International Journal of Livestock Research, Vol. 15 (7) Jul' 2025
http://www.ijlr.org
eISSN: 2277-1964
NAAS Score:4.31

Original Research

Variation in Serum Immunoglobulin, Histamine, and Cytokines Response between Resistant Garole and Susceptible Sahabadi Sheep Infected with *Haemonchus contortus*

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How to cite this paper
Bordoloi, G., Brahma, A., Das, S., Hembram, A., Rai, S., & Jas, R. (2025). Variation in Serum Immunoglobulin, Histamine, and Cytokines Response between Resistant Garole and Susceptible Sahabadi Sheep Infected with Haemonchus contortus. *International Journal of Livestock Research*, *15*(7), 11–23.

Received : May 05, 2025
Accepted : Jul 20, 2025
Published : Jul 31, 2025

Abstract

The immune response of resistant Garole sheep to Haemonchus contortus infection was compared with susceptible Sahabadi sheep. Each breed of sheep was divided into infected (n = 6) and control (n = 6) groups, and the infected groups were orally infected with H. contortus larvae. Faecal egg counts (FEC), and concentrations of immunoglobulin (IgA and IgE), histamine and cytokines (IFN- γ and IL-4) in serum were measured in all the experimental sheep. Faecal egg count was significantly (P < 0.05) lower in Garole sheep compared to Sahabadi sheep. Serum IgA and histamine concentration increased (P < 0.05) in resistant Garole sheep compared to Sahabadi sheep, with a non-significant difference in IgE concentration. Significantly (P<0.05) greater levels of serum IFN- γ and IL-4 were observed in resistant Garole sheep than in the susceptible Sahabadi sheep. Therefore, it can be concluded that these immunological parameters played an important role in the comparative resistance of Garole sheep.

Keywords: Garole Sheep, Host Resistance, *Haemonchus contortus*, Immune Response.

Introduction

Gastrointestinal (GI) nematodes constitute one of the most important limiting factors in small ruminant livestock productivity in both the tropical and temperate regions of the world (Emery *et al*., 2016; Arsenopoulos *et al*., 2021) including India (Jas and Ghosh, 2009; Singh *et al*., 2013). Amongst the nematode parasites *Haemonchus contortus* is known as the most pathogenic and predominant parasite, particularly in sheep and goat (Emery *et al*., 2016; Jas *et al*., 2017). Control of GI nematode infections including haemonchosis has, since long, been primarily dependent on the use of anthelmintics (Kamaraj *et al*., 2011). This method of control using anthelmintic treatment has exerted a strong selection pressure on the parasites with consequent emergence of anthelmintic resistant strains of the parasite (Mickiewicz *et al*., 2021) all over the world. Moreover, there is increasing global concern about the chemical residues in animals and animal products. Hence, there is intensive global effort towards lessening the dependence on anthelmintics through alternative means of controlling GI parasites, particularly of sheep (Arsenopoulos *et al*., 2017).

Evidence of variation in sheep for resistance/resilience to GI nematodes was first documented by Stewart *et al* (1937). There exists strong to weak protective immune response of ruminants to GI nematodes (Karrow *et al*., 2014) and susceptible sheep have been reported to have a delayed response as compared to resistant (Ingham *et al*., 2008). One of the factors that may play a role in resistance of sheep to GI parasites is production and specificity of antibodies, particularly IgA and IgE (Shakya *et al*., 2011). Relative amounts of IgA in serum and in the gut are associated with FEC, which would allow for an easily accessible assessment of resistant animals (Martínez-Valladares *et al*., 2005). These studies suggest that IgA in mucus may damage parasitic larvae and are consistent with reports that increased mucus IgA levels are associated with reduced FEC (Rocha *et al*., 2005) and reduced worm length (Strain and Stear, 2001).

Extracellular parasite infection typically leads to production of local and systemic antibodies by plasma cells, infiltration of mast cells and eosinophils, and differentiation of CD4+ T-helper lymphocytes into T-helper type-2 (Th-2) cells (Lacroux *et al*., 2006) and Th-2 type immune response is associated with resistance to GI nematode infection (Shakya *et al*., 2011) whereas, Th-1 type immune response is related to susceptibility in animals. An interaction between Th-1 / Th-2 and Treg genes has been suggested to modify the immune response to GI nematodes instead of the unequivocal Th-1 for susceptibility and Th-2 for resistance (Hassan *et al*., 2011; Arsenopoulos *et al*., 2017; Brahma *et al*., 2023).

Garole sheep of West Bengal, India have been found to be resistant to naturally occurring GI nematodes as well as experimental *H. contortus* infection (Bordoloi *et al*., 2012; Ghosh *et al*., 2012; Brahma *et al*., 2023) based on faecal egg count. In the present study the immunological response in terms of serum immunoglobulin, histamine and cytokine concentration of resistant Garole sheep experimentally infected with *H. contortus* was compared with the susceptible Sahabadi sheep with a view to determine the immune components responsible for comparative resistance in Garole sheep.

Materials and Methods

Ethics Approval

The entire experimental design and protocol for the present work on sheep were approved by the Institutional Animal Ethical Committee of the West Bengal University of Animal and Fishery Sciences, Kolkata(763/GO/Re/SL/03/CPCSEA) approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) under the Ministry of Fisheries, Animal Husbandry and Dairying; Government of India.

Selection of Experimental Animals

Based on phenotypic characteristics 60 Garole sheep in the age group of 4 to 6 months were randomly selected from two villages under the block Joynagar-I in South 24 Parganas district (West Bengal), the native tract of this sheep. Similarly, 40 Sahabadi sheep of 4 to 6 months old were also randomly selected from two villages under the block Katwa -I in Purba Bardhhaman district. Faecal egg counts (FEC) in terms of eggs per gram (EPG) of faeces of all the selected sheep of both the breeds were estimated at monthly interval for a period of one year by Modified

McMaster technique (Soulsby, 1982). Out of 60 Garole sheep 20 sheep showed consistently low EPG (mean EPG < 150) and they were considered as comparative resistant. On the other hand, none of the selected Sahabadi sheep showed consistently low FEC and showed a variable EPG (mean EPG > 500). Finally, twelve Garole sheep showing consistently low faecal egg count throughout the entire study period and thirteen Sahabadi sheep were procured from the respective owners and the animals were then brought to the university's animal house following the standard protocol.

Maintenance and Grouping of Experimental Animals

All the procured sheep of both the breeds were maintained under intensive system of management in the experimental animal house of the university for two months before they were used in the study. The experimental sheep of each breed were divided into infected group (n = 6) and control group (n = 6) and one Sahabadi sheep were used as donor animal. Sheep were kept in five separate enclosures on concrete floor in the experimental animal house. Pre-existing GI parasites, if any, after coprological screening were eliminated by treatment with Fenbendazole (Panacur®, Intervet) @ 5mg/kg body weight. Thereafter, all possible precautions were taken to exclude extraneous parasitic infections during the experiment. The animals were provided with adequate green fodder (only tree leaves) and recommended quantity of concentrate feed with provision for *ad libitum* clean drinking water.

Artificial Infection of Experimental Sheep

Apparently, adult female *H. contortus* were obtained from abomasums of sheep, which were slaughtered at the local abattoir at New Market in Kolkata. Then the eggs were separated by triturating all the female worms and the eggs were then cultured in sterile sheep faecal powder following the method described by Anon. (1971) and incubated at 27°± 1°C for 10 days in a Biological Oxygen Demand (B.O.D.) incubator. After 10 days, the third stage infective larvae (L_3) of *H. contortus* were harvested in lukewarm water, washed thrice in distilled water (D.W.) and stored in D.W. at 4°C till further use.

Faecal sample of the donor sheep was examined thrice on alternate days by standard sedimentation and salt floatation techniques (Soulsby, 1982) before giving the infection. The donor sheep was weighed and orally infected L_3 of *H. contortus* @ 500 L_3/kg body weight after overnight withdrawal of feed and water (Brahma *et al*., 2023). The infected sheep was allowed to take feed and water only after four hours of artificial infection. After patency, total faecal sample of donor sheep was collected and cultured by standard method (Anon. 1971) to harvest infective larvae of *H. contortus*. After obtaining sufficient number of larvae, faecal samples of all the sheep were examined thrice on alternate days by standard techniques (Soulsby, 1982) prior to giving the infection. Body weights of all the 24 animals were recorded individually and the sheep of infected groups of both the breeds were experimentally infected with *H. contortus* larvae, as stated earlier in case of donor sheep.

Faecal Egg Count

Faecal samples of all the infected sheep were qualitatively examined daily by salt floatation technique (Soulsby, 1982) from second week post-infection onwards to determine the prepatent period of the infection. After the infection became patent in all the infected sheep, quantitative examination of faecal samples of all the infected sheep was done by Modified McMaster technique (Soulsby, 1982) to determine the difference in FEC in terms of egg per gramme of faeces (EPG) from 18 DPI at three days interval for nine occasions.

Serum Concentration of IgA, IgE, Histamine, Cytokines INF-γ and IL-4

To study the difference in serum concentration of different immune component between the two breeds of sheep experimentally infected with H. contortus, blood samples (3 ml / animal) were collected from all the animals and subsequently serum samples were separated following the standard protocol from 0 DPI to 42 DPI at weekly interval.

Concentrations of immunoglobulins (IgA and IgE), histamine and cytokines (IL-4 and IFN-γ) in serum were estimated by using commercial ELISA kits based on sandwich ELISA (Bethyl Laboratories, USA) from 0 DPI up to 42 DPI at weekly interval. The kits contained micro-ELISA plates precoated with antibodies specific to ovine

IgA, IgE, histamine and cytokines IFN-γ or IL-4. The serum concentrations of above parameters were measured following the protocols of the kits. The absorbance of the ELISA plates was measured at 450 nm in ELISA Reader (Rayto, India). The concentrations of above immunological parameters were determined by comparing the mean OD values of test samples with the standard curve prepared in the present assay. The serum concentrations of IgA, IgE, histamine, IL-4 and IFN-γ were expressed in µg/ml, µg/ml, ng/ml, pg/ml, and pg/ml, respectively.

Statistical Analysis

All the parameters for each group on different post-infection days were compared (Analyze-Compare Means) for obtaining the mean value along with standard error (S.E.). Then they were analyzed separately i.e. between groups and between post-infection days, by Duncan method (One- way- ANOVA) and the significance (p- value) was recorded at 5 % ($p<0.05$) level and 1 % ($p<0.01$) level. Further, the changes in respect of all the parameters in the infected group of Garole and Sahabadi sheep were calculated in terms of percentage in relation to the respective mean value in the control group. The percent changes were analyzed between the two groups and the significance was recorded as above.

The complete statistical analyses were done with the help of Statistical Package for Social Scientists (SPSS), Windows Version 20.0.

Results and Discussion

Faecal Egg Count as a Phenotypic Indicator of Resistance

Following artificial infection with *H. contortus* in all the infected Garole sheep, the first egg in the faeces was detected on 17 DPI. Whereas Sahabadi sheep started excreting eggs in the faeces on 16 DPI. After the infection attained patency in all the sheep of both breeds, FEC in terms of EPG was determined from 18 DPI and the results have been presented in Fig.1. Throughout the course of the experiment, the FEC was consistently higher in Sahabadi sheep than the Garole sheep. Faecal samples of two Garole sheep showed no eggs of *H. contortus* on 27 DPI and subsequently, on 30 DPI rest of the infected Garole sheep became free of infection both on quantitative and qualitative faecal examination, whereas all the infected Sahabadi sheep recorded consistently higher EPG till the end of the experiment on 42 DPI.

Table 1: Changes in serum IgA concentration (µg/ml) due to haemonchosis in two breeds of sheep

DPI	Garole sheep			Sahabadi sheep		
	Infected	Control	P value	Infected	Control	P value
0	$27.52^{d} \pm 1.55$	25.18 ± 0.91	0.229	$18.58^{c} \pm 0.59$	19.71 ± 0.26	0.118
7	$50.75^{bx} \pm 1.97$	$25.05^{y} \pm 0.45$	0	$38.06^{ax} \pm 4.60$	$19.32^{y} \pm 0.22$	0.004
14	$56.19^{ax} \pm 2.03$	$26.05^{y} \pm 0.67$	0	$32.06^{bx} \pm 1.71$	$20.3^{y} \pm 0.58$	0
21	$47.27^{bx} \pm 1.83$	$25.39^{y} \pm 0.63$	0	$28.03^{bx} \pm 0.57$	$19.48^{y} \pm 0.58$	0
28	$37.94^{cx} \pm 1.07$	$25.04^{y} \pm 0.99$	0	$18.64^{cy} \pm 0.59$	$20.08^{x} \pm 0.19$	0.049
35	$37.23^{cx} \pm 0.44$	$25.78^{y} \pm 0.52$	0	$17.93^{cy} \pm 0.37$	$20.52^{x} \pm 0.58$	0.006
42	$27.32^{d} \pm 0.98$	25.45 ± 0.25	0.173	$17.24^{cy} \pm 0.40$	$19.71^{x} \pm 0.38$	0.002
P value	0	0.92		0	0.425	

N.B. Values bearing superscripts x, y in a row and a, b, c,.in a column differs significantly (P < 0.05)

Faecal egg count (FEC) is the most practicable and principal measurement for evaluation of resistance status in sheep undergoing similar parasite challenge (Lobo *et al*., 2009) and it is directly related with intensity of infection. The FEC in Garole sheep in the present study remained consistently lower than the Sahabadi sheep throughout the course of experiment. These findings agreed to those of Saddiqi *et al* (2010) and Shakya et al. (2011), who reported that resistant animals had lower FEC than susceptible ones. Such variations were also recorded between Young Menz lambs and Horro lambs (Rege *et al*., 2002), Sabi and Dorper ewes (Matika *et al*., 2003), Black Belly and INRA 401 (Gruner *et al*., 2003), Wool and Hair lambs (Notter *et al*., 2003), Canaria Hair and Canaria sheep (González *et al*., 2008), Rhön and Merinoland sheep (Gauly *et al*., 2002), Dorper and Katahdin sheep (Vanimisetti *et al*., 2004), and Suffolk and Texel lambs (Good *et al*., 2006).

Table 2: Changes in serum IgE concentration (µg/ml) due to haemonchosis in two breeds of sheep

DPI	Garole sheep			Sahabadi sheep		
	Infected	Control	P value	Infected	Infected	P value
0	$4.10^{d} \pm 0.02$	4.10 ± 0.03	0.982	$4.57^{e} \pm 0.03$	4.51 ± 0.03	0.056
7	$4.55^{bx} \pm 0.03$	$4.12^{y} \pm 0.03$	0	$5.11^{bx} \pm 0.04$	$4.51^{y} \pm 0.04$	0
14	$4.81^{ax} \pm 0.06$	$4.14^{y} \pm 0.03$	0	$5.24^{ax} \pm 0.02$	$4.50^{y} \pm 0.04$	0
21	$4.41^{bcx} \pm 0.03$	$4.15^{y} \pm 0.09$	0	$5.21^{ax} \pm 0.04$	$4.50^{y} \pm 0.05$	0
28	$4.30^{c} \pm 0.10$	4.16 ± 0.05	0.242	$4.95^{cx} \pm 0.05$	$4.47^{y} \pm 0.04$	0
35	$4.34^{cx} \pm 0.07$	$4.13^{y} \pm 0.02$	0.022	$4.75^{dx} \pm 0.03$	$4.48^{y} \pm 0.04$	0
42	$4.25^{cd} \pm 0.02$	4.15 ± 0.05	0.141	$4.56^{e} \pm 0.02$	4.51 ± 0.03	0.209
P value	0	0.519		0	0.974	

N.B. Values bearing superscripts x, y in a row and a, b, c,in a column differs significantly (P < 0.05)

In the present study absence of *Haemonchus* eggs in faecal sample of infected Garole sheep after 30 DPI indicates expulsion or rejection of adult worms whereas, all the infected Sahabadi sheep showed consistently higher EPG till the termination of the study. Earlier studies also showed that there was spontaneous elimination of *H. contortus* infection in Garole sheep after 24 to 33 DPI (Bordoloi *et al*., 2012; Michael *et al*., 2020; Brahma *et al*., 2023). Mucosal mast cells are granular leucocytes having specific receptor for IgE binding on its surface and when these cells bind with specific IgE, the granules release specific mediators such as histamine, serotonin, prostaglandins, and 5-hydroxy tryptamine (Abraham and St John, 2010) which cause type I hypersensitivity resulting expulsion of parasites. The hyperplasia of mucosal mast cells and globule leucocytes are involved in rejection of challenge infection of *H. contortus* in sheep (Amarante *et al*., 2005). Association of parasite specific antibodies (IgA, IgG_1 and IgE) in the abomasal mucosa are also suggested in the response against GI nematodes including *H. contortus* (Amarante *et al*., 2005; McRae *et al*., 2014). In the present study the spontaneous elimination of adult *Haemonchus contortus* were elucidated in response to the serum level of antibodies (IgA and IgE), inflammatory mediators (histamine) and cytokine (IFN-γ and IL-4) in Garole sheep.

Table 3: Changes in serum histamine concentration (ng/ml) due to haemonchosis in two breeds of sheep

DPI	Garole sheep			Sahabadi sheep		
	Infected	Control	P value	Infected	Control	P value
0	$12.67^{cd} \pm 0.41$	9.97 ± 0.07	0.054	$9.51^{e} \pm 0.17$	9.66 ± 0.11	0.498
7	$17.34^{b} \pm 3.35$	9.78 ± 0.12	0	$24.61^{bx} \pm 0.24$	$9.69^{y} \pm 0.07$	0
14	$24.73^{ax} \pm 0.36$	$10.04^{y} \pm 0.14$	0	$27.15^{ax} \pm 0.58$	$9.69^{y} \pm 0.03$	0
21	$25.09^{ax} \pm 0.34$	$10.04^{y} \pm 0.06$	0	$12.72^{cx} \pm 0.39$	$9.88^{y} \pm 0.09$	0
28	$15.52^{bcx} \pm 0.22$	$10.04^{y} \pm 0.06$	0	$11.29^{dx} \pm 0.18$	$9.68^{y} \pm 0.05$	0
35	$14.85^{bcx} \pm 0.32$	$9.94^{y} \pm 0.13$	0	$9.94^{e} \pm 0.19$	9.68 ± 0.17	0.34
42	$10.24^{d} \pm 0.14$	9.83 ± 0.08	0.05	$9.72^{e} \pm 0.22$	9.92 ± 0.1	0.413
P value	0	0.369		0	0.291	

N.B. Values bearing superscripts x, y in a row and a, b, c,in a column differs significantly (P < 0.05)

Table 4: Changes in serum IFN- γ concentration (pg/ml) due to haemonchosis in two breeds of sheep

DPI	Garole sheep			Sahabadi sheep		
	Infected	Control	P value	Infected	Control	P value
0	$161.40^{f} \pm 0.65$	156.40 ± 5.37	0.406	$132.60^{f} \pm 0.51$	131.80 ± 1.83	0.684
7	$247.10^{cx} \pm 0.31$	$153.60^{y} \pm 2.82$	0	$139.60^{fx} \pm 0.40$	$131.80^{y} \pm 0.37$	0
14	$245.48^{dx} \pm 0.23$	$162.20^{y} \pm 3.23$	0	$188.00^{ex} \pm 0.32$	$130.40^{y} \pm 0.40$	0
21	$289.32^{ax} \pm 0.26$	$154.80^{y} \pm 1.43$	0	$278.00^{ax} \pm 0.71$	$130.60^{y} \pm 0.68$	0
28	$255.53^{bx} \pm 0.37$	$153.20^{y} \pm 3.48$	0	$251.60^{bx} \pm 14.54$	$131.60^{y} \pm 1.21$	0
35	$228.76^{ex} \pm 0.70$	$157.00^{y} \pm 4.70$	0	$233.60^{cx} \pm 0.68$	$131.60^{y} \pm 0.24$	0
42	$156.47^{g} \pm 0.47$	152.40 ± 0.51	0.855	$212.80^{dx} \pm 0.37$	$132.00^{y} \pm 0.71$	0
P value	0	0.491		0	0.833	

N.B. Values bearing superscripts x, y in a row and a, b, c in a column differs significantly (P < 0.05)

Table 5: Changes in serum IL - 4 concentration (pg/ml) due to haemonchosis in two breeds of sheep

DPI	Garole sheep			Sahabadi sheep		
	Infected	Control	P value	Infected	Control	P value
0	$147.50^{c} \pm 6.24$	145.00 ± 5.44	0.771	$93.16^{b} \pm 0.42$	94.24 ± 1.80	0.573
7	$238.26^{ax} \pm 20.86$	$144.14^{y} \pm 1.19$	0.002	$115.51^{ax} \pm 7.37$	$95.52^{y} \pm 0.96$	0.027
14	$236.87^{ax} \pm 11.97$	$143.61^{y} \pm 2.29$	0	$117.01^{ax} \pm 7.44$	$95.49^{y} \pm 0.59$	0.02
21	$199.40^{bx} \pm 3.72$	$144.14^{y} \pm 2.18$	0	$106.22^{abx} \pm 1.62$	$94.33^{y} \pm 0.74$	0
28	$162.85^{cx} \pm 3.07$	$143.28^{y} \pm 1.02$	0	$98.66^{b} \pm 2.43$	95.12 ± 0.65	0.197
35	$144.75^{c} \pm 5.44$	144.44 ± 0.73	0.956	$97.57^{b} \pm 1.40$	94.77 ± 0.51	0.098
42	$139.78^{c} \pm 3.20$	142.68 ± 0.63	0.401	$97.95^{b} \pm 1.25$	96.06 ± 0.64	0.215
P value	0	0.996		0.001	0.798	

N.B. Values bearing superscripts x, y in a row and a, b, c,.....in a column differs significantly (P < 0.05)

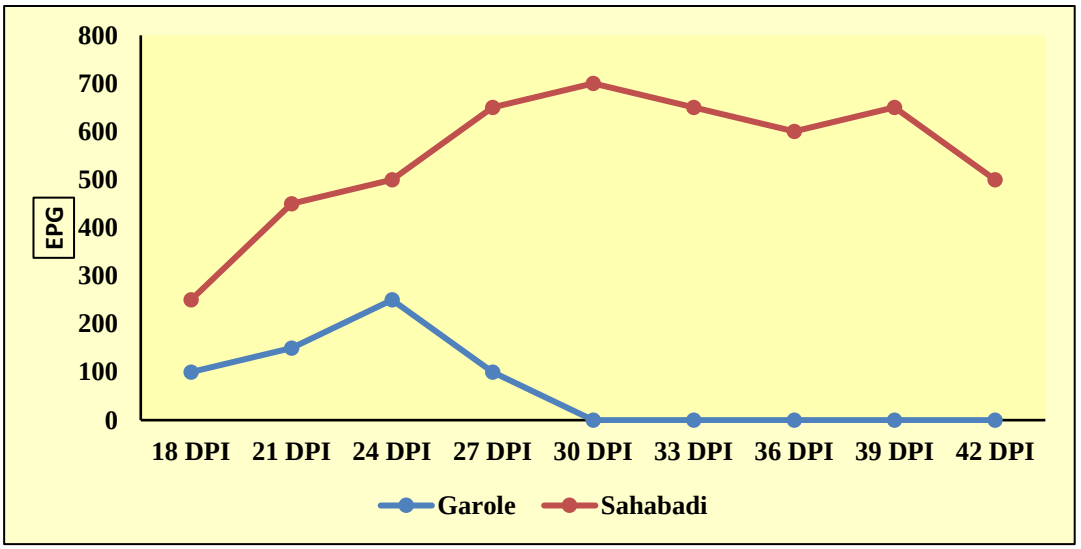


Fig. 1: Faecal egg count of Garole and Sahabadi sheep artificially infected with *Haemonchus contortus*

Serum Immunoglobulin Concentration

Serum immunoglobulin (IgA and IgE) concentrations increased significantly ($P < 0.01$) in the infected groups of both Garole and Sahabadi sheep from 7 DPI onwards, which however restored towards the pre-infection value on 42 DPI. The values of serum IgA and IgE in the infected Garole sheep remained consistently ($P < 0.01$) higher than the control group from 7DPI to 35DPI (Table -1). In the infected Sahabadi sheep serum IgA and IgE concentration was significantly ($P < 0.01$) higher from 7 DPI to 21 DPI compared to the uninfected control sheep. Comparison in serum concentration of immunoglobulin (IgA and IgE) between breeds were measured in terms of percent increase/decrease in the values due to *H. contortus* infection. Significantly ($P < 0.01$) higher value of serum IgA was observed in infected Garole sheep than the infected Sahabadi sheep from 14 DPI onwards till the end of the experiment (Fig. 2). Comparison of the two breeds revealed that there was no significant ($P > 0.05$) difference in serum IgE values in terms of per cent change on different post-infection days except on 21DPI when there was significantly ($P < 0.01$) higher value in infected Sahabadi sheep than that of Garole sheep (Fig. 2).

Serum Histamine Concentration

Serum histamine concentration increased significantly ($P < 0.01$) in the infected groups of both Garole and Sahabadi sheep due to experimental haemonchosis. The concentration of serum histamine increased significantly ($P < 0.01$) in the infected Garole sheep from 14 to 35 DPI compared to control group. In Sahabadi sheep, on comparison with the respective control group the values of serum histamine increased significantly ($P < 0.01$) in the infected group from 7 to 28 DPI (Table - 3). The percent increase in serum histamine concentration was significantly ($P < 0.01$) higher in Sahabadi sheep on 7 and 14 DPI and from 21 DPI onwards the value was significantly ($P < 0.01$) higher

in Garole sheep till the end of the experiment (Fig. 3).

Antibody attached with specific antigen also binds with the Fc receptor of inflammatory cells (mucosal mast cells) and causes degranulation with the release of inflammatory mediators such as histamine. In the present study serum IgA and IgE increased significantly ($P < 0.05$) in the infected sheep of both the breeds which might have caused degranulation of mucosal mast cells resulting increased concentration of histamine. Histamine along with other inflammatory mediators produce type-I hypersensitivity reaction in abomasum and help in the expulsion adult worms. Histamine concentration increased in susceptible Sahabadi sheep during the earlier phase of infection but this increased histamine could not damage or eliminate the parasites. Whereas, in resistant Garole sheep histamine concentration increased significantly ($P < 0.05$) from 21 DPI compared to susceptible sheep and this histamine along with other inflammatory mediators, cytokines and specific antibodies might have created an intense reaction in abomasum resulting expulsion of adult worms from 24 DPI as observed in the present study.

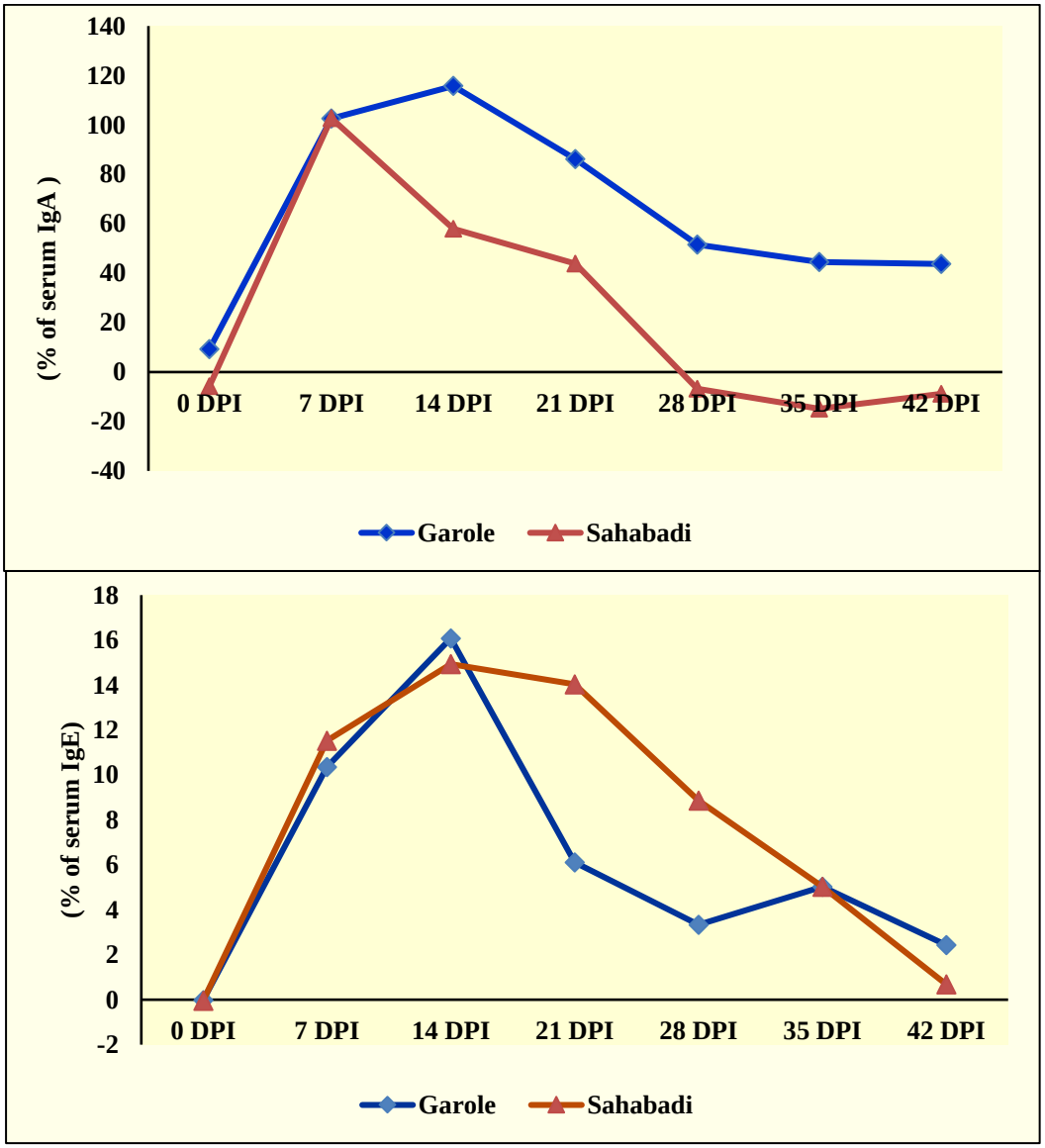


Fig. 2: Variation in serum immunoglobulin concentration (IgA% and IgE%) between Garole and Sahabadi sheep due to experimental haemonchosis

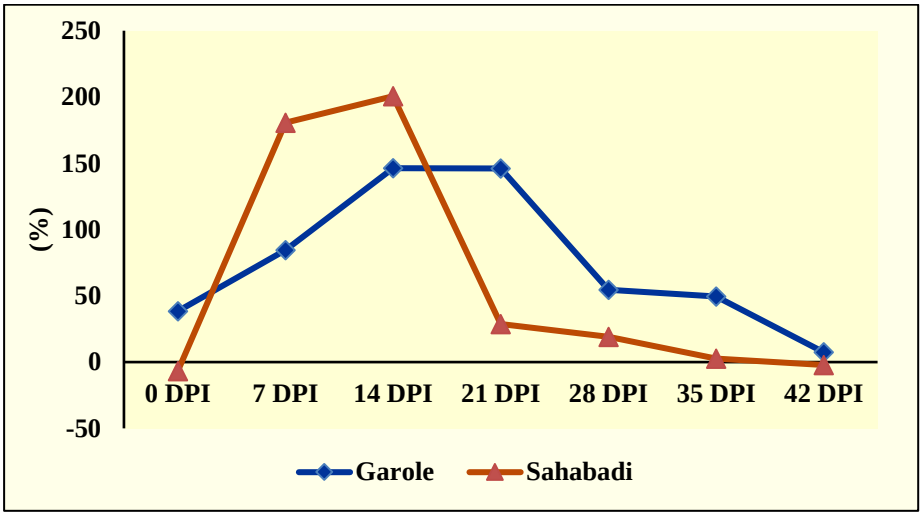
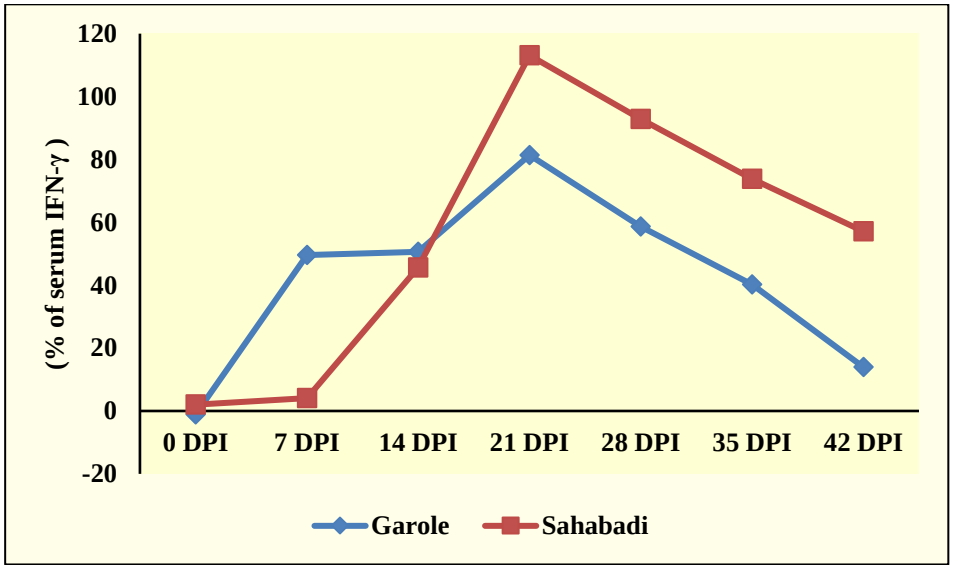


Fig. 3: Comparative changes in serum histamine concentration (ng/ml) between Garole and Sahabadi sheep infected with *Haemonchus contortus*



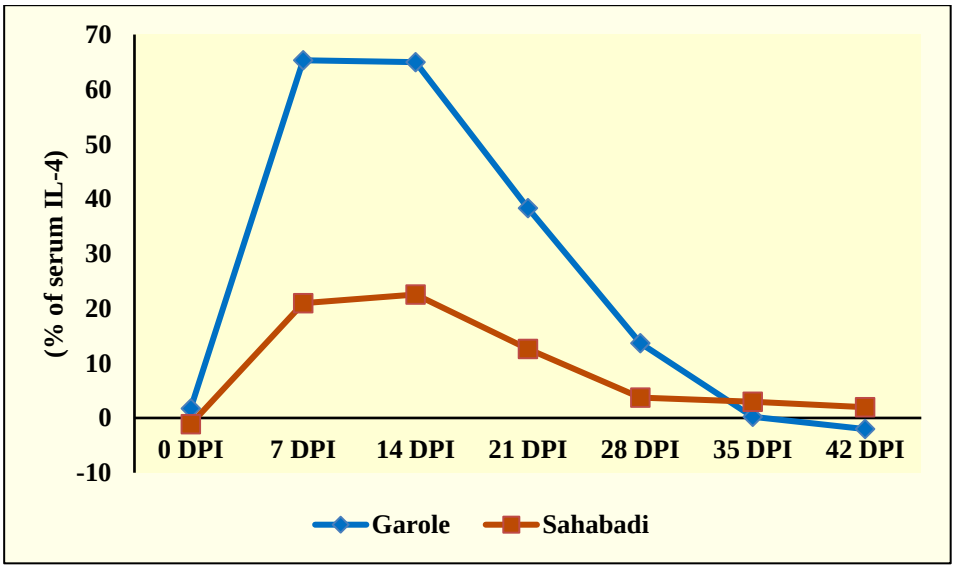


Fig. 4: Changes in serum cytokine concentration (IFN-γ% and IL-4%) between Garole and Sahabadi sheep infected with *Haemonchus contortus*

One of the factors that may play a role in resistance of sheep to GI parasites is production and specificity of antibodies, particularly IgA and IgE (Shakya *et al*., 2011). Immunoglobulin A is the second most common antibody in serum. Immunoglobulin E, on the other hand, has very low serum concentrations and is primarily found attached to high affinity receptors on immune cells, mainly mast cells and basophils (Abraham and St John, 2010). These activated IgE-bound cells may provide an additional mechanism to damage invading parasites and facilitate their expulsion (McRae *et al*., 2014). Immunoglobulin A (IgA) has been reported to be helpful for strong immune response and its concentration is reported more in resistant animals (Brahma *et al*., 2022). Level of IgA rises in many GI nematode infections of sheep and is associated with increased host resistance (González-Garduño *et al*., 2021). In the present study, the serum IgA level increased significantly ($P < 0.05$) in the infected sheep both resistant Garole and susceptible Sahabadi sheep. In response to parasitic antigen specific IgA might have increased at the site of infection (abomasum) leading to higher concentration in infected sheep of both the breeds. In the resistant Garole sheep, the value of IgA was significantly ($P < 0.05$) higher than the Sahabadi sheep throughout the experimental period. Inflammatory cells and parasite specific IgA in abomasum were inversely associated with *Haemonchus* worm burdens and EPG, indicating the cells and IgA may harm parasite growth or fecundity in sheep (McRae *et al*., 2014). Many workers have reported the elevated level of serum IgE antibodies during infection with *H. contortus* (Kooyman *et al*., 2000), and *T. circumcincta* (Pettit *et al*., 2005) and it was associated with host resistance. In the present study serum IgE concentrations increased in the infected sheep compared to the control group but no significant difference was observed between the resistant Garole and susceptible Sahabadi sheep as reported earlier by Michael *et al* (2020). Although there was no significant ($P > 0.05$) difference in serum IgE concentration between the resistant and susceptible sheep but it could be assumed that IgE played an important role in infected resistant Garole sheep to eliminate the parasites from the abomasum. Parasite specific IgE attached with eosinophils helps in the expulsion of parasites and eosinophil count increased significantly in resistant Garole sheep compared to susceptible Sahabadi sheep as reported by Michael *et al* (2020).

Serum Cytokines Concentration

Serum cytokine (IFN-γ and IL-4) concentrations increased significantly ($P < 0.01$) in the infected groups of both Garole and Sahabadi sheep. In the infected group Garole sheep serum IFN-γ concentration was consistently higher ($P < 0.01$) than the control group from 7 DPI to 35 DPI and in infected Sahabadi sheep the values were significantly ($P < 0.01$) higher from 7 DPI to 42 DPI compared to the control group (Table - 4). On comparison between the two breeds of sheep it was observed that the percent increase in serum IFN-γ remained significantly ($P < 0.01$) higher in Garole sheep than the Sahabadi sheep only on two occasions (7 and 14DPI). On all other occasions during the entire experiment the value remained significantly ($P < 0.01$) higher in Sahabadi sheep (Fig. 4). Infected Garole sheep had significantly ($P < 0.01$) higher values of serum IL-4 from 7 to 28 DPI (Table - 5) compared to control group. Whereas, in infected Sahabadi sheep serum IL-4 concentration increased significantly ($P < 0.01$) from 7 to 21 DPI compared to control group of sheep. Comparison between breeds revealed that infected Garole sheep had significantly higher percentage of increase in serum IL-4 values compared to infected Sahabadi sheep from 7 to 28 DPI (Fig. 4).

Cytokines are important mediators of cell mediated immune response and the role of several cytokines, secreted from both Th1 as well as Th2 cells in resistance against GI nematodes have been studied (McRae *et al*., 2015). It is generally agreed that Th1 cytokine associated with susceptibility and Th2 cytokines associated with resistance (Lacroux *et al*., 2006). Therefore, in the present study one Th1 cytokine (IFN-γ) and one Th2 cytokine (IL-4) were estimated and compared between the resistant Garole and susceptible Sahabadi sheep. Response of Garole sheep in terms of concentration of IFN-γ in the serum was significantly ($P < 0.01$) higher on 2 occasions i.e., 7 and 14DPI than the Sahabadi sheep. The concentration of serum IL-4 was significantly ($P < 0.01$) higher in the Garole sheep in comparison with the Sahabadi sheep till 28DPI.

Stimulation of Th1 lymphocytes occurs at the early stage of helminth infection and this stimulation is caused by IL-12 produced by dendritic cells (Henry *et al*., 2008) and these activated Th1 cells might have produced IFN-γ which was found to be significantly ($P < 0.01$) higher in resistant Garole sheep on 7 and 14 DPI in the present study. With the progression of the infection, the helminth parasites cause strong polarization of Th 2 response which cause downregulation of Th1 (Jankovic *et al*., 2004).

Higher concentration of IL-4 has been reported in resistant breed of sheep compared to susceptible breed (Anthony *et al.*, 2007; Gossner *et al*., 2013) as observed in the present study. Increased concentration of IL-4 and IFN-γ in the

cell culture supernatant stimulated with *H. contortus* somatic antigen was observed in resistant Garole sheep compared to susceptible Sahabadi sheep (Michael *et al*., 2020). Increased expression of IL-13, IL-5, and IL-4 occurs in lymph node cells of resistant wool sheep infected with *Trichostrongylus colubriformis* (Pernthaner *et al*., 2005). In younger wool sheep of a different breed, Lacroux *et al* (2006) reported increased IL-13, IL-5, and IL-4 with no difference in IFN-γ, IL-12, IL-10, or TNFα in abomasal lymph tissues compared to the susceptible sheep. The IFN-γ gene regulate immune responses to infections (Charon, 2004). Association between IFN-γ gene and resistance to nematode infection in Texel breed of sheep has been proposed by Sayers *et al* (2005). Increased concentration of serum IFN-γ as well as increased mRNA expression of *IFN-γ* gene have been reported in resistant sheep compared to susceptible sheep (Brahma *et al*., 2023). Recently, interaction between Th1/Th2 and T-reg genes has been suggested to modulate immune response against GI nematode parasites instead of straightforward Th1 or Th2 immunity responsible for susceptibility or resistance (Arsenopoulos *et al*., 2017). As reported earlier by Michael *et al* (2020) and Claerebout *et al* (2005) an interaction between Th1 and Th2 type immune responses have been found to be involved in resistance against *H. contortus* in Garole sheep.

Conclusion

In the resistant Garole sheep, increased level of histamine, IgA, IFN-γ and IL-4 was observed between 14 DPI to 28 DPI compared to susceptible Sahabadi sheep and after 27 DPI all the infected Garole sheep became free of *H. contortus* infection. Therefore, it can be concluded that those immune components might be responsible for comparative resistant in Garole sheep against *H. contortus* also have played some role in elimination of adult worms.

Acknowledgements

The authors thankfully acknowledge the financial assistance by the Project entitled “All India Network Programme on Gastrointestinal Parasitism” funded by the Indian Council of Agricultural Research, New Delhi, India.

Author contributions: All authors contributed to the study conception and design. **RJ:** Conceptualization, Project administration, Writing – original draft. **GB:** Methodology, Investigation, Data curation. **SR:** Methodology, Investigation. **AH:** Methodology. **SD:** Investigation and Data analysis. **AB:** Writing – review and editing. All authors approved the final version of this manuscript.

Conflict of Interests

There is no conflict of interest.

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