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# Correlates of resistance to gastrointestinal nematode infection in Nigerian West African dwarf sheep

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ABSTRACT

**Objective:** To investigate correlates of resistance to GI nematode infection in Nigerian West African dwarf (WAD) sheep. **Methods:** Thirty three sheep were randomly assigned to two groups, A ($n$=27) which were used for experimental infections, and B ($n$=6) which served as uninfected control. Each infected animal received weekly escalating infections with infective larvae (60% *Haemonchus contortus (H. contortus)* and 40% *Trichostrongylus colubriformis (T. colubriformis)* for 4 weeks. The responses of all the infected and control sheep were assessed by faecal egg count (FEC), worm burden (Wb), packed cell volume (PCV), body weight (Bwt), and body condition score (BCS). On the basis of their individual faecal egg output, Lambs in group A with epg$\leqslant$1 000 on any sampling day were classified as low faecal egg count (LFEC) phenotype ($n$ = 16), those with epg between 1 000 and 10 000 as intermediate ($n$=5) and lambs with epg > 10 000 as high feacal egg count (HFEC) phenotype ($n$=6). **Results:** The difference between the FEC classes was highly significant ($P$=0.001). The BCS and weight gained at the end of the experiment by the control and LFEC sheep was significantly higher ($P\leqslant$0.05) than those of the intermediate and HFEC phenotypes. There was a significant and negative correlation between the parasitological measures and the trio of BCS, PCV and Bwt of sheep. **Conclusions:** The result of the study indicated that the FEC, weight gain, PCV, and BCS are correlates and potential selection criteria of GI nematode resistant WAD sheep.

## 1. Introduction

In the prevailing absence of new anthelmintics or commercially available vaccines as solutions to the problems of gastrointestinal (GI) nematode parasitism and anthelmintic resistance in small ruminants in the tropics, potential option lies with the development of selective breeding schemes for GI nematode resistant animals. Such a scheme will be based on selection for resistance using indicator traits/phenotypic markers and correlates of GI nematode resistance. Parameters such as FEC and PCV have been found to be repeatable, heritable and responsive to selection[1]. These markers of GI nematode resistance are the basis for identifying quantitative trait loci (QTL) associated with resistance to the parasites which could be used in a genetic marker-assisted selection scheme[2]. Consequently, phenotypic markers and correlates of GI nematode resistance such as FEC are valuable tools and therefore widely used in selective breeding programmes for parasite resistant sheep and goats[3]. Heritability of resistance to GI nematode infection in sheep as measured by FEC has been estimated to vary between 0.22 and 0.43[4].

Faecal egg count (FEC) and PCV have been identified and validated as reliable phenotypic markers and correlates of host resistance and resilience both to experimental *Haemonchus contortus (H. contortus)*[5] and natural GI nematode infections in Nigerian WAD goats[6]. However, there is little or no information on the resistance and resilience status of the Nigerian WAD sheep to these parasites as well as reliable phenotypic markers and correlates of such resistance and resilience. This study was therefore, designed to investigate the resistance and resilience status of the Nigerian WAD sheep with a view to identifying reliable phenotypic markers and correlates of host resistance and resilience to infection with *H. contortus* and *Trichostrongylus colubriformis (T. colubriformis)* which can be used as selection criteria for GI nematode resistant sheep.

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## 2. Materials and method

### 2.1. Experimental animals

Thirty three (33) male WAD sheep aged between 8 and 9 months and purchased from local markets around Nsukka, Nigeria were used for the study. At the time of acquisition, the sheep had no apparent infection but were routinely treated against ectoparasites (fleas, lice and ticks), GI nematodes and coccidia. The sheep were also vaccinated against peste des petit ruminants (PPR) using the tissue culture rinderpest vaccine (TCRV, NVRI, Vom, Nigeria). All treatment and vaccination procedures were completed within the first week of acquisition. They were fed daily on fresh cut and carry grass and legume supplemented with concentrate mixture. Water was provided *ad libitum*. The sheep were acclimatized for 8 weeks in fly-proof pens with concrete floors before the commencement of experimental infection.

### 2.2. Experimental design

After the 8 weeks of acclimatization period, the sheep were randomly assigned to two groups, (A, $n$=27 and B, $n$=6) and then distributed to their appropriate pens. Animals in group A were placed on a regimen of weekly escalating infections[5] of 500, 1 000, 2 000 and 4 000 infective larvae (L3) of a mixed culture of *H. contortus* (60%) and *T. colubriformis* (40%) per animal starting from day 0 (wk 1) to day 21 (wk 4) of the study. Animals in group B were used as uninfected control.

### 2.3. H. contortus and T. colubriformis infections

Infective larvae (L3) of local strains of *H. contortus* and *T. colubriformis* were harvested from faecal cultures prepared using faeces collected from donor sheep harbouring mixed infections with these two nematodes[7]. They were preserved in a refrigerator and used within 2 weeks of recovery from cultures. Prior to administration of the larvae to the experimental sheep, larval identification and counts were performed to determine the proportion of *H. contortus* and *T. colubriformis* larvae in each larval suspension and the inocula prepared from it. The estimated dose of L3 for each week was administered orally to each sheep via a stomach tube

### 2.4. Faecal egg and worm counts

Faecal egg count per gram of faeces, were determined daily from day 15 post infection until patency, after which it was carried out twice weekly on freshly collected faecal samples from individual sheep using centrifugal floatation in saturated salt (NaCl) solution and where appropriate by the modified McMaster technique[8]. At the termination of the experiment on day 59 post infection, all the sheep were humanely sacrificed, and their abomasal and small intestinal worm counts carried out according to Hansen and Perry[9].

### 2.5. Allocation of sheep to infection classes

Segregation of the sheep into their respective response phenotypes namely, low FEC (LFEC) phenotype and high FEC (HFEC) phenotype was based on their individual faecal egg output as described by Waruiru[10] with slight modification. Lambs whose FEC did not exceed 1 000 epg on any sampling day were classified as 'LFEC phenotype' those that exceed 10 000, as 'HFEC phenotype and lambs with FEC between 1 000 and 10 000 as 'intermediates.

### 2.6. Haematology

1 mL of Blood was collected weekly from the jugular vein of each of the experimental sheep into vacutainer tubes (Trittau, Germany) containing heparin (4 μL/mL of blood) as anticoagulant from D0. This was used to determine the PCV using the microhaematocrit method[11].

### 2.7. Body weight determination

Each sheep was weighed on D0 for the determination of body weight using a weighing balance and thereafter, weekly as described by Fakae[12].

### 2.8. Body condition scoring

The body condition scores of each animal was determined on D0 and thereafter, weekly by feeling the level of muscling and fat deposition over and around the vertebrae in the loin region[13].

### 2.9. Statistical analysis

Statistical analysis was conducted using SPSS version 15 for Windows. Parameters recorded on more than a single day were analyzed by repeated measure ANOVA in General Linear Model (GLIM). Where data conformed to normal distributions, analysis was by ANOVA in GLIM on raw values and the results were summarized as arithmetic means with standard errors of the mean (SEM). Where data did not conform to normal distribution, an appropriate logarithmic transformation was adopted prior to analysis and all residuals for ANOVA checked for appropriate normal distribution. Correlations between variables were analyzed by Pearson's moment correlation test for parametric data or Spearman's Rank Order Test for non-parametric data. Probabilities ($P$) of 0.05 or less were considered significant.

## 3. Results

### 3.1. Faecal egg count

The mean pre-patent period as shown by the occurrence of strongyle eggs in faeces was 22.4 ± 1.3 d (range: 22-23 d). Table 1 shows the mean FEC/gram of faeces segregated into low, intermediate and high FEC phenotypes. Analysis of log10 (FEC + 1) by rm ANOVA gave a highly significant difference in the FECs within the phenotypes ($P$<0.001). There was also a highly significant effect of time on the FEC phenotypes ($P$<0.001) as the FECs increased significantly with time.

### 3.2. Worm burden

Mean ± S.E.M *H. contortus* and *T. colubriformis* burdens are shown in Table 1. Analysis by one-way ANOVA indicates that the difference between the mean worm burdens of the LFEC phenotype (102.89 ± 18.80) and the HFEC phenotype (237.13 ± 54.63) was significant ($P$=0.028). Similar analysis showed that the LFEC sheep also had significantly lower

($P$=0.021) *T. colubriformis* burden (640.78 ± 84.38) than the HFEC phenotype (1441.00 ± 199.49).

### 3.3. Body weight gain

Analysis by One-way ANOVA showed that the weight gained at the end of the experiment by the control and LFEC sheep was significantly higher ($P$=0.001) than those of the HFEC phenotypes. The mean gains (± SEM) in body weight over D0 body weights at the end of the experiment by the control, LFEC, intermediate and HFEC phenotypes were 3.68 ± 0.29, 2.82 ± 0.49, 2.11 ± 0.32 and 1.12 ± 0.68, respectively (Table 1).

### 3.4. Packed cell volume

Figure 1 shows the changes in the PCV of sheep in the control group, LFEC, intermediate and HFEC phenotypes. The PCV of the control and infected groups were comparable until D35 after which the PCV of the intermediate and HFEC phenotypes began to fall steadily with terminal mean PCV of 22.33 ± 1.67 and 25.00 ± 0.32 respectively. The LFEC and control sheep had terminal mean PCV of (27.22 ± 0.70)% and (30.00 ± 1.41)% respectively.

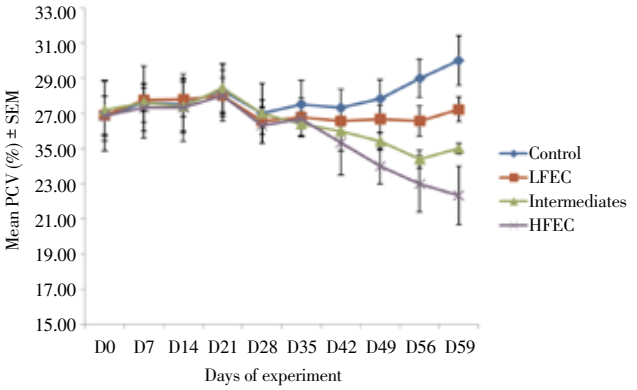


**Figure 1.** Mean packed cell volumes (%) of WAD sheep given mixed escalating *H. contortus* and *T. colubriformis* infections.

### 3.5. Body condition score

The BCS fluctuated differently among the control and the infected sheep (Figure 2). The result indicates a gradual and steady rise in the BCS among members of the control group throughout the course of the experiment. The BCS of the infected sheep showed initial rise up to day 21 post infection. Thereafter, the LFEC group maintained their body condition to the end of the study whereas among the intermediate and HFEC phenotype there was a continuous but gradual decline up to day 42 following which the BCS of the HFEC phenotype sheep dropped sharply to the end of the study. The terminal (D59) mean (± SEM) BCSs of the sheep were 4.17 ± 0.17, 3.18 ± 0.23, 2.67 ± 0.62 and 2.17 ± 0.48 respectively for the control, LFEC, intermediate and HFEC phenotypes. Analysis by rm ANOVA showed infection had a significant effect on the BCS particularly from D35 of the experiment ($P$=0.05). Also, the main effect of time on the BCS of the FEC class was highly significant ($P$<0.001).

**Table 1**
Mean FEC/gram of faeces, worm burden and body weight gains of WAD sheep given mixed escalating *H. contortus* and *T. colubriformis* infections.

| | Control | FEC response phenotypes | | |
|---|---|---|---|---|
| | | LFEC | Intermediate | HFEC |
| FEC/gram of feaces | 0 | 304.15±80.52 | 1 762.91±108.03 | 3 784.33±139.46 |
| *H. contortus* | 0 | 102.89±18.80 | 156.80±48.19 | 371.00±76.00 |
| *T. colubriformis* | 0 | 640.78±84.38 | 1 456.20±310.25 | 1 415.67±223.74 |
| Body weight gain | 3.37±0.36 | 2.99±0.33 | 1.07±0.64 | 0.50±0.32 |

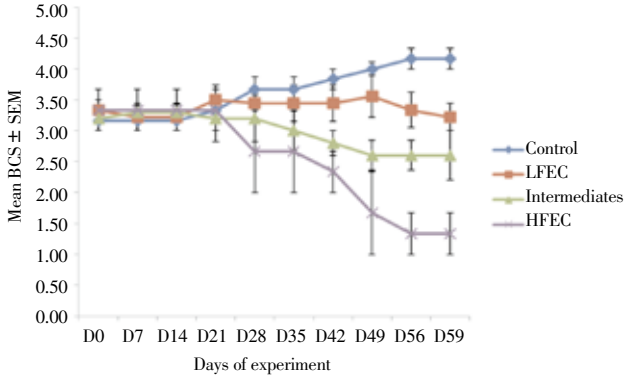


**Figure 2.** Mean body condition scores of WAD sheep given mixed escalating *H. contortus* and *T. colubriformis* infections.

### 3.6. Relationships between worm burden and other measures of infection

There was a highly significant positive correlation between log10 (Wb + 10) and the average of D56 and D59 FECs ($rp$=0.861, $P$=0.001, $n$=27). Whereas, log10 (Wb + 10) had a significant negative correlation with the average of D56 and D59 PCV ($rp$=−0.464, $P$=0.026, $n$=27) and BCS ($rs$=−0.576, $P$=0.004, $n$=27). There was also a highly significant positive correlation between the Bwt and the BCS [$rp$=0.698, $P$=0.001, $n$=27] of the infected sheep. The relationship between log10 (Wb + 10) and Bwt was negative but not significant ($rp$=−0.367, $P$=0.085, $n$=27).

## 4. Discussion

Three response phenotypes were readily recognizable in this study, namely, Low FEC, intermediate and High FEC phenotypes. Sheep belonging to the LFEC phenotype had significantly lower FEC than those of the HFEC phenotype. There was also a dichotomy in the worm burdens of HFEC and LFEC phenotypes. Such variability in FEC and Wb was described by Chiejina[5] and Fakae[14] in humid zone ecotype of Nigerian WAD goats and Chiejina[15] in savanna ecotype of the Nigerian WAD goats experimentally infected with their native strains of *H. contortus*. This pattern of responsiveness has also been confirmed[6] to occur under natural acquired field infections with mixed GI nematodes. These workers regarded this variability and the dominance of strong responder phenotypes as evidence of strong innate resistance of Nigerian WAD goats to *H. contortus* in

particular and GI nematodes in general.

In the present study, the abomasal worms (*H. contortus*) recovered at necropsy was significantly lower in the LFEC phenotype than in the HFEC phenotype. Likewise, the intestinal worm (*T. colubriformis*) burden was significantly higher in the HFEC phenotypes than in the LFEC phenotypes. It is noteworthy in this study that resistance to *H. contortus* was accompanied by resistance to *T. colubriformis*. This observation is consistent with previous studies[6,16] that reported good correlation between resistance to *H. contortus* and *T. colubriformis* in 'INRA 401' breed of sheep and the Nigerian WAD goats respectively.

Parameters such as FEC and PCV have been found to be repeatable, heritable and responsive to selection[1,3]. These markers of GI nematode resistance are the basis for identifying quantitative trait loci (QTL) associated with resistance to the parasites which could be used in a genetic marker-assisted selection scheme[2]. Heritability of resistance to GI nematode infection in sheep as measured by FEC has been estimated to vary between 0.22 and 0.43[4]. FEC and PCV have also been identified and validated as reliable phenotypic markers and correlates of host resistance and resilience both to experimental *H. contortus*[5,14] and natural GI nematode[6] infections in Nigerian WAD goats.

In the present study, FEC and Wb correlated strongly and negatively with the measures of host pathology studied, namely BCS, Bwt and PCV. Days 56 and 59 FEC, BCS and PCV gave a good prediction of intensity of the infection as evidenced by the strong positive correlation between the infection intensity (Wb) and FEC and equally strong negative correlations between Wb and BCS and between Wb and PCV. However, BCS on D56 and D59 most accurately predicted the infection intensity as assessed by Wb at necropsy in the Nigerian WAD sheep. The strong positive correlation between BCS and body weight further underscore its value as a potential parameter for assessing the GI nematode infections on the performance of small ruminants. Generally, BCS is considered to be the best and simplest indicator of available fat reserves which can be used by the animals in periods of high energy demand, stress or suboptimal nutrition[19] which are characteristics of GI nematode infections[20].

In conclusion, this study strongly suggests that FEC, BCS, body weight gain and PCV are reliable measures of the intensity of mixed *H. contortus* and *T. colubriformis* infections and therefore can be used as selection criteria for GI nematode resistant Nigerian WAD sheep. However, further work, including an examination of genetic parameters such as heritabilities and genetic correlations among the traits examined is recommended to support this conclusion.

## Conflict of interest statement

We declare that we have no conflict of interest.

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