04 and 174.90±1.07 mg/dl, respectively (Table 02). The mean values of cholesterol were increased in groups having high worms load and the difference was highly significant ($P<0.01$) between all the groups. The correlation between EPG and cholesterol was 0.992. This correlation was positive and highly significant ($P<0.01$) (Table 03). The significantly higher concentrations of cholesterol in infected animals may be attributed to the parasitic stress resulting in increase of epinephrine and corticosteroids output. The elevated level of cholesterol in infected animals also reflects negative energy balance created by heavy load of parasitic burden, leading to enhanced lipolysis. The elevation of serum cholesterol has also been reported in buffaloes infected with *Toxocara vitulorum* (Hayat *et al.*, 1999) [8].

Table 2: Mean values of biochemical parameters in different groups

Groups	Glucose (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G Ratio	Cholesterol (mg/dl)
Group-I	64.30±0.69 ^a	7.40±0.03 ^a	4.10±0.03 ^a	3.30±0.06 ^c	1.20±0.03 ^a	134.90±1.09 ^e
Group-II	58.00±0.40 ^b	7.20±0.02 ^b	3.80±0.02 ^b	3.40±0.00 ^c	1.10±0.00 ^b	145.90±0.69 ^d
Group-III	51.80±0.38 ^c	6.90±0.03 ^c	3.20±0.03 ^c	3.70±0.03 ^b	0.90±0.01 ^c	156.10±0.51 ^c
Group-IV	44.30±0.88 ^d	6.70±0.03 ^d	2.60±0.03 ^d	4.10±0.03 ^a	0.60±0.00 ^d	166.20±1.04 ^b
Group-V	36.50±1.00 ^e	6.20±0.03 ^e	2.10±0.07 ^e	4.10±0.03 ^a	0.50±0.03 ^e	174.90±1.07 ^a
	**	**	**	**	**	**

**Highly significant ($P<0.01$)

Mean bearing different superscript within column differ significantly

Table 3: Correlation of egg per gram (EPG) with biochemical parameters (n=100)

		Glucose (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G ratio	Cholesterol (mg/dl)
Egg per gram	Pearson correlation	-0.989**	-0.939**	-0.983**	0.890**	-0.974**	0.992**

**Correlation is highly significant at the 0.01 level ($P<0.01$)

The mean values of ALT in group-I, group-II, group-III, group-IV and group-V were 12.80±0.78, 17.30±0.34, 21.70±0.25, 29.10±0.79 and 37.00±1.03 IU/L, respectively (Table 04) and mean values of AST in group-I, group-II, group-III, group-IV and group-V were 70.10±2.26, 89.20±4.36, 117.80±1.33, 148.90±3.73 and 176.30±2.73 IU/L, respectively (Table 04). The mean values of ALT and

AST were elevated in groups having high worms load and highly significant ($P<0.01$) difference was observed between all the groups for mean values of ALT and AST. The correlation of EPG with ALT and AST were 0.970 and 0.942, respectively (Table 05). These correlations were positive and highly significant ($P<0.01$). Rise in enzymatic levels in haemonchosis may be attributed to the damage to abomasal

mucosa by these parasites similar to that described by Charleston (1965) [4] who reported deep invasion of muscular layer of abomasal mucosa in sheep by *H. contortus* larvae. The elevation of ALT and AST level indicates some disruptive activities or of altered membrane permeability (Sharma *et al.*, 2001) [18]. Significant increases in the activity of these serum enzymes in *H. contortus* infected goats were observed in the present study. Similar findings were also reported by Bordoloi *et al.* (2012) [3] and Sharma *et al.*

(2001)[18] in sheep infected with *H. contortus*.

In conclusion, the *Haemonchus contortus* infection in goats is responsible for hypoproteinaemia and hypoalbuminemia, decreased glucose levels, increased levels of cholesterol. Increased levels of alanine aminotransferase and aspartate aminotransferase enzymes in groups with higher worms load also suggesting of its adverse effect on the liver. Treatment should be recommended to goats which come under group-II and above.

Table 4: Mean values of enzymes of protein metabolism in different groups

Groups	Alanine aminotransferase (ALT) (IU/L)	Aspartate aminotransferase (AST) (IU/L)
Group-I	12.80±0.78 ^c	70.10±2.26 ^e
Group-II	17.30±0.34 ^d	89.20±4.36 ^d
Group-III	21.70±0.25 ^c	117.80±1.33 ^c
Group-IV	29.10±0.79 ^b	148.90±3.73 ^b
Group-V	37.00±1.03 ^a	176.30±2.73 ^a
	**	**

**Highly significant ($P<0.01$)

Mean bearing different superscript within column differ significantly

Table 5: Correlation of egg per gram (EPG) with enzymes of protein metabolism (n=100)

		Alanine aminotransferase (ALT) (IU/L)	Aspartate aminotransferase (AST) (IU/L)
Egg per gram	Pearson correlation	0.970**	0.942**

**Correlation is significant at the 0.01 level ($P<0.01$)

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