Indian J Anim Health (2025), 64(1) - Special Issue: 131-139
DOI: https://doi.org/10.36062/ijah.2025.15124

Research Article

Comparative parasitological and haemato-biochemical responses between resistant and susceptible Garole sheep infected with *Haemonchus contortus*

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Abstract

Parasitological and haemato-biochemical response of experimental *Haemonchus contortus* infection was compared between the resistant and susceptible lines of Garole sheep. Nine resistant and susceptible Garole sheep (female sheep of 16 to 18 months old) were selected out of screening of 60 animals on the basis of faecal egg count (FEC). Garole sheep (n=18) were divided into three groups; infected resistant (n=6), infected susceptible (n=6) and uninfected control (n=6). Sheep of infected groups were orally fed with infective larvae (L_3) of *H. contortus*, and FEC was recorded from 18 to 40-day post infection (DPI). Haemato-biochemical and clinical observations were monitored from 0 DPI to 40 DPI at 10 days interval. In resistant sheep FEC was significantly (P <0.01) lower than the susceptible group during the study period with spontaneous elimination of adult worms from 33 to 40 DPI. Haematological values (Hb -11.28 g/dL, PCV - 33.28%, TLC - 16.25x10^3/cmm, eosinophils - 1350.33/cmm, lymphocytes - 8.38x10^3/cmm and neutrophils - 6.28 x10^3/cmm) was significantly (P<0.05) higher in resistant group than the susceptible group (Hb - 9.50 g/dL, PCV - 27.25%, TLC - 9.22x10^3/cmm, eosinophils - 619.41/cmm, lymphocytes - 5.24 x10^3/cmm and neutrophils - 3.07 x10^3/cmm) on 30 DPI. Significant (P <0.01) decline in total protein and albumin levels was more pronounced in infected susceptible sheep (total protein - 4.94 g/dL, albumin - 2.55 g/dL) compared to resistant group (total protein - 6.36 g/dL, albumin - 3.29 g/dL) on 30 and 40 DPI. Anaemia developed in susceptible sheep on 20 DPI to 40 DPI and body condition was deteriorating from healthy condition with the progress of haemonchosis. Resistant sheep was able to withstand the pathogenic effect of *H. contortus* infection in terms of haemato-biochemical and clinical observations.

Keywords: Garole sheep, Haemato-biochemical parameters, *Haemonchus contortus*, Host resistance

Highlights

- Significantly low FEC was recorded in resistant Garole sheep
- Spontaneous elimination of *H. contortus* infection from resistant sheep
- Significantly higher Haemato-biochemical values in resistant sheep
- Different types of leucocytes were higher in resistant sheep
- Resistant sheep with haemonchosis were clinically fit

INTRODUCTION

Gastrointestinal nematode (GINs) infections are causing severe production losses in small ruminants including sheep and goat production worldwide (Jas and Ghosh, 2009). Over dependence on anthelmintics in recent years has led to the emergence of resistance in nematode population against available commercial anthelmintics (McRae *et al.*, 2014). Therefore, alternative control approach to manage anthelmintic resistance in worm population is the need of the hour. Use of genetically resistant animals against *H. contortus* selected on the basis of phenotypic markers (*viz.*, parasitological, haematological, biochemical, and immunological parameters) in breeding programs will have significant effect on infection epidemiology and re-infection rate (Saddiqi

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et al., 2012). Resistance to internal parasites can be assessed by observing how infected sheep respond to either experimental (Albers *et al*., 1987) or natural parasite challenges (Bisset *et al*., 1992). Various traits can be utilized to measure the resistance and resilience to GI parasites (Bishop, 2012). Faecal egg count (FEC) can effectively determine the resistance or susceptibility level in different sheep breeds, both within and between breeds (Eady *et al*., 1996). Haematocrit value is also a valuable marker when blood-sucking parasites like *H. contortus* are prevalent (Gauly and Erhardt, 2001). Total plasma protein and albumin levels (g/dL) may be other important determinants for the evaluation of resistance status as the GI tract infections with *H. contortus* can lead to hypoproteinaemia (Amarante *et al*., 1999, Bricarello *et al*., 2004). Haematological and serum-biochemical analyses are important and reliable indicators for assessing an animal's health status and assessment of the severity of damage to host caused by parasite (Otesile *et al*., 1991). The present study was designed to compare various haemato-biochemical and clinical parameters in Garole sheep experimentally infected with *Haemonchus contortus* for determining the resistant and susceptibility status of the animals.

MATERIALS AND METHODS

Selection and maintenance of experimental sheep: Sixty Garole sheep in the age group of 4 to 6 months and of both sexes were selected from the two villages under the block Kultali in the South 24 Parganas district of West Bengal. Per-rectal faecal samples were collected from all the selected sheep and FEC in terms of eggs per gram of faeces (EPG) were measured by modified McMaster technique (Soulsby, 1982) at monthly interval for 12 months. Garole sheep showing persistently low FEC (mean EPG<150) were considered as resistant and Garole sheep showing variable FEC (mean EPG>500) were considered as susceptible. Finally, nine (9) female Garole showing low EPG and another nine (9) female sheep showing comparatively higher mean EPG were selected for experimental studies. In the present study, only female sheep were selected for artificial infection to avoid the effect of sex on resistance and susceptible status of animals.

The selected sheep were maintained through intensive system of management and dewormed before giving the experimental infection. Experimental animals were divided into 3 groups; infected resistant (n=6); infected susceptible (n=6) and uninfected control [n=6 (resistant sheep = 3, susceptible sheep = 3)].

Experimental infection of Garole sheep with H. contortus: Adult female *H. contortus* worms were collected from the abomasums of slaughtered sheep. Eggs were separated from the worms and cultured following the standard procedure using sterile sheep faecal powder (Soulsby, 1982). After 7 days, infective third-stage (L_3) larvae were harvested from the coproculture and stored in distilled water at 4°C for further use. Faecal samples of all the experimental sheep were examined two times at three days interval to ascertain the infection free status of animals and the body weight of individual animals was also recorded. The experimental animals of both groups (resistant and susceptible group) were orally fed with infective larvae of *H. contortus* @ 500 L_3 per kg b. wt after withdrawing the feed and water to the animals.

Parasitological parameters: Following *H. contortus* infection in the experimental animals, the first day of eggs appeared in the faeces of the infected animals, was determined as prepatent period of infection. Faecal samples were regularly examined after 2 weeks of infection to determine the prepatent period in the infected group.

After development of the experimental infection, modified McMaster technique was used to measure FEC in terms of eggs per gram (EPG) of faeces as described by Soulsby (1982) in different days post infection (DPI); (18, 21, 24, 27, 30, 33, 35 and 40).

Haematological parameters: Haemoglobin (Hb), packed cell volume (PCV), total leucocyte count (TLC) and differential leukocytes counts (DLC) were estimated in sheep of all the three experimental groups from 0 to 40 DPI at 10 days interval. Haemoglobin (Hb; g/dL) concentration was estimated by cyanomethanoglobulin method (Jain, 1993) and PCV (%) was estimated by capillary tube micro centrifuge method, and TLC value (x 10^3/mm^3) was estimated using haemocytometer according to the method described by Jain (1993). Differential leukocytes count (DLC) were estimated after counting first 100 different type leukocytes blood cell randomly from Leishman's stained blood smear and each type of cells represent the percentage of that cell type, then TLC value was multiplied by the percentage of cells to determine different types of cells and value express as number of cells per cubic millimeter (Jain, 1993).

Biochemical parameters: Plasma protein and albumin concentration were studied on 0, 10, 20, 30 and 40 DPI using commercially available kits (Autospan, Arkray

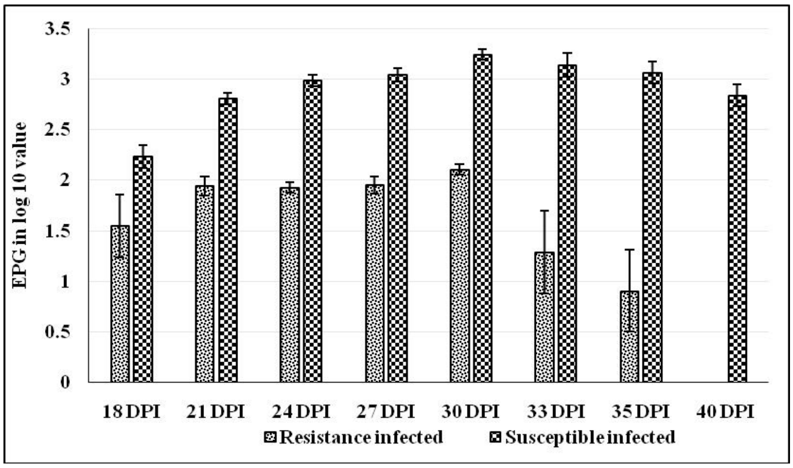

Fig. 1. Faecal egg count on different days post infection in resistant and susceptible Garole sheep experimentally infected with *Haemonchus contortus*

Health care, India) as per the manufacturer's instructions, and value of plasma protein and albumin were expressed as g/dL. Globulin concentration was indirectly measured by subtracting albumin value from total protein value in this study.

Clinical observation: Clinical observations of haemonchosis like anemia studied by FAMACHA score (Kaplan *et al.*, 2004) and body condition score (Phythian *et al.*, 2012) and bottle jaw condition were examined, in experimental animal.

Statistical analysis: To obtain the value of standard error (S.E.) along with mean value all the parameters for each group on different post infection days were compared by Analyse-Compare means. The significance (P-value) was recorded at 5% (P<0.05) level and 1% (P<0.01) level by separate analysis of parameters (between post-infection days) using the Duncan method (One-way- ANOVA). The complete statistical analyses were done with the help of Statistical Package for Social Scientist (SPSS), Windows Version 20.0.

RESULTS

Prepatent period and FEC: Following experimental infection with *H. contortus* in resistant Garole sheep, the first egg in the faeces was detected on 16 DPI (in one animal), 17 DPI (in two animals), and 18 DPI (in three animals). So, the mean prepatent period of infection in the resistant animal was 17.33 days. Whereas in susceptible Garole sheep, it was 16 DPI (in three animals), 17 DPI (in two animals), and 18 DPI (in one animal). Thus, the mean prepatent period of the infection in susceptible sheep was 16.66 days. However, the difference in prepatent period between the resistant and susceptible animals was not significant.

From the mean faecal egg count (FEC) between 18 to 40 DPI, it was found that EPG of infected susceptible sheep was significantly (P<0.001) higher than the infected resistant animals. No faecal eggs were detected in two infected resistant sheep after 33 DPI in their faecal samples, and in one sheep after 35 DPI, and finally after 40 DPI rest of the infected resistant sheep became free of infection. However, all the infected susceptible Garole sheep showed variable number of FEC during the study period (Fig.1).

Haematological changes: No significant difference in Hb concentration was recorded between infected resistant sheep and the uninfected control during the entire study period (Table 1). However, in infected susceptible Garole sheep, the Hb concentration reduced significantly (P<0.01) from 20 DPI onwards till the end of the study as compared to the infected resistant sheep as well as the control sheep.

Packed cell volume was found to be reduced in both the infected groups of sheep. In resistant group, packed cell value (PCV) reduced to non-significant (P>0.05) level compared to the uninfected control group during the study period. On the other hand, the PCV value of infected susceptible sheep declined significantly (P<0.05) from 20 DPI onward still the end of the study, compared to the resistant and control group (Table 1). In infected susceptible Garole sheep, PCV value reduced significantly (P<0.05) from 10 DPI onwards till the end of the study than the pre-infection value (0 DPI value) but in the resistant group significant reduction (P<0.05) was observed only on 20 DPI compared to the 0 DPI's value.

In resistant Garole sheep infected with *H. contortus*, TLC value increased significantly (P<0.01) from 10 DPI to 30 DPI compared to 0 DPI's value (Table 1). Whereas, in infected susceptible sheep, the TLC value increased significantly (P< 0.01) from 10 DPI to 20 DPI compared to the pre-infection value. Significant (P<0.05) variation in TLC was recorded between the uninfected control sheep and infected resistant and susceptible Garole sheep during the study period. In infected resistant sheep TLC value was significantly (P < 0.01) higher than the uninfected control sheep from 10 to 30 DPI, and from 30 to 40 DPI compared to the infected susceptible Garole sheep, which had

Table 1. Haematological changes due to *Haemonchus contortus* infection among the different experimental groups of Garole sheep

Variables	DPI	Control	Infected Resistant	Infected Susceptible	P-value
Hb (gm/dL)	0	12.38±0.33	12.57±0.56	12.35^{a} ±0.21	0.914
	10	12.13±0.37	12.18±0.61	11.51^{b} ±0.30	0.521
	20	12.10^{x} ±0.38	11.79^{x} ±0.54	10.37^{yc}±0.13	0.016
	30	12.30^{x} ±0.38	11.28^{x} ±0.50	9.50^{yd}±0.21	0.000
	40	12.25^{x}±0.34	11.48^{x}±0.43	8.65^{ye}±0.23	0.000
	P-value	0.975	0.455	0.000	
PCV (%)	0	34.25±0.40	34.20^{a} ± 0.16	34.25^{a} ±0.40	0.993
	10	33.76^{x} ±0.29	33.85^{xab}±0.16	32.88^{ya}±0.21	0.018
	20	34.01^{x}±0.15	33.36^{xb}±0.16	30.01^{yb} ±0.80	0.000
	30	34.00^{x}±0.18	33.28^{xb}±0.16	27.25^{yc} ±0.48	0.000
	40	34.15^{y}±0.17	33.36^{yb}±0.31	26.96^{xc}±0.63	0.000
	P-value	0.473	0.015	0.000	
TTLC (10^{3}/mm^{3})	0	8.96±0.09	9.15^{d}±0.21	8.66^{c}±0.22	0.194
	10	9.32^{y}±0.07	13.33^{xb}±0.53	13.99^{xa}±0.65	0.000
	20	9.14^{y}±0.25	10.77^{xc}±0.35	10.22^{xb}±0.15	0.002
	30	9.01^{y} ±0.22	16.25^{xa}±0.73	9.22^{ybc}±0.14	0.000
	40	9.12^{xy}±0.16	9.66^{xcd}±0.49	8.55^{yc}±0.29	0.110
	P-value	0.647	0.000	0.000	N = 6

Values bearing superscripts; x, y… letters in a row within the DPI differs significantly (P<0.05) among the different groups. Values bearing superscripts; a, b, c…. in a column within a group differs significantly (P< 0.05) among different DPIs.

significantly (P<0.01) higher TLC only on 10 DPI compared to control sheep.

Significant effect of experimental haemonchosis was observed on different types of leukocytes except basophil in the infected sheep (Table 2). Eosinophils count was found to be significantly (P<0.05) higher in infected resistant sheep compared to the pre-infection value as well as uninfected control sheep during the different post-infection days and on 30 and 40 DPI compared to infected susceptible sheep. In susceptible sheep eosinophils number increased significantly (P<0.01) only on 10 and 20 DPI compared to the pre-infection value. Lymphocytes counts were significantly (P <0.01) higher in infected resistant sheep on 10 and 30 DPI compared to uninfected control sheep as well as pre-infection value, and only on 30 DPI compared to the infected susceptible group. Lymphocytes numbers increased significantly (P<0.01) from 10 to 30 DPI compared to the pre-infection value in infected susceptible sheep (Table 2). Changes in neutrophils

Table 2. Changes in different types of leucocytes count due to *Haemonchus contortus* infection among the different experimental groups of Garole sheep

Variables	DPI	Control	Infected Resistant	Infected Susceptible	P-value
Eosinophils (per mm^{3})	0	447.66 ±22.06	442.08^{b} ±17.72	432.16^{c} ±21.36	0.865
	10	465.83^{y} ±3.57	805.25^{xb} ±114.82	935.08^{xa} ±121.90	0.010
	20	422.16^{y} ±11.19	789.83^{xb} ±151.27	765.91^{xyab}±131.02	0.072
	30	466.33^{y} ±31.30	1350.33^{xa} ±212.09	619.41^{ybc} ±117.75	0.001
	40	454.33^{y} ±29.99	738.00^{xb} ±129.27	403.33^{yc} ±82.65	0.041
	P-value	0.630	0.003	0.005	
Lymphocytes (10^{3}/mm^{3})	0	4.58^{x}±0.11	4.83^{xc}±0.19	4.03^{yc}±0.14	0.006
	10	4.72^{y}±0.09	6.61^{xb}±0.53	7.45^{xa} ±0.51	0.001
	20	4.65±0.20	5.15^{c} ±0.32	5.36^{b}±0.13	0.122
	30	4.52^{y}±0.15	8.38^{xa}±0.36	5.24^{yb}±0.26	0.000
	40	4.58±0.13	4.97^{c}±0.38	4.79^{bc}±0.18	0.585
	P-value	0.893	0.000	0.000	

Cont. Table 2.

Table 2., Cont. ...

Variables	DPI	Control	Infected Resistant	Infected Susceptible	P-value
Neutrophils	0	3.60±0.83	3.56^{c}±0.10	3.77^{b}±0.11	0.329
(10^3/mm^3)	10	3.81^{y}±0.15	5.65^{xab}±0.31	5.08^{xa}±0.12	0.000
	20	3.94^{xy}±0.17	4.55^{xbc}±0.19	3.79^{yb}±0. 24	0.049
	30	3.86^{y}±0.16	6.28^{xa} 0.81	3.07^{yc}±0.27	0.001
	40	3.84^{x}±0.19	3.74^{xc}±0.13	3.07^{yc}±0.21	0.019
	P-value	0.644	0.000	0.000	
Monocytes	0	268.75±2.80	288.16^{b}±8.34	286.50^{b} ±26.49	0.649
(per mm^3)	10	279.50^{y}±2.14	466.83^{xa}±34.82	463.91^{xa} ±30.52	0.000
	20	274.25±7.58	251.83^{b}±25.13	238.50^{bc} ±21.58	0.448
	30	252.75^{xy}±23.05	317.75^{xb}±33.39	215.08^{ybc} ±19.24	0.040
	40	273.08±33.68	274.50^{b}±23.36	212.33^{c}±16.92	0.181
	P-value	0.875	0.000	0.000	
Basophils	0	45.00±20.12	60.83±19.34	41.58±18.62	0.759
(per mm^3)	10	30.83±19.50	65.25±29.58	33.66±21.30	0.541
	20	28.83±18.25	51.41±23.09	44.91±20.09	0.732
	30	28.33±18.25	56.08±35.91	15.33±15.33	0.514
	40	30.33±19.22	43.33±19.47	42.08±18.88	0.871
	P-value	0.970	0.980	0.805	N = 6

Values bearing superscripts; x, y… letters in a row within the DPI differs significantly (P<0.05) among the different groups. Values bearing superscripts; a, b, c…. in a column within a group differs significantly (P<0.05) among different DPIs.

number did not show any regular pattern in the infected groups of animals. Neutrophil counts were found to be significantly (P <0.01) higher in infected resistant sheep from 20 DPI to 40 DP compared to susceptible sheep and on 10 and 30 DPI compared to uninfected control sheep.

Monocyte numbers were found to be increased significantly (P <0.001) in both infected resistant and susceptible sheep after *H. contortus* infection only on 10 DPI compared to control sheep. On 30 DPI infected resistant sheep had significantly (P<0.05) higher numbers of monocytes than the infected susceptible sheep. Basophil numbers did not differ significantly (P>0.05) among the different groups of animals.

Biochemical changes: Total protein and serum albumin concentration were reduced in both the infected groups as compared to the uninfected control sheep from 20 to 40 DPI (Table 3). In infected susceptible sheep, total protein and albumin concentration was significantly (P<0.001) lower than the infected resistant sheep on 30 and 40 DPI (Table 3). Globulin concentration reduced significantly (P<0.001) only on 20 and 30 DPI in resistant group, and from 20 to 40 DPI in infected susceptible sheep compared uninfected control sheep. It was interesting to note that there was no significant difference in total protein and serum globulin concentration in the infected resistant sheep during the entire study period.

Clinical changes: Anaemia is an important clinical sign of haemonchosis and it was studied in infected

Table 3. Changes in protein concentration due to *Haemonchus contortus* infection among the different experimental groups of Garole sheep

Variables	DPI	Control	Infected Resistant	Infected Susceptible	P-value
Total	0	7.17±0.08	7.21±0.08	7.24^{a} ±0.19	0.391
Protein	10	7.07±0.08	6.77±0.22	6.67^{a}±0.21	0.436
(g/dL)	20	7.11^{x}±0.09	6.23^{y}±0.20	5.95^{yb}±0.22	0.001
	30	7.24^{x}±0.19	5.82^{y}±0.13	5.14^{zc}±0.13	0.000
	40	7.14^{x}±0.12	6.36^{y}±0.27	4.94^{zc}±0.22	0.000
	P value	0.884	0.417	0.000	
Albumin	0	3.07±0.07	3.80^{a}±0.03	3.68^{a} ±0.10	0.528
(g/dL)	10	3.68 ±0.08	3.58^{a} ±0.11	3.48^{a} ±0.12	0.484
	20	3.64^{x} ±0.09	3.27^{yb} ±0.11	3.00^{yb}±0.06	0.001
	30	3.27^{x}±0.08	2.88^{yc}±0.05	2.60^{zc}±0.11	0.000

Cont. Table 3.

Table 3., Cont. ...

Variables	DPI	Control	Infected Resistant	Infected Susceptible	P-value
	40	3.66^{x} ±0.05	3.29^{yb}±0.11	2.55^{zc}±0.16	0.000
	P value	0.964	0.000	0.000	
Globulin (g/dL)	0	3.47±0.09	3.44±0.11	3.41^{a}±0.15	0.945
	10	3.38 ±0.11	3.18 ±0.24	3.33^{a}±0.10	0.673
	20	3.53^{x}±0.16	2.95^{y}±0.17	2.88^{yab}±0.22	0.06
	30	3.52^{x}±0.13	2.94^{y}±0.12	2.53^{zb}±0.10	0.000
	40	3.47^{x}±0.10	3.07^{xy}±0.20	2.66^{yb}±0.22	0.025
	P value	0.93	0.291	0.003	N = 6

Values bearing superscripts; x, y… letters in a row within the DPI differs significantly ($P < 0.05$) among the different groups. Values bearing superscripts; a, b, c…. in a column within a group differs significantly ($P < 0.05$) among different DPIs.

Table 4. Changes in clinical parameters due to *Haemonchus contortus* infection among the different experimental groups of Garole sheep

Variables	DPI	Control	Infected Resistant	Infected Susceptible	P value
FAMACHA	0	1.33±0.21	1.33^{c}±0.21	1.16^{c}±0.16	0.791
	10	1.33±0.21	1.50^{bc}±0.22	1.33^{c}±0.21	0.821
	20	1.16^{y}±0.16	2.00^{xab}±0.00	2.16^{xb}±0.16	0.000
	30	1.33^{z}±0.21	2.16^{ya}±0.16	3.66^{xa}±0.21	0.000
	40	1.16^{y}±0.16	1.50^{ybc}±0.22	$4.16^{x\,a}$±0.30	0.000
	P-value	0.924	0.015	0.000	N = 6
BCS	0	2.66±0.21	2.66±0.21	2.50^{a} ±0.22	0.821
	10	2.83±0.16	2.50±0.22	2.33^{ab} ±0.21	0.236
	20	2.83^{x}±0.16	2.33^{xy}±0.21	2.00^{yabc}±0.25	0.046
	30	2.33^{x}±0.21	2.16^{xy}±0.16	1.66^{ybc}±0.21	0.075
	40	2.33^{x}±0.21	2.50^{x} ±0.22	1.33^{yc}±0.21	0.003
	P-value	0.184	0.517	0.007	N = 6

Values bearing superscripts; x, y… letters in a row within the DPI differs significantly ($P<0.05$) among the different groups. Values bearing superscripts; a, b, c…. in a column within a group differs significantly ($P<0.05$) among different DPIs.

sheep by FAMACHA score. In infected resistant sheep, mild anaemia was observed only on 30 DPI as per FAMACHA score (2.166±0.166). Whereas, in infected susceptible group anemia were observed on 20 to 40 DPI and severe anaemia was recorded on 40 DPI (4.166±0.307) but no signs of anemia were observed in control group till 40 DPI (Table 4). Weight loss was indirectly assumed from the body condition loss in the experimental animal and was expressed in terms of body condition score (BCS). In susceptible sheep, BCS score was very low on 30 and 40 DPI compared to infected resistant and control group. Whereas in resistant sheep body condition loss was mild compared to control group only on 30 DPI (Table 4). No oedema was observed during experimental study in any animals.

DISCUSSION

Faecal egg count (FEC) was found to be significantly ($P<0.01$) lower in infected resistant sheep compared to the susceptible sheep during the study period. Significantly reduced FEC has also been recorded earlier in many resistant sheep by many authors (Shakya *et al.*, 2011; Escribano *et al.*, 2019; Hernández *et al.*, 2020; Thorne *et al.*, 2022). Faecal egg count is the most important phenotypic indicator of host resistance against gastrointestinal nematodes (GINs), and heritability of FEC was recorded as 0.12 and 0.44 in resistant sheep (Miller and Horohov, 2006; Cruz-Tamayo *et al.*, 2020; Weaver *et al.*, 2023). In resistant Garole sheep, significantly lower FEC was also observed compared to susceptible Sahabadi sheep (Michael *et al.*, 2020) and susceptible Garole sheep (Brahma *et al.*, 2023). Eggs of *H. contortus* were not found in the faecal sample of infected resistant Garole sheep from 33 to 40 DPI, indicating spontaneous elimination of adult worms, and similar observation was also recorded earlier where rejection of adult worms was observed between 24 to 33 DPI (Bordoloi *et al.*, 2012; Michael *et al.*, 2020; Brahma *et al.*, 2023). Elimination of adult worms might be due to severe immunological reaction in resistant sheep (MacKinnon *et al.*, 2010; Albuquerque *et al.*, 2019; Weaver *et al.*, 2021).

Anaemia with reduced Hb and PCV is the most common symptom in *H. contortus* infection in animals and thus Hb and PCV are also important markers for resilience against *H. contortus*, a potent hematophagous nematode (Gauly and Erhardt, 2001; Vanimisetti *et al.*, 2004). Therefore, a more or less stable haematocrit value, particularly Hb and PCV, has been reported in resistant animals infected with *H. contortus* (Miller *et al.*, 1998; Bricarello *et al.*, 2002). In the present study too significantly ($P <0.01$) lower values of Hb and PCV have been recorded in infected susceptible sheep compared to resistant sheep in which those value did not reduce significantly compared to uninfected control sheep. The results of the present study were in agreement with many authors (Michael *et al.*, 2020) who recorded significantly higher Hb and PCV in resistant Garole sheep compared to susceptible Sahabadi sheep.

Total leukocyte count increased in both the infected groups of sheep in response to experimental haemonchosis. Different types of leucocytes are important component of the immune system and increased number of leucocytes in the infected animals might be due to host's immune response to the artificial *H. contortus* infection. Higher TLC values have been recorded in sheep and goat infected with different types of gastrointestinal helminths including *H. contortus* (Jas *et al.*, 2008; Huang *et al.*, 2016). It was interesting to note that total leucocytes count including number of lymphocytes, eosinophils, monocytes and neutrophils increased significantly ($P<0.01$) in resistant sheep from 10 DPI and was highest on 30 DPI after which elimination of adult parasites was started from the abomasum of resistant sheep indicating their role in host resistance against *H. contortus*. Whereas in susceptible sheep number of different types of leucocytes increased only on 10 DPI in response to *H. contortus* infection and then started reducing significantly ($P<0.01$) from 20 DPI compared to resistant sheep. Lymphocytes and neutrophils are the primary cells of inflammatory reaction which also occurs due to biting and blood sucking activity of *H. contortus* in the abomasum. Therefore, significantly higher number of lymphocytes and neutrophils in resistant sheep might be due to better immune response to *H. contortus* infection compared to susceptible sheep. Eosinophils are the primary immune cells that target parasites, especially non-phagocytizable organisms such as helminths (Behm *et al.*, 2000) and considered to be important elements that respond against nematode infestations (Balic *et al.*, 2006). Elevated levels of eosinophils have been extensively observed in animals infected with *H. contortus* and strongly associated with immunity (Huang *et al.*, 2016). Increased number of eosinophils as observed in infected resistant sheep compared to susceptible sheep was also reported earlier by other authors (Travers and Rothenberg, 2015; Michael *et al.*, 2020).

Gastrointestinal helminths, particularly nematode parasites, cause protein-losing gastro-enteropathy leading to loss of plasma protein through the damaged intestinal mucosa (Soulsby, 1982). Significantly reduced plasma protein, including plasma albumin and globulin has been recorded in infected animals compared to uninfected animals by many workers (Bricarello *et al.*, 2002; Jas *et al.*, 2008; Bordoloi *et al.*, 2012). In the present study, reduction of concentration of total protein, albumin, and globulin was more in infected sheep compared to the uninfected control sheep. It was promising to record that serum protein concentration was significantly ($P<0.01$) higher in infected resistant sheep compared to infected susceptible sheep and this might be due to that the resistant animals have the better ability to reduce the pathogenic effects of *H. contortus* infection. Significantly higher serum protein concentration in resistant sheep might also be due to a smaller number of parasites in the abomasums as indicated by the significantly lower FEC compared to susceptible sheep.

Negative impact of *H. contortus* was more severe in infected susceptible sheep in terms of anaemia as indicated by FAMACHA score and low body condition score compared to infected resistant sheep, which had the better ability to combat the pathogenic effect of parasite and to eliminate the parasites after certain period, as observed in the study.

Within breed resistance against *H. contortus* has been observed in Garole sheep as determined by faecal egg count. Haemato-biochemical impact of *H. contortus* infection was more severe in susceptible sheep in terms of anaemia and hypoproteinaemia. Resistant sheep was able to withstand the negative impact of *H. contortus* infection with better immunological response as evidenced by increased number of different types of leucocytes.

Conflict of interest: The authors have no relevant financial or non-financial interests to disclose. The authors declare that no funds, grants, or other support were received from any organization during the preparation of this manuscript.

Author's contribution: SD, AH, SB: Methodology, Investigation, Data curation; SP, SB: Data analysis; AN, SSK: Resource; JM, SP: Writing - review and editing; RJ: Conceptualization, Project administration, Writing - original draft. All authors approved the final version of this manuscript.

Data availability statement: The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval: This experimental study was approved by the Institutional Animal Ethics Committee (IAEC) of West Bengal University of Animal and Fishery Sciences (WBUAFS), which has the approval (763/GO/Re-S/ReRc-L/03/CCSEA/48/2022-23) of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CCSEA) under the Ministry of Fisheries, Animal Husbandry and Dairying; Government of India.

ACKNOWLEDGEMENTS

The financial assistance for conducting this study was received from the West Bengal University of Animal & Fishery Sciences through the Indian Council Agricultural Research-Development Grant for UG and PG studies.

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Received - 18.11.2024, Accepted - 16.05.2025, Published - 22.05.2025(Online), 01.06.2025 (Print)

Section Editor: Dr. C. K. Jana, Editorial Board Member