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Resistance of Black Bengal goat to *Haemonchus contortus*

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Abstract

Artificial challenge trial and field trial were carried out in two populations of Black Bengal goat in Bangladesh to evaluate the resistance to *Haemonchus Contortus*-a major gastrointestinal nematode in goat. Artificial challenge trial revealed that there was no difference in susceptibility of *H. contortus* in Black Bengal goat of hilly region (BBH) and Black Bengal goat of western region (BBW). In absence of parasitic infection, growth rate of kids of both populations were almost equal. Field trial was conducted with kids of BBW population to find out the within breed variation. Numerically, there was variation among the individuals for parasitic load however the results were statistically insignificant ($p > 0.05$). At individual level, the parasite load ranged from 100 to 400 eggs per gram (epg). The location of villages, age and sex of kids did not influence on the parasitic load of kids. There was no interaction for location, age and sex for body weight at day 8. The Packed Cell Volume and Hemoglobin values differed significantly ($p < 0.01$) due to age of kids at day 8 and 28 after deworming. The results indicate that Black Bengal goat can be considered as resilience to *H. contortus*.

Key words: Black Bengal goat, *Haemonchus contortus*, FEC, resistance

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Introduction

Gastrointestinal parasitism is an important limiting factor for deteriorating the health and productivity of livestock including goat. Many gastrointestinal parasites are found in goat. These usually include *Haemonchus, Oesophagostomum, Ostertagia, Chabertia, Nematodirus, Trichuris, Moniezia* and *Fasciola*. *H. contortus* is also known as red stomach worm, wire worm or barber's pole worm which is very common gastrointestinal parasite and one of the most pathogenic nematodes of goats. Like other parasites, *Haemonchus contortus* causes economic losses in terms of mortality, stunted growth, and loss of bodyweight gain, decreased milk and meat production (Silvestre *et al*., 2000; Sahlu *et al.,* 2009). The standard treatment to control the parasites has been the use of anti-helminthes drugs. However, parasites have attained resistance to anti helminthes drug in many cases which is an increasing problem. Livestock producers and breeders are now trying to develop breeds that have resistance to infectious diseases. In this regard, they are trying to exploit the within and between breed genetic variations in resistance to infectious diseases. There is well-documented evidence for within and between breed genetic variations in resistance to infectious diseases, such as gastrointestinal nematode infections, diseases due to mycotoxins, bacterial diseases including foot root and mastitis, ectoparasites such as flies and lice, and scrapie, the small ruminant transmissible spongiform encephalopathy. Within-breed variation, disease resistance in many cases is a heritable trait. This offers the opportunity to select animals for enhanced resistance to the disease. The feasibility of this approach has been experimentally demonstrated and breeding programs for selecting commercial animals that enhanced resistance are being successfully established, especially for sheep as compared to goat (Gray *et al.,* 1995; Bishop *et al.*, 2007; McManus *et al.*, 2014).

Goat is an important ruminant species in Asia and Africa, which represents 95% of the total goat population. It is also important ruminant species in Bangladesh as it stands second in number next to cattle. Bangladesh possesses 16.02 million goats distributed all over the country (BBS, 2010). More than 90% of the goat population in Bangladesh is comprised of Black Bengal goat having some variants in color and size; the majority of remainder is imported Indian breeds and their crosses (Husain, 1993; Afroz *et al.*, 2010). Black Bengal goat is the only recognized breed of livestock in Bangladesh. Geographically and genetically, Black Bengal goat can be divided into three populations, viz., (i) Black Bengal West (BBW) - the goat that found in the Western region of the country, (ii) Black Bengal Central (BBC) – the goat found in the central of the country, and finally (iii) Black Bengal East (BBH) - the goat found in the hilly region of eastern part of the country (Husain, 1993). Published reports revealed the differences in morphology and gene constituents in the mentioned three populations of Black Bengal goat (Nozawa *et al.,* 1984; Husain, 1993).

H. contortus is the most frequent gastrointestinal nematode found in Black Bengal goat of Bangladesh as reported by a number of investigators (Kamal *et al.,* 1993; Howlader *et al.*, 2002; Hassan *et al.*, 2011; Nahar *et al.,* 2012; Akanda *et al.,* 2012). The common practice to control this parasite is to use of anti helminthes drugs. No attempt has ever been made in Bangladesh to investigate within and between breed variations in Black Bengal goat for resistance to *H contortus* to develop *H contortus* resistant Black Bengal goat. We, therefore, undertook this study to identify the population of Black Bengal goat having resistant to *H contortus* based on artificial challenge trial and field trial.

Materials and Methods

Experimental sites and populations of goat: The experimental sites were in two locations Viz., Natore representing western part of the country and Bandarban Hill district representing hilly region of the eastern part of the country as shown in Figure 1.

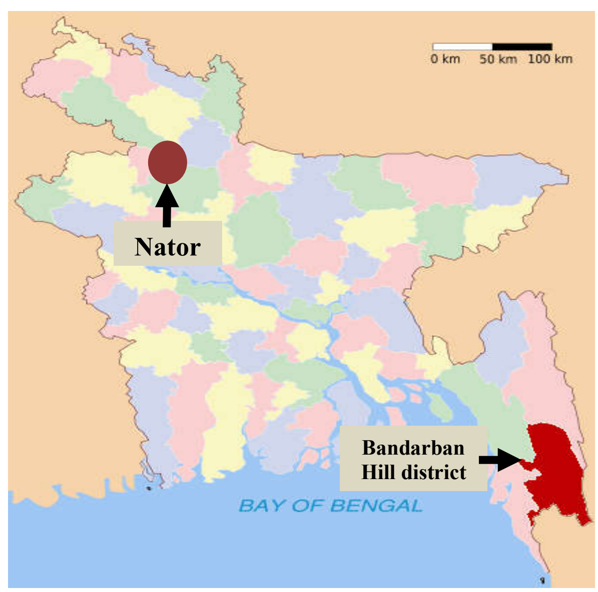

Figure 1. Experimental sites indicated by red colour in the map.

The GPS coordinates of three locations were N: 24°07.163′; E: 89°03.997′, and N: 24°47.523; E: 091°43.893 for Natore and Bandarban Hill district respectively. Ecology of two locations is different. Bandarban Hill district is hilly region of the country which is rich in forest and soil is sandy. Natore is flood -fed area which is rich in plain land and soil is clay loom. Natore is rich in fruit trees and leaves of those fruit trees are available to goat. Experimental animals (Black Bengal goat) were selected from those two regions.

***Management of kids for artificial challenge trial*:** Artificial challenge trial was conducted from April to July 2011 in Natore. Goats selected from two regions where Natore represented BBW goat and Bandarban Hill district represented BBH goat, respectively (Plate 1). 20 BBH kids and 20 BBW Kids of 4 to 5 months of both sexes were used for this trial.

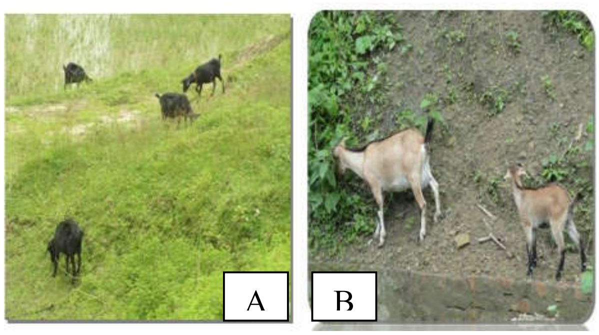

Plate 1. Black Bengal goats are under natural grazing condition. "A" represents BBW goat of Natore district and "B" represents BBH goat of Bandarban Hill district.

The BBH kids were transferred to Natore where both BBW and BBH kids were reared. 15 days was given for acclimation of BBH kids. Then 40 kids were distributed to 10 farmers, 2 BBW kids and 2 BBH kids for each farmer. All the kids were kept in confined condition and fed only jack fruit leaves and concentrate mixture. Jack fruit leave was fed at ad libitum and concentrate mixture was fed at the rate of 50 g/d/kid. Concentrate mixture consisted of 3 parts crushed maize grain and 1 part crushed mustard oil cake. Deworming was done by Ivermectin @ 1ml/10 kg body weight of kid. Faeces were examined at weekly interval to ensure for complete deworming of kids. Two week interval was allowed between complete deworming that is indicated by zero FEC and inoculation of Larvae of stomach worm artificially. Larvae of *H. contortus* were administered artificially to kids which was counted as day 0. On day 0 a total of 5000 L3 larvae of *H. contortus* were administered to each kid. Kids were monitored to ensure that the material had been swallowed and not regurgitated. Body weight was measured, faeces samples and blood samples were collected for further processing at day 0, 28, 35 and 42.

***Management of kids for field trial*:** The population of field trial was selected on the basis of results of artificial challenge trial and facilities available. BBW population was selected for the field trial. So, this trial was conducted in Natore in three villages using 200 BBW kids for the period from August 2012 to September 2014. The age of kids ranged from 3 to 6 months old. 75 kids, 79 kids and 46 kids were used from Nagar (Village-1), Udbaria (village-2) and Moukhora (village-3) village respectively. The distance of villages was 20 to 25 km; but those had the same environment. The kids were dewormed by Invermectin @ 1ml/10 kg body weight of kid. Faeces and blood were collected after 7 days i.e. at day 8 and body weight was recorded on the same days. Body weight of kids were recorded, faeces samples and blood samples were again collected after 28 days (termed day 28).

***Faecal sample collection and examination*:** Faecal samples were collected by a two-finger procedure directly from the rectum of experimental kids. About 5gm fresh faecal samples were collected and preserved in 10% formalin contained in suitable airtight containers viz., screw-cap bottles. Each vial was marked with the unique identification number similar to kid identification number. During sample collection, information on sex and age of kids was enlisted. Samples were kept in refrigerator at 4°C temperature until examined. Faeces were examined for presence of eggs, cysts and larvae of GIN parasites. Species was determined as *H. contortus* after Zajac and Conboy (Zajac *et al.,* 2012). Eggs per gram (EPG) of experimental kids was determined by McMaster method. In this method, a known volume of feces (4gm) was thoroughly suspended in a volume of (56ml) saturated salt solution. The suspension was stirred through a 150 mm mesh sieve to remove the course particles. A portion of the suspension was withdrawn with the help of Pasteur pipette and allowed to run into the chambers of the McMaster slide. The slide was kept to stand for 5 minutes to allow the eggs to float. The eggs in the two chambers were counted using low power objectives (10x). The number of eggs per gram of feces was calculated by using the following formula:

Egg/gm = [no. of egg counted x (T/V)]/F

T = Total volume of faeces/flotation solution mixture,
V = Volume of aliquot examined in slide, and
F = grams of faeces used. The sensitivity was 50.

***Estimation of packed cell volume and hemoglobin value*:** Blood samples were collected for hematological assessment like Packed Cell Volume (PCV) and Hemoglobin (Hb) from jugular vein of kids in EDTA coated venoject tube. PCV was determined by the micro-hematocrit method. The result of the PCV was expressed in percentage (%). Hemoglobin value was determined by the use of Sahli's acid haematin method and the result was expressed in g/dl.

***Statistical analysis*:** Since the FEC data were not normally distributed, i.e. they were positively skewed, logarithmic transformation were applied before analysis. The descriptive statistics viz., arithmetic mean, variance, standard error, student t-test etc. were carried out by following the described procedure of Snedecor and Cochran (1980). Data were transformed into $\log_{10}$ (n+1) formulae (where n is the number of egg per gram feces), and then analysis of variance was done by using Generalized Linear Model procedures of computer package of SAS (SAS Institute Inc. USA, 1998).

Results

***Artificial challenge trial*:** In artificial challenge trial, only 4 kids out of 20 developed Haemonchosis after larval inoculation in BBW population and FEC ranged from 200 to 900 epg. There is no evidence of parasitic development in rest of the 16 kids. On the other hand, only 2 kids in BBH population developed haemonchosis and FEC was 500 and 900 epg, respectively. The rest 18 kids had no parasitic development. The FEC of individual kid has been present in Figure 2. Figure 2 reveals that both populations had almost similar susceptibly to *H. contortus*, though BBH was slightly better than BBW population.

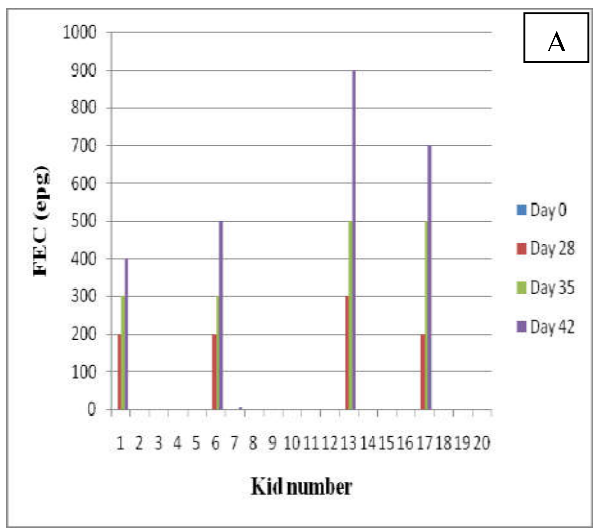

Figure 2A. Faecal egg count of BBW kids during artificial challenge trial.

However, the difference in FEC between populations was insignificant ($p > 0.05$). In absence of parasitic infection, changes in body weights of kids from both populations were almost equal throughout the experimental period (Figure 3). The PCV% remained constant in BBH population throughout the experimental period. The PCV% of kids of BBW population was slightly lower on day 35 than that of day 8 and day 42 in (Figure 4).

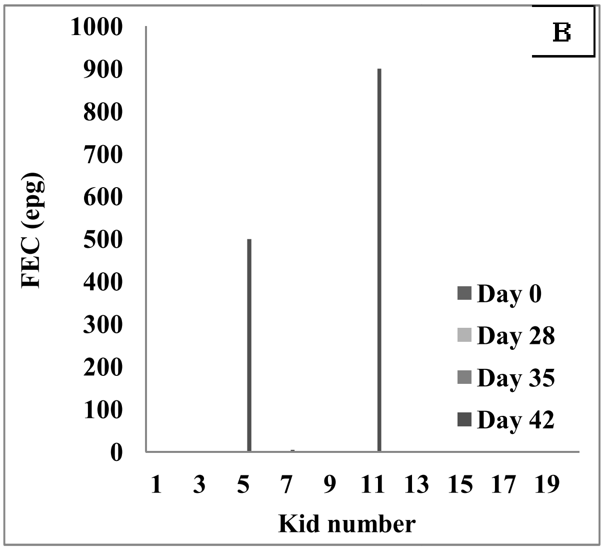

Figure 2B. Faecal egg count of BBW kids during artificial challenge trial.

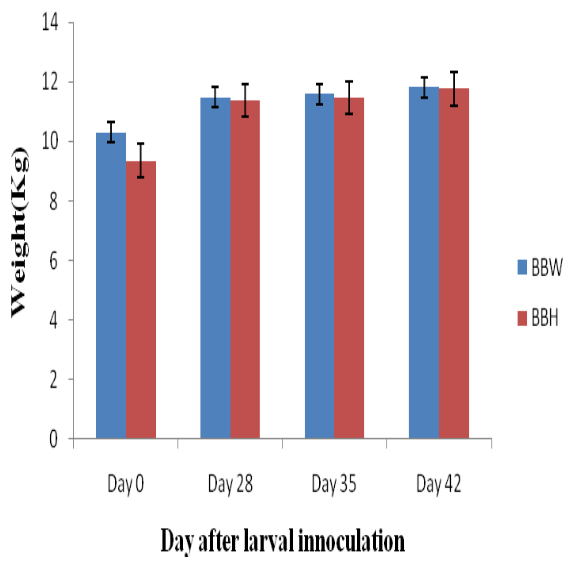

Figure 3. Body weight of kids from both BBH and BBW population during artificial challenged trial.

However, the difference in body weight and PCV% in both populations was insignificant (p > 0.05). The Hb value of both populations increased continuously from day 0 to day 35, and then decreased slightly in both populations as shown in Figure 5. The difference in Hb value between two populations was non-significant (p > 0.05).

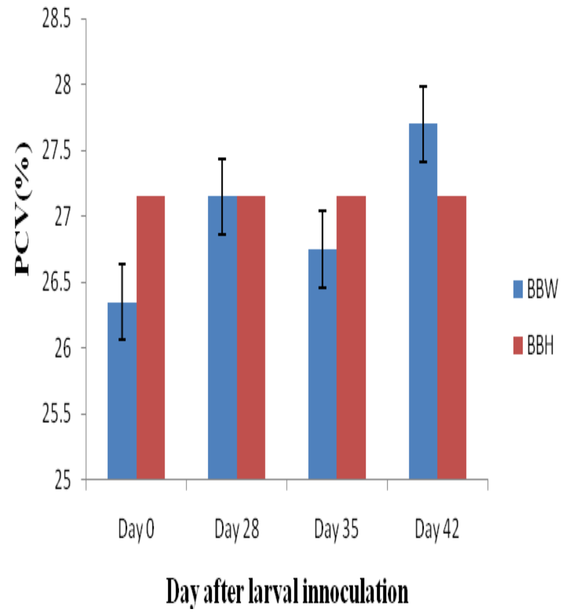

Figure 4. PCV% of kids of BBH and BBW population during artificial challenge trial.

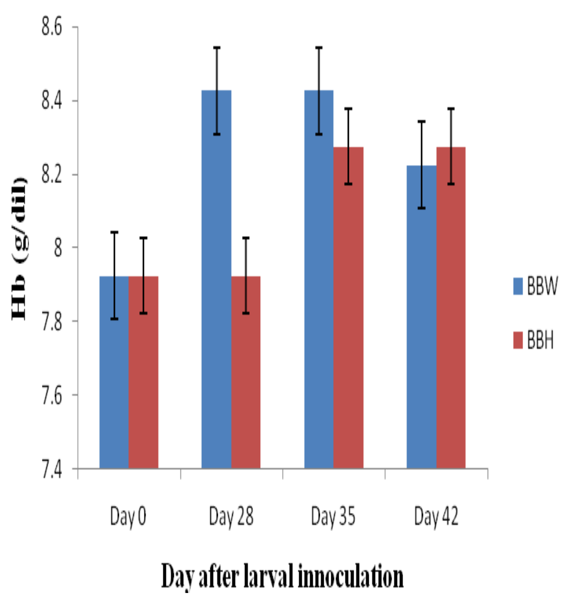

Figure 5. Hb values of kids of BBH and BBW kids during artificial challenge trial.

Field trial: Field trial was conducted with 200 kids of BBW population to find out the within breed variation. The percentage of kids infected by *H. contortus* at day 28 in three locations within BBW population has been presented in Table 1.

Table 1. FEC of BBW kids at day 28 in different villages during field trial.

Parameter	Day 8			Day 28			
	Village 1	**Village 2**	**Village 3**	**Village 1**	**Village 2**	**Village 3**	**Overall**
Number of kid tested	75	46	79	75	46	79	200
Number of kid infected	0	0	0	19	6	3	28
% of kid infected	00.00	00.00	00.00	25.33	13.04	3.80	14.00
Average FEC (EPG)	00.00	00.00	00.00	226.32[a] ± 21.46	166.67[b] ± 42.15	133.33[c] ± 33.37	203.57 ± 18.17
Maximum FEC (EPG)	00.00	00.00	00.00	400	300	200	400
Minimum FEC (EPG)	00.00	00.00	00.00	0	0	0	0

The percentages of infected kids were 25.33, 13.04 and 3.80 for village -1, village -2 and village -3, respectively and overall infection rate was 14%. Average FEC was 226.32 ± 21.46, 166.67 ± 42.15, 133.33 ± 33.37 and overall mean was 203.57 ± 18.17 for village -1, village -2 and village -3, respectively. The maximum FEC was only 400 in all locations. Only 9 out of 127 male kids developed Haemonchosis and 19 out of 73 female kids developed Haemonchosis. The average FEC was 3.66 ±0.09 and 3.69 ± 0.10 for male and female kids, respectively. The difference of FEC between two sexes were insignificant ($p > 0.05$). The relative parasite load of male and female kids has been presented in Figure 6. The average FEC was 3.63±0.11, 3.80±0.12, 3.76±0.15 and 3.50±0.17 for kids of 6 months, 5 months, 4 months and 3 months of age, respectively. The differences of FEC of kids for four aged groups were insignificant ($p > 0.05$). The relative parasitic load of different aged groups of kids has been presented in Figure 7. The parasite load in terms of FEC was analyzed statistically for finding out the differences for location, sex and age (Table 2).

Table 2. ANOVA of FEC for location, sex and age of kids at day 28 during field trial.

Source	DF	Sum Square	Mean Square	F Value	Pr > F	Significant
Village	2	2.75512425	1.37756212	1.66	0.1930	NS
Sex	1	0.02079748	0.02079748	0.03	0.8744	NS
Age	3	1.93542906	0.64514302	0.78	0.5080	NS
Location X sex	2	5.65723848	0.94287308	1.14	0.3431	NS
Location X age	6	0.62742305	0.31371152	0.38	0.6858	NS
Age X sex	3	0.61777840	0.20592613	0.25	0.8626	NS

The differences in FEC for location, sex and age were found to be insignificant ($p > 0.05$). Similarly interaction between location x age, location x sex and sex x age for FEC was also found insignificant ($P > 0.05$). The performances (body weight, PCV and Hb values) of kids at day 8 and 28 during field trial have been presented in Table 3. The performance data was analyzed statistically for location, age and sex, and for interaction between location x age, location x sex and age x sex. The body weight at day 8 and day 28 after deworming varied significantly among 4 aged groups and 2 sexes ($p < 0.01$). But there was no interaction for location X age, location X sex and age X sex for body weight at day 8. Interaction was observed only between age x sex at day 28. The PCV and Hb values differed significantly ($p < 0.01$) due to age of kids at day 28 after deworming whereas location and sex has no effect on the PCV and Hb values of kids.

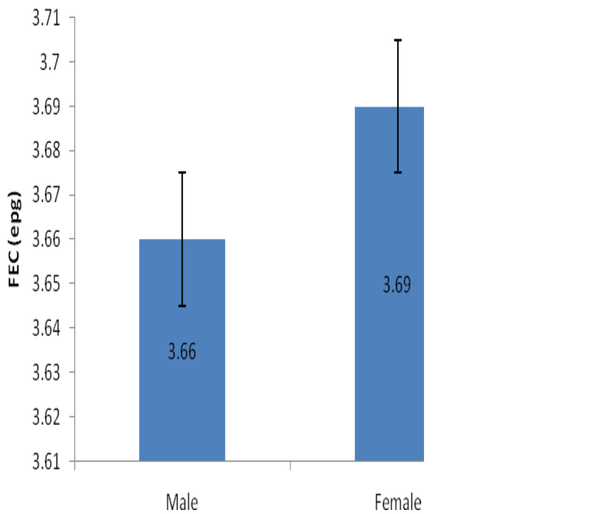

Figure 6. FEC of male and female kids during field trial.

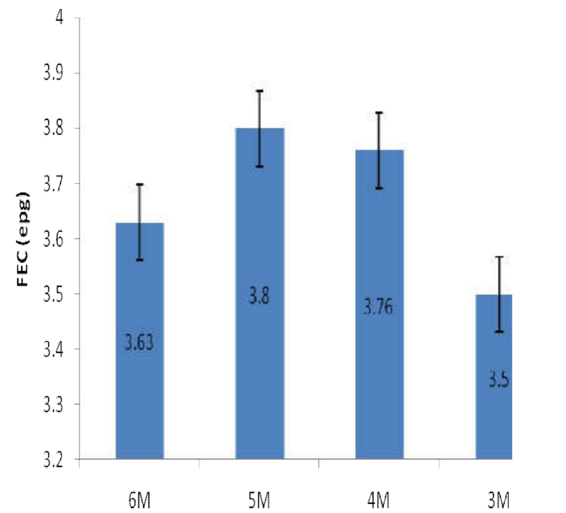

Figure 7. FEC of kids from different age groups during field trial.

Table 3. Least square mean for body weight, PCV and Hb values of kids at day 8 and 28 during field trial. Figures in the parentheses indicate the number of observation.

Parameter	Day 8			Day 28		
	Village 1	Village 2	Village 3	Village 1	Village 2	Village 3
Body weight (kg)	8.28 ±0.20 (75)	8.45 ±0.18 (46)	8.39 ±0.14 (79)	8.99[a] ±0.25 (75)	9.20[c] ±0.23 (46)	8.91[b] ±0.18 (79)
Haemoglobin (g/dil)	-	-	-	7.58[c] ±0.15 (75)	8.02[a] ±0.13 (46)	7.87[b] ±0.10 (79)
Packed Cell Volume (%)	-	-	-	26.36[c]±0.32 (75)	26.91[a]±0.28 (46)	26.62[b]±0.22 (79)

Discussion

Artificial challenge trial is a recognized method for detecting the relative resistance of parasite specially *Haemonchus contortus* between two breeds. FEC is common parameter to measure relative resistance between/among breeds. In addition to body weight, haematological parameters like HB, PCV etc are also measured. The field trial is done to detect within breed variation for parasite resistance. The artificial challenge trial and field trial have been conducted to find out the between and within breed resistance to *H. contortus* of small ruminant in many countries of Europe, Australia, Africa, Asia and USA (Pralomkarn *et al.*, 1997; Baker *et al.*, 2001; Amarante *et al.*, 2004; Cezar *et al.*, 2010; Yin *et al.*, Chiejina *et al.*, 2011; Babar *et al.*, 2015; Tasfaye *et al.*, 2015).

FEC and PCV are considered as valuable measures for finding whether breeds have resistance against internal nematodes. Ideally, there should be low FEC and higher PCV in genetically resistant animals. In present study, the most kids of BBW and BBH populations had no Haemonchosis during artificial challenge trial, indicating equal resistance of both populations to *H. contortus.* Field trial conducted on BBW revealed very low level of infestation (FEC on an average 200 epg and maximum FEC was only 400 epg) in few kids (only 14% kids developed Haemonchosis). In absence of *H. contortus* infestation, PCV of BBW and BBH population remained almost constant. Such results were observed for FEC and PCV values for sheep and goat by the scientists (Pralomkarn *et al.*, 1997; Costa *et al.*, 2000; Baker *et al.*, 2001; Vanimisetti *et al.*, 2004; Rout *et al.*, 2011 Chiejina and Bahnke, 2011; Mandonnet *et al.,* 2001; Babar *et al.*, 2015). Costa *et al.* (2000) studied the variability between and within breeds with respect to nematode. Eggs per gram counts (EPG), Packed cell volume (PCV) and hemoglobin (Hb) was studied in 36 yearling female goats of the Caninde, Bhuj and Anglo-Nubian breeds, exposed to *H. contortus*. Nematode-free goats were turned to a contaminated paddock in late February. After that fecal egg per gram counts (EPG), packed cell volume (PCV) and hemoglobin (Hb) were determined at 2-week intervals up to Week 18. The EPG, transformed as [log (EPG C 75)], varied ($p < 0.01$) between goats within breeds and between weeks of exposure, but not between goat breeds ($p > 0.05$). PCV and Hb were affected by goat breeds ($P < 0.05$), by goats within breeds ($p < 0.01$) and by weeks of exposure ($p < 0.01$). Anglo-Nubians had higher ($p < 0.01$) PCV and Hb than Caninde, Bhuj had intermediate values. There were two EPG rises; one between Weeks 6 and 10 and the other between Weeks 14 and 16. The within breed variability was marked during the EPG rise on Week 6, when individual egg counts ranged from 130 to 2500. The EPG rises coincided with drops in Hb. PCV presented a similar trend, though not as marked. *Haemonchus* was responsible for more than 95% of nematode eggs counted. Baker *et al.* (2001), Vanimisetti *et al.* (2004), Rout *et al.* (2011), Chiejina and Behnke (2011), Mandonnet *et al.* (2001) and Babar *et al.* (2015) also reported similar trend in FEC and PCV values in their studies with goat and sheep in tropical and sub-tropical condition. They also mentioned that body weight gain and Hb value were correlated with FEC i.e. parasitic load. Body weight gain was higher in kids with low level of parasite infection and Hb values were also high in such a case. The findings of the present study also matched with their findings. In absence of *H. contortus* infection in most of the kids, the PCV values and Hb values remained constant and kid's growth was smooth.

Results and discussion made so for indicates that kids of both populations of Black Bengal goat were infected by *H. contortus.* However, the difference among the populations for parasitic load was statistically insignificant ($p>0.05$). Moreover, both populations had low level of parasitic load in term of susceptibility. The kids could maintain the normal growth even after infected by parasite. Black Bengal goat, therefore, can be considered as resilience to *H. contortus.*

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