Assessment of genetic resistance and abomasal tissues expression of Yichang white goat experimentally challenge with *Haemonchus contortus* infection

AI Omar¹, MBB Alam², TNT Thi², M Kamaruzzaman³, MI Ali², TW Binegde⁴, X Du²,⁵, S Zhao²*

¹National Engineering Laboratory for Animal Breeding, Key Lab of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Agriculture, College of Animal Science and Technology, China Agricultural University, Beijing 100193, P. R. China; ²Key Lab of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, P. R. China; ³The State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China; ⁴School of Veterinary Medicine, Wollega University; PO Box: 395, Nekemte, Ethiopia; ⁵Key Laboratory of Agricultural Bioinformatics of Hubei Province, College of Informatics, Huazhong Agricultural University, Wuhan 430070, PR China.

Abstract

Our previous association study revealed the mutation in candidate immune genes (*NOD1* & *NLRP9*) was significantly associated with FEC of *Haemonchus contortus* infection in Yichang white goats, but the relative expression of mRNA of those genes associated with resistance to *H. contortus* was not investigated. Aim of the current experiment was to evaluate the susceptible and resistant individuals to nematode infection within the population of Yichang white goat (YWG) and assess the differential level of mRNA expression of those candidate genes in the abomasal tissues of susceptible and resistant goats. Fecal egg count (FEC) was determined using a modified McMaster technique, and the hematological parameter was measured by Mindray auto hematology analyzer. Phenotype data were collected and analyzed using a generalized linear model with SAS statistical program. Field investigate revealed that the prevalence (76%) with maximum parasite load (734.34±84.21epg) of *H. contortus* occurred in August within the experimental flock. FEC in resistant group (103.38±1.20epg) and susceptible group (1180.25±43.53epg) demonstrated the presence of two distinct goat populations within this breed. Four resistant and four susceptible goats were selected from each group. The parasite infection was established by artificially challenge with 5000 infective L₁ larvae of *H. contortus*. Abomasal tissues were collected from all experimental goats after 42 days of post-infection. FEC, Body weight, packed cell volume, and hemoglobin value were significantly different (*P* < 0.01) between resistant and susceptible group of goats. Quantitative real-time PCR in abomasal tissues revealed that the expression level of mRNA for *NOD1* (*P* < 0.00001), *IFNG* (*P* < 0.0001), *NLRP9*, *TLR8*, *IL32*, (*P* < 0.001) and *IGF1* (*P* < 0.01) was higher in resistant goat compared to susceptible, except *SFTPDA1*. These findings revealed the presence of genetic resistant individuals to *H. contortus* within the goat breed and expression of *NOD1* and *NLRP9* genes proved the positive finding of our previous study. Presence of genetic resistant individuals in Yichang white goat YWG breed could be a good candidate for selective breeding and highly expressed genes related to resistant could be used as biomarkers to develop *H. contortus* resistant goat population.

Key words: Goat, *Haemonchus contortus*, challenge trail, gene expression, genetic resistance

*Corresponding Author:* shzhao@mail.hzau.edu.cn
Introduction

Goat is one of the most valuable domestic animals among livestock species. It was domesticated about 11,000 years ago in the Asian region and is recognized as an important source of milk, meat, skin, fiber and great source of income for small farmers, and shepherds in marginal areas of developing countries (Chessa et al. 2009; Colli et al. 2015). Presently, goat production becomes a profitable business to the farmers especially the rural people who efficiently contribute to the economy of the country. However, goat production is threatened by the gastrointestinal parasitic diseases particularly in the tropical and subtropical regions of Asia and Africa. Among the gastrointestinal parasites, *Haemonchus contortus* is the predominant nematode that affected goat production severely under natural grazing condition (Yin et al. 2013; Ma et al. 2014; Omar et al. 2016).

*H. contortus* is a common blood-feeding nematode responsible for high morbidity and mortality in the flocks of high temperate and rainfall areas. Each adult parasite sucks around 0.05 to 0.07 ml of host blood daily thereby resulting in loss of around 250 ml blood infected by a total of 5000 parasites and can cause severe anemia and finally death (Clark et al. 1962; Rodriguez et al. 2015; Omar et al. 2016). Treatment with anthelminthic is a common practice for control of parasitic infestation in sheep and goats worldwide. This practice is leading to develop the widespread anthelminthic resistance to the nematode. In this situation, developing resistant goat breeds to gastrointestinal nematode could be the alternative strategy for controlling this problem (Getachew et al. 2015). Genetic variation for resistance to nematode infection varies among breeds and within breed even between individuals and this is controlled by several genes that have been recognized for sheep and goats (Mandonnet et al. 2001; Chauhan et al. 2003; Rout et al. 2011; Traoré et al. 2017). The proliferation and activation of mast cells, eosinophils, and globules leukocytes of the abomasal mucosa contributed to this genetic resistance against nematodes (Bambou et al. 2013). A number of studies described the genetic resistance to GIN of different sheep breeds, and it is mainly associated with the immune responses of hosts. However, few studies have been conducted on the immune response of goats to *H. contortus* infection (Baker et al. 2001; Chiejina & Behnke 2011; Omar et al. 2019; Kurukulasuriya et al. 2018). Several goats and sheep breeds are naturally resistant to gastrointestinal parasites infection and evaluating the relative resistance of goat breeds using artificial challenge trial should be considered as a way towards mapping of genes controlling internal parasite (Getachew et al. 2015). By the relative expression analysis, it has been proved that resistant animals express hundreds of genes involved in their immune response (Diez-Tascón et al. 2005). Subsequently, it was hypothesized that the expression of immune genes would increase the resistant capability to the parasite and would possibly be used as biomarkers for producing *H. contortus* resistant goat breeds.

China has many native goat breeds rearing for meat, milk and wool purpose scattered in the different agro-ecological postural zone and commonly infected by nematode species particularly *H. contortus* (Omar et al. 2019; Ma et al. 2014). Yichang white goat is the native goat breed of China and it has been observed a greater degree of resistance to *H. contortus* infection and great inconsistency of FEC within this breed (Omar et al. 2016; Omar et al. 2019; Alam et al. 2019) thus making it for perfect candidate for selective breeding program to improve the resistance to nematode infection. Our previous study (Omar et al. 2019) revealed some novel polymorphisms in nucleotide-binding oligomerization domain containing 1 (*NOD1*) and NLR family pyrin domain containing 9 (*NLRP9*) genes that were significantly associated with disease resistant trait, particularly *H. contortus* (Omar et al. 2019). However, several investigators also reported similar results for dopamine receptor binding 1 (*DRB-1*), insulin-like
Based on the fact that Yichang white goat (YWGs) are commonly reared under natural grazing condition and semi-intensive system with comparable levels of exposure to gastrointestinal parasites.

**Screening the animals for parasitic load:** For the evaluation of the parasitic load in the term of fecal egg count (FEC) expressed as eggs per gram of feces (epg), goats were selected from natural grazing condition and confirming no deworming history for the past one year within the flock. FEC was performed by the rapid modified Macmaster technique (Gordon & Whitlock 1939; Zajac & Conboy 2012) in every month from March 2016 to December 2016 to assess the parasitic load of *H. contortus* infection in the herd for goats age 6 month to above and to judge the resistance or susceptibility based on low and high responder of FEC of YWGs in our studied population. The numbers of epg was calculated as:

\[ \text{Egg/gm} = \frac{[\text{no. of egg counted} \times (T/V)]}{F} \]

Where, T is the total volume of the mixture of feces and flotation solution, V is the total volume of solution examined on the slide, and F is the grams of feces used. The sensitivity of the assay was 50 eggs per gram of feces; each observed egg corresponded to 50 epg.

An initial sample of 81 Yichang white goats of 9 to 12 month of age were selected from a flock of 450 goats and FEC, body weight, circulating hemoglobin levels (Hg), and pack cell volume (PCV), white blood cell (WBC) and red blood cell (RBC) were determined to assess the susceptibility of parasites infection. Based on their FEC, goats were divided into two groups viz. resistant and susceptible group. Goats with High FEC group (FEC > 500) was considered as a high responder and assigned to be more susceptible group and goats with Low FEC (FEC < 500) was considered as a low responder and assigned to be the more resistant group to *H. contortus*. FEC, circulating hemoglobin (Hg), packed cell volume (PCV), Red Blood Cells (RBC), White Blood Cells (WBC) and animals body weight (BW) from resistant and susceptible goats were determined for selecting goats for the challenge experiment. Finally, 4 resistant goats (lowest number
of EPG count) and four susceptible goats (highest number of EPG count) with an average age of 305 ± 10 days were selected for the artificial challenge.

**Parasitological technique:** To procure L3 larvae for infection, adult female *H. contortus* was collected from the abomasum of the infected goats from the nearby abattoir. The female worms were washed and crushed using mortar and pestle to liberate eggs. Filtration was made to avoid debris, and eggs were collected by sedimentation process using slow centrifugation at 100 rpm for 2 min. Coprocultures were maintained under laboratory conditions (with 25°C and 80% humidity) and moistened and aerated daily for eight days (Ojeda-Robertos *et al.* 2017). A culture of third-stage (infective stage) larvae (L3) in vitro condition was recovered following the slandered Baerman technique (Hansen & Perry 1994; Ojeda-Robertos *et al.* 2017). Harvested larvae were counted in each of five 20ul aliquots, and larval density (larvae/ml) was estimated. After harvesting, larvae were stored in deionized water at 4°C and used within one month to infect experiment animals.

**Nematode challenge trial:** Eight goats (4 resistant and 4 susceptible) were selected to dry lot for challenge trial and treated with broad-spectrum anthelmintic viz., Ivermectin (0.25 mg/kg body weight) and 15 days later, with levamisole (8 mg/kg body weight) to ensure the animals were completely dewormed (Bambou *et al.* 2013; Kurukulasuriya *et al.* 2018). In dry lot, each goat were fed 30 g/kg BW of balance feed including GIN-free fresh grass per day on a dry-matter basis, and a concentrated mixture was provided twice per day at a rate of 1.5% of BW containing 18% crude protein and 10.6UJ/kg of metabolizable energy on a dry matter basis (Ojeda-Robertos *et al.* 2017). When all experimental goats achieved a FEC of zero ensured by microscopic examination, resistant and susceptible goats were inoculated with a single dose of 5,000 third-stage (L3) larvae of *H. contortus* (Notter *et al.* 2003; Getachew *et al.* 2015; Kurukulasuriya *et al.* 2018). The day of inoculation was considered to be day 0, and FEC, PCV, Hg, and BW were determined weekly for six weeks from the day of inoculation.

**Abomasal tissue preparation for RNA extraction:** Animals were sacrificed following the proper procedure and by national humane euthanasia guidelines at 42 days post-infection. After slaughtering the animals, gastrointestinal tract and abomasum were removed from the digestive tract. The abomasal wall was then cut along the greater curvature and washed with phosphate buffer solution (PBS) at room temperature. A 2cm² section of tissue, including the full thickness and 1-fold of the abomasal wall, was removed from the fundic region (MacKinnon *et al.* 2009). Approximately 100 gm samples were weighted, immediately frozen by liquid nitrogen and stored at -80°C until extraction of total RNA.

**Candidate gene selection for mRNA expression:** Candidate genes (*NOD1*, *NLRP9*, *TLR8*, *SFTPA1*, *IGF-1*, *IL32*, *IL33* and *IFNG*), that were significantly associated with FEC trait of resistance to *H. contortus* infection in goat, were selected based on our previous association study and reports of other investigators for such study (Bressani *et al.* 2014; Alim *et al.* 2016; Asif *et al.* 2016). Oligonucleotide primers were designed to see the mRNA expression for our selected genes using the coding sequences (CDS) of caprine mRNA following NCBI primer design web program (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and optimized with the primer 7 software to avoid self-priming and primer-dimers. The primers used for Quantitative Reverse-Transcriptase Polymerase Chain Reaction (qRT-PCR) are presented in Table 1.

**RNA extraction and quantitative real-time PCR (qRT-PCR):** Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s recommended procedure. Quantity of RNA was assessed using a Nano Drop ND2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and gel electrophoresis was used to ensure the quality of RNA (MacKinnon *et al.* 2009). Quantitative real-time PCR (qRT-PCR) was performed...
to assess the expression of our selected candidate genes (Table 1) using cDNA generated from approximately one µg of RNA using a standard Prime Scipt™ RT reagent kit with gDNA eraser (Perfect Real Time, TAKARA Bio, Inc.). CFX-96 Bio-Rad thermal cycler with SYBR green real-time PCR master mix (Toyobo Co., Ltd., Osaka, Japan) was used for qRT-PCR. The qRT-PCR protocol was a single cycle of denaturation at 95°C for 5 minutes, 45 cycles of denaturation at 95°C for 20 seconds, annealing at 58°C for 20 seconds, and extension at 72°C for 15 seconds. Beta-actin (ACTB) was used as a housekeeping gene to normalize the samples (Rebouças et al. 2013).

Table 1. Primers information for the measurement of mRNA expression by a quantitative reverse-transcriptase polymerase chain reaction.

| Gene Name | Primer | Sequence 5' to 3' | Product length | Ref. Sequence no. |
|-----------|--------|-------------------|----------------|-------------------|
| NOD1      | F      | CTCCCACACCTCGATGTC | 105            | XM_018047107.1   |
|           | R      | GCAGACGGTCCTGAGCC |                |                   |
| NLRP9     | F      | ACTTTGATCGATCTGAGC | 109            | XM_005692931.2   |
|           | R      | GCATCCATATGGAGAGGCT |               |                   |
| SFTPA1    | F      | TCCCAGAAAGCTGGAGAA | 126            | NM_00109728.2    |
|           | R      | CCTGTAGCAGAGGATTA |                |                   |
| TLR8      | F      | AAGGCTCTGGATACC   | 135            | XM_013976677.2   |
|           | R      | ACATCGCAAGGATAGCTTCC |              |                   |
| IL32      | F      | AAGTCACGCCTCCAGGGA | 139            | XM_013974707.2   |
|           | R      | GATGGAATATCGAAGGCTCT |              |                   |
| IL33      | F      | CAGGGGAAATATCAAAATAAGA | 196     | XM_013965943.2   |
|           | R      | TTCTGTTGATCCACACTTTC |              |                   |
| IGF1      | F      | TCTCAAGCCCAACAGTCAG | 196            | XM_005680538.3   |
|           | R      | GTAACGTGCAGAGGCAAGG |                |                   |
| IFNG      | F      | AAGTCCTTGACGGAGGTCT | 158            | NM_001285682.1   |
|           | R      | CTTCATCTCCAAGGCTGAGT |               |                   |
| β-actin   | F      | GCACTTCGAGGAGAGATGG | 233            | NM_001101        |
|           | R      | GCACTTCGAGGAGGCTAGG |                |                   |

Note: F = Forward, R = Reversed

Evaluation of gene expression in the artificial challenge trial: Differences in gene expression between resistant and susceptible goats in the challenge trial were tested using the $2^{-ΔΔCT}$ method in SAS (Livak & Schmittgen 2001; Ling 2012). To compare gene expression in resistant and susceptible goats, Student’s t-tests and a significance level of $P < 0.05$ were carried out using Graph Pad Software Prism7 (San Diego, CA USA).

Statistical analysis: The data of FEC were not normally distributed among the samples and exhibited positive skewness. So, a logarithmic transformation [log$_{10}$(FEC+25)] was therefore applied before analysis (Rout et al. 2011; González-Garduño et al. 2013) to minimize the heterogeneity of variance and increase normality of the FEC distribution. Analysis of FEC carried out using a repeated measures analysis of variance with the GLIMMIX Procedure of SAS version 9.2 (SAS Inst., Inc., Cary, NC, USA). Remaining
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quantitative variables (RBC, WBC, PCV, and Hg) were assumed to be normally distributed and least-square means (LSM) and stander error (SE) for measured variables were analyzed with a one-way ANOVA and the means were compared with a Duncan test in the version of SAS 9.2 (Ojeda-Robertos et al. 2017). P-values lower than 0.05 were considered to be statistically significant.

Results

Evaluation of fecal egg count: FEC of H. contortus infected goats from March to December 2017 demonstrated the natural stage of parasitic infection in YWG of our studied area (Figure 1A). The mean of FEC was much higher from July to August and slightly reduced in September. The mean of FEC was the highest in August whereas the lowest was recorded in March 2017. The prevalence of H. contortus infection was gradually higher from the March to July and reached the highest in August (76%) in the experimental herd of YWG (Figure 1B). Prevalence was also higher in July and October but it was comparatively lower in August 2017 (Figure 1B). A similar trend was observed for the prevalence of H. contortus infection in December (47%) and March (49%) 2017, respectively (Figure 1B).

Descriptive statistic between resistant and susceptible group of goats: Resistant (low responder) and susceptible (high responder) group of goats were assessed based on their FEC. The mean of FEC for the resistant group of goats was 103.38±1.20 epg with the maximum number of eggs for the individual goat was recorded as 450 epg. Similarly, the highest number of FEC was 3800 epg in susceptible goats with the average 1180.25±43.53 epg (Table 2). The mean of circular blood parameter like hemoglobin (Hg) and pack cell volume (PCV) significantly varied (P>0.05) between the resistant and susceptible group. Red Blood Cells (RBC) count and White Blood cells (WBC) count were almost similar between two groups of goats (Table 2). Mean of body weight (BW) was almost similar between two groups of a goat.

Artificial challenge trial: After the goats were classified as a resistant and susceptible group, artificial challenge trial was carried out with 5000 L3 larvae of H. contortus, and the comparison of parasitic infection between two groups are presented in Figure 2. The descriptive statistic for FEC confirms the expected right skewness of FEC distribution. During the challenge trial, FEC gradually increased from day 14 through day 42 and was higher in the susceptible group. In the resistant group, it rised very slowly than that of susceptible group during the experimental period (Figure 2A). The difference among FEC means at 28, 35, and 42 days after challenge was significant (P>0.01) between a resistant and susceptible group of YWG goat.

Moreover, the initial BW of resistance and susceptible goats were almost the same. Body weight of resistant goat was a little bit higher during the challenge trial and maintains their weight gain throughout the trial period after post infection. The average daily gain was
negative in susceptible goats, and it slightly declined after the day of 35 post-infection (Figure 2B).

Table 2. Fecal egg count, body weight and hematological parameters comparison between resistant and susceptible of YWG populations.

| Parameters                  | Resistance group, n=33 | Susceptible group, n=48 |
|-----------------------------|------------------------|-------------------------|
|                             | Low FEC <500           | High FEC >500           |
| Fecal Egg Count (egp)       | Mean±SE Range          | Mean±SE Range           |
|                             | 103.38±1.20* 0 - 450  | 1180.25±43.53 600 - 3800 |
| Body-weight (Kg)            | 19.04±1.37 11.0-33.0   | 18.03±1.69 10.0-34.0    |
| Hemoglobin (Hg), (g/dl)     | 9.81±0.92* 9.0 - 13    | 7.51±0.65 5.30 – 8.2   |
| Packed Cell Volume (PCV), (%)| 31.02±0.56* 27.0 – 35.0| 25.83±0.69 18.0 – 29.0 |
| Red Blood Cells (RBC), (10^{12}/L)| 10.92±0.62 9.8- 11.3 | 9.98±0.32 9.10-10.80 |
| White Blood Cells (WBC), (10^{9}/L)| 10.65±0.27 10.5-10.9 | 10.95±0.27 10.72-11.23 |

*Significant (p < 0.01), based on assuming unequal variances Student’s t-test.

Figure 2. Means for (A) fecal egg count (FEC), (B) body weight (BWT), (C) packed cell volume (PCV), and (D) haemoglobin (Hg) level in resistant and susceptible groups of YWG at 0, 7, 14, 21, 28, 35 and 42 days after artificial challenge with 5000 infective L_{3} larvae of Haemonchus contortus cultured under in vitro condition. Day 0 was the day of inoculation with the L_{3} larvae.
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The PCV was higher in the resistant group compared to that of the susceptible group and remained unchanged till day 21 of post-infection, but slightly declined from the day 28 to day 42. In the susceptible groups, PCV began to decline gradually at days 28 and continued to decline through day 42 indicating the high level of parasitic infection (Figure 2C). In susceptible goats, Hg value began to decline at days 21 and continued to decline up to the end of the experimental period showing the high responding to parasite infection. On the other hand, Hg values in resistant goats did not decline as rapidly as susceptible goats (Figure 2D).

**Differential gene expression analysis:** The relative expression of NOD1, NLRP9, TLR8, SFTPA1, IGF1, IFNG, IL32, and IL33 genes at the mRNA level are presented in Figure 3.

![Figure 3](image_url)

**Figure 3.** Relative expression of mRNA in abomasal tissue for (A) NOD1 = Nucleotide binding oligomerization domain containing 1 (B) TLR8 = Toll like receptor 8, (C) IL33 = Interleukin 33, (D) NLRP9 = NLR family pyrin domain containing 9, (E) SFTPA1 = , (F) IFNG = Interferon gamma, (G) IGF1 = Insulin like growth factor 1 and (H) IL32 = Interleukin 32 genes measured by Quantitative Reverse-Transcriptase Polymerase Chain Reaction (qRT-PCR) in resistant and susceptible goats. Black and Gray color bars represent resistant and susceptible goats respectively. *(p < 0.01) **(p < 0.001), *** (p < 0.0001), ****(p < 0.00001) based on assuming unequal variances Student’s t-test. Error bars represent standard error of the mean (SEM).
Relative expression of mRNA in resistant goats was higher than that of susceptible goats for NOD1 (P < 0.00001) and very high for IFNG (P < 0.0001). Similarly, relative levels of mRNA expression for NLRP9, TLR8, IL32, (all P < 0.001) and for IGF1 (P < 0.01) also significantly high in resistant goats. On the other hand, SFTPA1 showed a higher expression in susceptible goat, but resistant and susceptible goats did not differ in expression for IL33 (Figure 3).

Discussion
Gastrointestinal nematode (GIN) infection is the foremost constraints for the small ruminant production and resulting in economic losses especially in sheep and goats (McRae et al. 2014). The application of updated knowledge about the host genetic mechanism that is associated with resistance to GIN infection may help to improve parasite resistance or resilience of goat breed. To date, few studies have been conducted based on microarray-based gene expression to identify genes associated with resistance or susceptibility to GIN infection (Diez-Tascón et al. 2005; Keane et al. 2006). To our knowledge, this is the first study which directly compared YWG’s susceptibility to H. contortus infection within breed and population through the gene expression profiles. Our selective immune genes were differentially expressed when goats were experimentally challenged with infective L3 larvae of H. contortus in resistant and susceptible goats. The FEC is generally considered to be the main indicator of H. contortus infection in sheep and goats (Rodriguez et al. 2015; Omar et al. 2016; Kurukulasuriya et al. 2018). Previous reports revealed that resistant goats shed lower FEC and rapid recovery from initial elevations in FEC (Pralomkarn et al. 1997; Makun et al. 2008; Chiejina et al. 2015). This is the hypothesis that resistant goats are not fully free of the parasite but their worm burden is very low that may not affect their productivity with other relative advantages, due to their ability to cope with the internal parasite infection compared to susceptible goats.

The parasitic load can be expressed by the FEC, an effective way to the measurement for the parasite infection in goat. Host genetics significantly affect FEC, a vital phenotypic marker along with other parameters in goat that are resistant against H. contortus infection (Kim et al. 2015; Valilou et al. 2015). Variability of FEC gives a wide range of opportunity for selection and improvement of the H. contortus resistant goat breed within the population. The current study identified the variation for FEC within individuals of YWG population that might be caused by a genetic factor that was also supported by previous works (Omar et al. 2016; Omar et al. 2019). This is for the first time, the YWG goats have been evaluated for resistance to H. contortus using comparable experimental protocols and selection for artificial challenge trial on the basis of their FEC within the population followed by mRNA expression.

Our field investigation indicated the difference in parasite load between resistance and a susceptible group of YWG goats breed and identified resistant goats within this breed. This is in agreement to finding of Bhuiyan et al. (2017). This finding will help us to use this breed as a good candidate for exploring the genetic resistance by expression study to develop genetic markers for selective breeding program of nematode resistance goat population. Result of challenge trial with infective L3 larvae of H. contortus showed that resistant goats had lower FEC compared to that of the susceptible. Although the mean dose of L3 larvae was almost the same, approximately 239 and 265 larvae per kilogram of initial body weight for resistant and susceptible goat respectively. Susceptible goats showed approximately 60% higher FEC compared to that of resistant goats that were similar to previous finding (González et al. 2011). Additionally, the challenge trial also showed that resistant YWG had a tendency to delayed egg production and shed fewer eggs at day 28, 35, and 42 of post-infection. A similar result was also reported by several scientists on artificial challenge trial with infective L3 larvae of H. contortus between resistant and susceptible goats.
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(González et al. 2008; Kurukulasuriya et al. 2018). The phenotypic of the resistance group in the term of FEC indicates an anti-fecundity effect of the immune response against parasitic infection.

Hematological parameters especially PCV and Hg also are considered as the sign of health and a good indicator to recognize the resistance or susceptibility of parasite infection. Our study demonstrated that PCV and Hg value had a distinct difference between the resistant and susceptible goats after challenge with L3 larvae and the anemic condition was developed after five weeks of infection in the later group. Several investigators (Costa et al. 2000; Makun et al. 2008; Ameen et al. 2010; Kurukulasuriya et al. 2018) described that susceptible goats experienced progressively severe anemic condition during the experimental period. Similar pattern of change in the other hematological parameters such as PCV, RBC, and Hg after 5 weeks of infection with L3 larvae of H. contortus also occurred. It has also been reported that hematological parameters especially PCV and Hg had strong negative correlations with FEC or worm burden during the natural or artificial infection by H. contortus (Blackburn et al. 1991; Fakae et al. 2004; Kaplan et al. 2004; Khobra et al. 2012; Chiejina et al. 2015).

Increase in the BW could be a sign of good health and well-being in growing animals, and negative correlation has been observed between worm burden and BW gain in goat (Pralomkarn et al. 1997; Amarante et al. 2004; Burke & Miller 2004; Bishop 2012). Earlier studies implicated that increase the body weight, in contrast of EPG (egg per gram), has a significant effect to evaluate the status of resistance or susceptibility in sheep (Burke & Miller 2004; Mugambi et al. 2005). At the initial stage of infection, the body weight of our selected goats was almost similar for the resistant and susceptible group but the distinct difference of body weight was observed after four weeks of infection although all the goats of the resistant and susceptible group were treated with same doses of L3 larvae. It can be suggested that a decline in the weight gain of susceptible goats were more sensitive to H. contortus than that of resistant goats.

Based on the results of our experimental trial on FEC, BW, PCV and Hg, it can be clearly stated that a well-distinguished infection status was present in resistant and susceptible goats in YWG population. The pattern of infection status, confirms the consistent variation in genetic resistance to H. contortus within this YWG population. Abomasal tissues were utilized for the assessment of the relative expression of our candidate genes for evaluation of the expression pattern of those genes with the YWG’s, resistance to H. contortus infection. Several studies have been reported on our studied candidate genes with their expression in different tissues associated with human disease but very few reports are available on the goat (Tadaki et al. 2011; Castaño-Rodriguez et al. 2014; Lin et al. 2016; Naicy et al. 2017). This is for the first time we investigated those genes to understand their relative expression pattern in abomasal tissues of experimentally challenged goats with H. contortus. Polymorphisms of those genes have been established as candidate genes for resistant to H. contortus infection in goat breeds. Among the candidate genes, NOD1, NLRP9, TLR8, IFNG, IGF1 and IL32 showed significantly higher expression in resistant goats indicating the high potency of these genes to resistant proficiency to H. contortus infection. Positive expression of these genes in our current study alone with significant association results of previous reports to our studied candidate genes, resistant to H. contortus infection provides opportunity of developing biomarkers that are resistant to nematode infection particularly H. contortus in goats.

Additionally, SFTPA1 and IL33 showed a higher expression in susceptible goat and might be responsible for infection or promote susceptibility to Haemonchosis. Researchers reported that the patient with chronic obstructive pulmonary disease (COPD) showed decreased SFTPA1 expression compared to non-COPD (Lin et al. 2016). That report is also in
favor of the present study. Our finding provides an important experimental basis of information for future research on the function of those genes and helpful for further investigation on those genes as biomarkers to develop the Haemonchosis resistant goat breeds.

**Conclusion**

In conclusion, lower FEC and a higher level of PCV and Hg value of resistant YWGs indicate the potential genetic resource for a selective breeding program to develop *H. contortus* resistant goat population. Also, the mRNA expression suggests *NOD1, NLRP9, TLR8, IL32, IGF1*, and *TLR8* genes, having a significant effect on nematode infection, are related to *H. contortus* resistance and could be used as biomarkers to develop *H. contortus* resistant goat breed in future.

**Competing interests**

The Authors have declared that they have no competing interests exist.

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