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Description of gastrointestinal nematode infections by faecal egg count in a carpet wool sheep breed

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Abstract

The ongoing spread of anthelmintic resistance is causing an increasing failure of existing chemical control methods against gastro-intestinal nematodes (GIN). Breeding for genetic resistance of the host is among complementary investigated solutions to anthelmintic use. The most utilized trait used in traditional selection of sheep in favour of GIN resistance is faecal egg count (FEC), the number of eggs per gram of faeces. The aims of this paper are to describe the distribution of nematodes in different flocks of a carpet wool sheep breed to investigate the relationship between different traits related to gastrointestinal nematode resistance. We investigated 211 weaned lambs (age 4–6 months) from nine flocks of the Qizil sheep breed (~20 per flock) from the East Azerbaijan province, Tabriz, the Islamic Republic of Iran. Body weight, FEC, FAMACHA test and packed cell volume were measured. Nematode eggs were classified according to the species or group of species of origin: (1) Strongyles; (2) *Nematodirus* spp.; (3) *Trichuris* sp.; and (4) *Marshallagia marshalli*. We evaluated the correlation among traits and the distribution of the different nematode species among flocks.

Keywords

resistance, sheep, nematodes, parasites, regression, carpet wool breeds

1. Introduction

The number of sheep in the world is 1.2 billion, of which nearly 4 percent are raised in the Islamic Republic of Iran (FAO, 2017). The Qizil (or Ghezel) is a fat-tailed sheep breed mainly reared in the northwest regions of the Islamic Republic of Iran and in eastern and north-eastern regions of the Republic of Türkiye, with an estimated population size of 17 million heads, four of which are reared in the Islamic Republic of Iran (Daskiran and Ayhan, 2014). Qizil sheep are mostly reared on open pasture where gastrointestinal nematodes (GIN) are common. Anthelmintic treatments against GIN exist and are commonly applied to combat the infestation, but drug resistance is increasingly reported. McMahon *et al.* (2017) observed a reduced efficacy of benzimidazole, avermectins and moxidectin treatment in northern region of the Republic of Iceland. Drug resistance to levamisole and albendazole in sheep flocks of the Islamic Republic of Iran have been reported in 66 and 27 percent of

cases, respectively. Unfortunately, alternative methods of chemical control against GIN are not currently available. Moreover, environmental problems associated with chemical control of parasites are notably important for consumers (Charlier *et al.*, 2018).

Breeding for host genetic resistance is a potential GIN control strategy (Preston *et al.*, 2014). The number of eggs per gram of faeces (EPG) is frequently used as selection criteria for resistance or resilience to GIN. EPG has low heritability, but selective breeding has been shown to be effective in improving the trait and reducing EPG values. It is to be noted that the use of EPG in sheep breeding programmes has some limitations. One of the limitations is the need to have some infected animals to record variability in the trait. In addition, variations in natural GIN infection and environmental load depend on climatic conditions that may vary across pastures, seasons and years. Further, measurement of EPG is labour intensive. Therefore, the availability of genetic markers associated to GIN resistance/tolerance is highly desirable. Markers would permit assisted selection for genetic resistance by using a drug-free and less labour-intensive strategy (Atlija *et al.*, 2016) to be applied as a part of an integrated parasite management strategy for the control of GIN.

This investigation is part of the Coordinated Research Project sponsored by IAEA on “Genetic variation on the control of resistance to infectious diseases in small ruminants for improving animal productivity genetic resistance to nematodes in small ruminants.” Objectives of this paper are to describe the distribution of GIN groups in different flocks of a carpet wool breed, and to investigate the relationships among traits associated with GIN resistance.

2. Material and methods

Animals and phenotypes

This study was conducted during the month of May, when rainfall and humidity were seasonally high, and animals were thus at the highest risk of becoming contaminated with GIN (based on past anecdotal observations). We selected 211 individuals among weaned lambs (age 4–6 months) from nine flocks (approximately 20 individuals per flock) of the Qizil sheep breed in East Azerbaijan province, Tabriz, the Islamic Republic of Iran. Body weight, faecal egg count, FAMACHA (Faffa MAlan CHArt) test and packed cell volume (PCV) were measured twice at one-week intervals. Nematode eggs were classified into four groups according to the species or group of species of origin: (1) Strongyles (EPGO); (2) *Nematodirus* spp. (EPGN); (3) *Trichuris* sp. (EPGT); and (4) *Marshallagia marshalli* (EPGM). Photo S1 shows Strongyles (*Trichostrongylus* spp.) and *Nematodirus* spp. The sum of the mass of eggs from the four nematode groups in each lamb’s faeces are reported

as EPG. FAMACHA is an ordinal trait with five levels and is based on the colour of the inner eyelids of sheep. Sheep with a darker red colour are considered healthy and are assigned low scores, whereas sheep with lighter inner eyelids colour are assumed to be anaemic due to nematode infection and are assigned higher scores as the degree of redness decreases.



Source: Authors' own elaboration.

Photo S1: a) *Trichostrongylus* spp.; b) *Nematodirus* spp

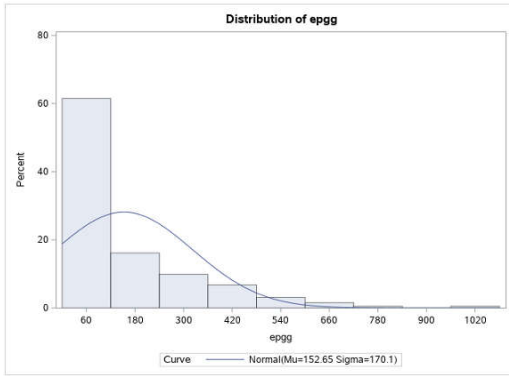
Statistical analyses

Correlation coefficients between traits were calculated by PROC CORR of SAS (SAS STUDIO). The distribution of traits was verified by PROC UNIVARIATE of SAS. Data of EPGO, EPGN, EPGT, EPGM and EPG were submitted to Box–Cox transformation by PROC TRANSREG to approximate a normal distribution (Figure 1). For these traits, analysis of variance was performed on the transformed data, while means are presented as non-transformed. Numbers equal to zero were replaced by 1 to enable transformation by the Box-Cox method. Distributions of PCV and FAMACHA are presented in Figure 2.

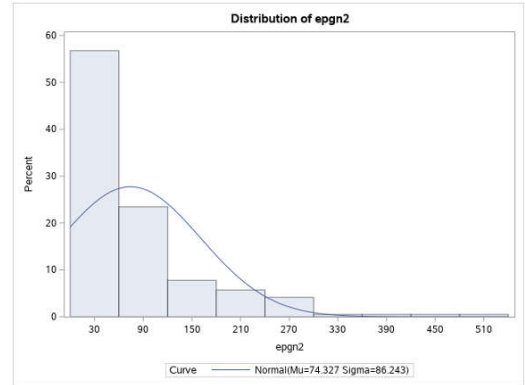
Initial analysis of variance was performed to investigate the effects of sex, flock, and live body weight (as linear covariate) on PCV, EPGO, EPGN, EPGT and EPG. Only the effect of flock resulted significant, so the other effects were removed from the model. FAMACHA scores were analysed by PROC FREQ of SAS with chi-square test.

We investigated the relationship between EPGO as dependent variable and FAMACHA as an independent variable using Proc REG of SAS. We estimated regression coefficients within each flock.

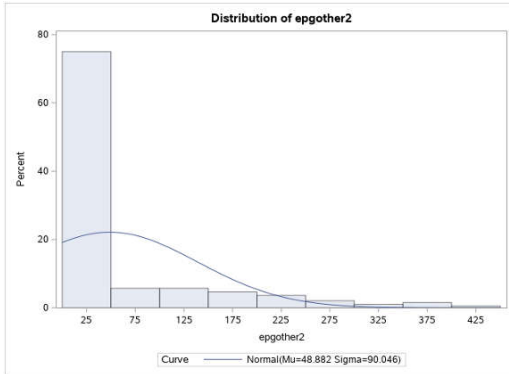
EPG



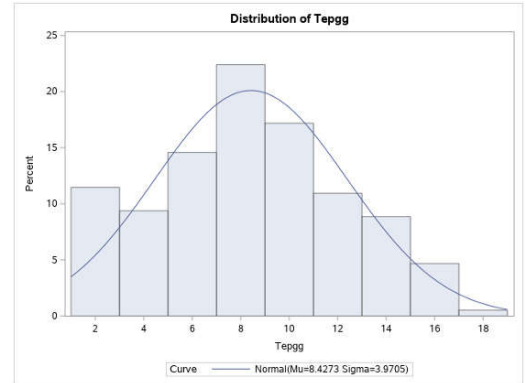
EPGN



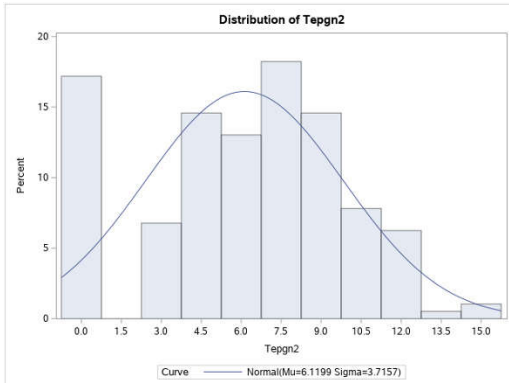
EPGO



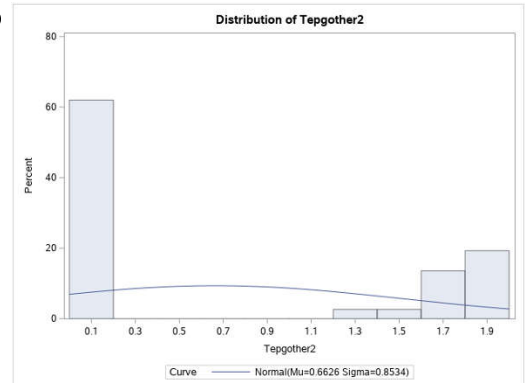
TEPG



TEPGN

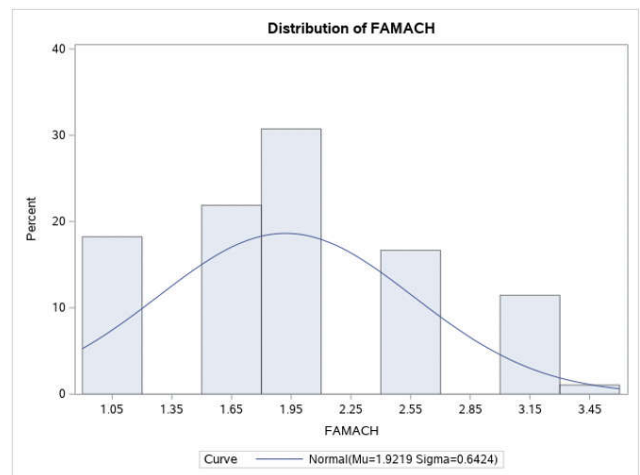
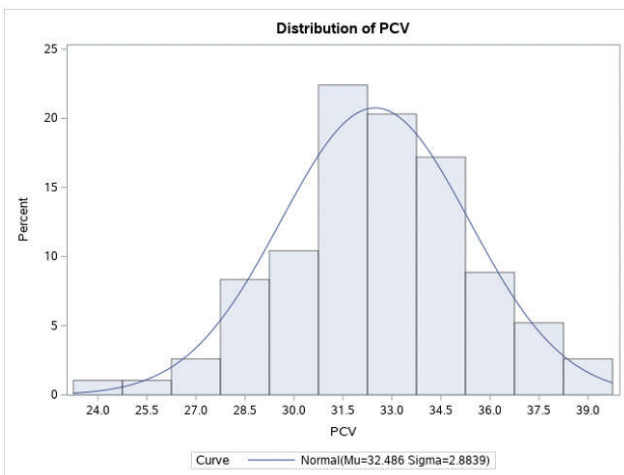


TEPGO



Source: Authors' own elaboration.

Figure 1: Non-transformed (EPG = overall eggs per gram of faeces; EPGN = EPG of *Nematodirus* spp.; EPGo = EPG of *Strongyles* spp.) and Box-Cox transformed (TEPG, TEPGN and TEPGo) distributions



Source: Authors' own elaboration.

Figure 2: Distribution of the PCV (left panel) and FAMACHA (right panel) traits

3. Results and discussions

Descriptive statistics of phenotypic traits are presented in Table 1. The Pearson correlation coefficient between EPG and PVC was 0.30 ($P < 0.0001$). Pearson and Spearman correlations between PCV and FAMACHA were -0.49 and -0.47, respectively ($P < 0.0001$).

Distribution of FAMACHA score among flocks is shown in Table 2. The chi-square test showed an association between the FAMACHA score and flock ($P < 0.03$).

The effect of flock resulted significant in all ANOVA tests run on the different traits. A comparison of means is presented in Figure 3. Previous studies have reported parasitic variability among flocks (Falzon *et al.*, 2013) and geographic areas (Papadopoulos *et al.*, 2003). Importantly, previous studies have observed that climatic conditions can have a great effect on the prevalence and severity of GI nematode parasites (Nabavi *et al.*, 2011).

Nabavi *et al.* (2011) attributed the greatest number of abomasal GIN infections among species to *Teladorsagia*

circumcincta, as we observed in Flocks 3 and 5 (Figure 4). There is high variability among flocks for overall level of infection and for the relative importance of the different GIN species (Figure 4). For example, Flocks 5 and 8 showed very different levels of EPG. This likely reflects differences in parasite load in the environment between these flocks. This might be due to differences in management factors (e.g. routine deworming, stall fed vs grazing, etc.) or in climatic conditions (e.g. rainy/dry weather, etc.) across flocks. For example, a positive correlation has been reported to exist between EPG and the mean humidity (Nematollahi *et al.*, 2020). Dominik and colleagues (Dominik, 2005) reviewed genetic variation within flocks of the same breeds for resistance to GIN, and Becker *et al.* (2020) observed phenotypic variation in natural helminth infection. In our study, flock genetic makeup is unlikely to contribute substantially to between flock phenotypic variation in EPG, considering the low heritability of the trait. Furthermore, this hypothesis would imply a high proportion of the genetic variance

Table 1: Descriptive statistics of the studied GIN related traits

Variable	n	Min	Max	Mean	SD
EPG#	192	4	1 071.50	152.65	170.1
EPGN	192	1	526.00	74.33	86.24
EPGM	192	1	219.75	26.94	42.91
EPGT	192	1	44.75	2.50	6.03
EPGO	192	1	447.25	48.88	90.05
PCV	192	23.50	39.25	32.49	2.88
FAMACHA	192	1	3.5	1.92	0.64

Source: Authors' own elaboration.

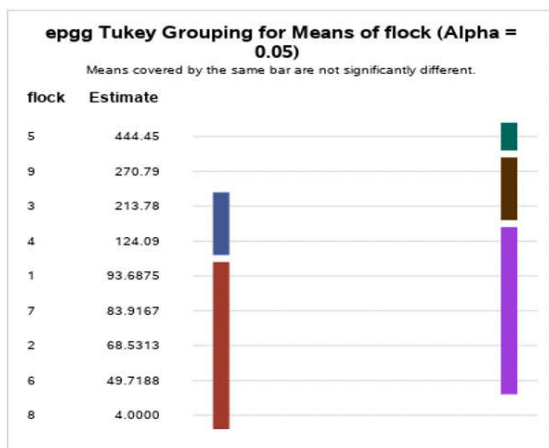
Note: PCV: Packed Cell Volume, EPGO: EPGal egg count for Strongyles including *Haemonchus contortus*, *Teladorsagia circumcincta*, *Ostertagia occidentalis*, *Trichostrongylus axei*, *Colubriformis*, *vitrinus*, and *rugatus*, EPGN: *Nematodirus* spp., EPGT: *Trichuris* sp., EPGM: *Marshallagia marshalli*. The sum of eggs in the four classes in each lamb's faeces were reported as EPG.

Table 2: Frequency of different FAMACHA scores in each flock and contribution in percent of each flock to the frequency across flocks

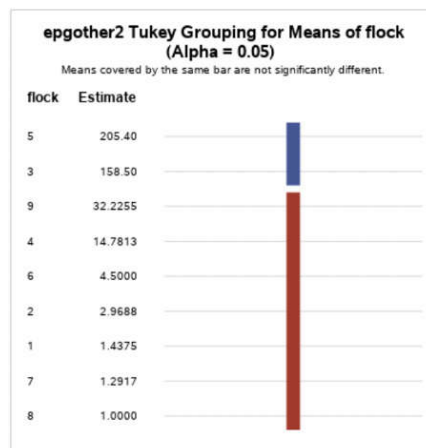
FAMACHA	Flock									
	1	2	3	4	5	6	7	8	9	Total
1	3	2	7	7	5	3	5	3	0	35
	1.56	1.04	3.65	3.65	2.60	1.56	2.60	1.56	0.00	18.23
1.5	7	9	5	7	2	1	3	3	5	42
	3.65	4.69	2.60	3.65	1.04	0.52	1.56	1.56	2.60	21.88
2	8	7	4	2	8	4	11	7	8	59
	4.17	3.65	2.08	1.04	4.17	2.08	5.73	3.65	4.17	30.73
2.5	1	2	2	4	4	7	6	3	3	32
	0.52	1.04	1.04	2.08	2.08	3.65	3.13	1.56	1.56	16.67
3	1	0	2	0	5	5	5	3	1	22
	0.52	0.00	1.04	0.00	2.60	2.60	2.60	1.56	0.52	11.46
3.5	0	0	0	0	1	0	0	1	0	2
	0.00	0.00	0.00	0.00	0.52	0.00	0.00	0.52	0.00	1.04
Total	20	20	20	20	25	20	30	20	17	192
	10.42	10.42	10.42	10.42	13.02	10.42	15.63	10.42	8.85	100.00

Source: Authors' own elaboration.

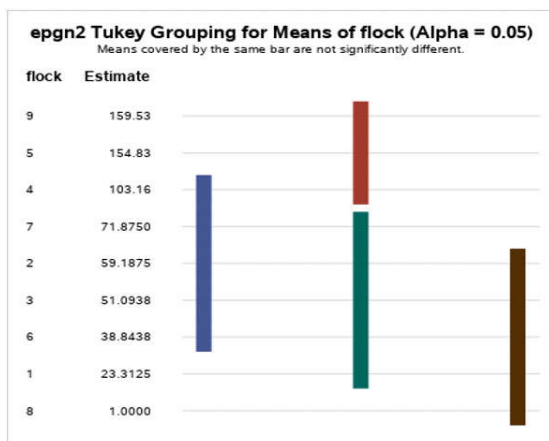
EPG



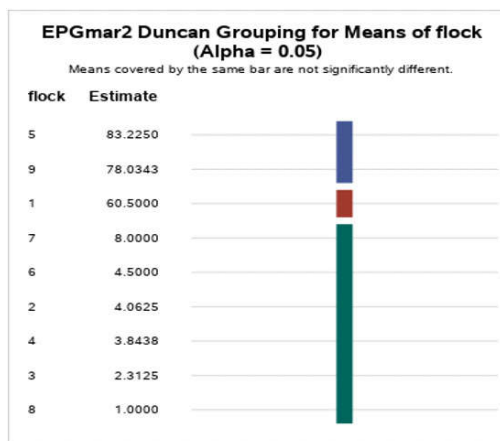
EPGO



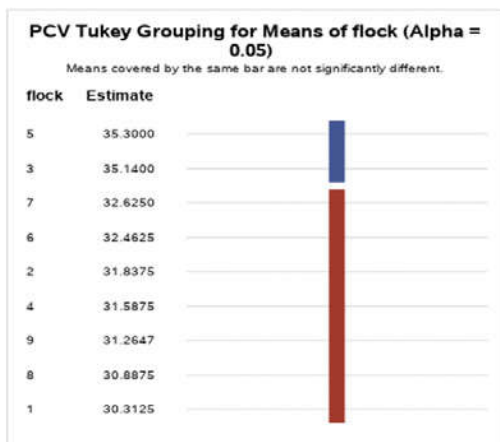
EPGN



EPGM



PCV



Notes: a Means covered by the same bar are not significantly different (Tukey grouping, alpha = 0.05). For example, for EPG, the mean for Flock 5 is significantly greater than for all other flocks. Flock 9 differs from all flocks except for Flock 3, although Flock 3 does not differ significantly from Flock 4. Meanwhile, the mean of Flock 4 does not differ from the means of Flocks 1,7, 2 and 6. Finally, the means of Flocks 1,7,2,6 and 8 do not differ significantly.

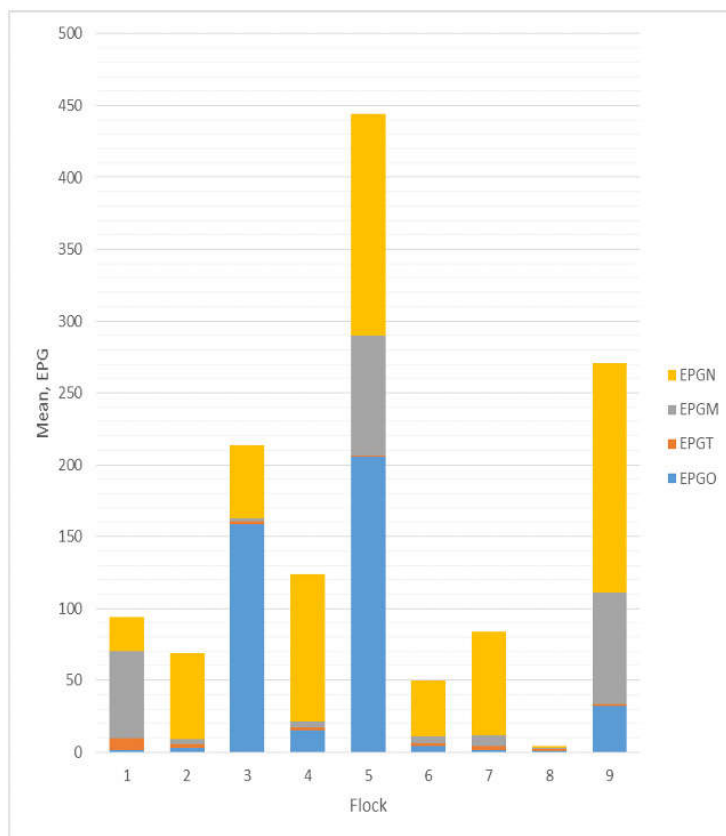
b **EPGO:** EPGal egg count for Strongyles including *Haemonchus contortus*, *Teladorsagia circumcincta*, *Ostertagia occidentalis*, *Trichostrongylus axei*, *colubriformis*, *vitrinus*, and *rugatus*, **EPGN:** *Nematodirus* spp., **EPGT:** *Trichuris* sp., **EPGM:** *Marshallagia marshalli*.

Source: Authors' own elaboration.

Figure 3: Comparison of means among flocks in the studied traits

to occur between flocks, an event occurring very rarely in populations for which no flocks have historically practiced formal directional selection for GIN resistance. Conversely, we cannot exclude the presence of a genetic component of individual response to parasite challenge, observing the high phenotypic variation of animals within flocks, particularly in those having high mean parasitic load. Interestingly, the frequency of different GIN species varied across flocks, independently on total parasite load.

For instance, EPGN was present in all flocks, and was the prevalent species in five flocks (2, 4, 6, 7 and 9), but not in Flocks 3 and 5, in which the prevalent species is EPGO. Also, EPGM was almost absent in Flock 3, which had an overall high parasite load, while it was in a high proportion for Flocks 5 and 9, which had the two highest overall parasitic loads, but also in Flock 1 that had a medium load. The reasons for these differences are unclear and may depend on local climatic conditions as indicated above.



Note: **EPGO:** EPGal egg count for Strongyles including *Haemonchus contortus*, *Teladorsagia circumcincta*, *Ostertagia occidentalis*, *Trichostrongylus axei*, *colubriformis*, *vitrinus*, and *rugatus*, **EPGN:** *Nematodirus* spp., **EPGT:** *Trichuris* sp., **EPGM:** *Marshallagia marshalli*.

Source: Authors' own elaboration.

Figure 4: GI distributions among flocks

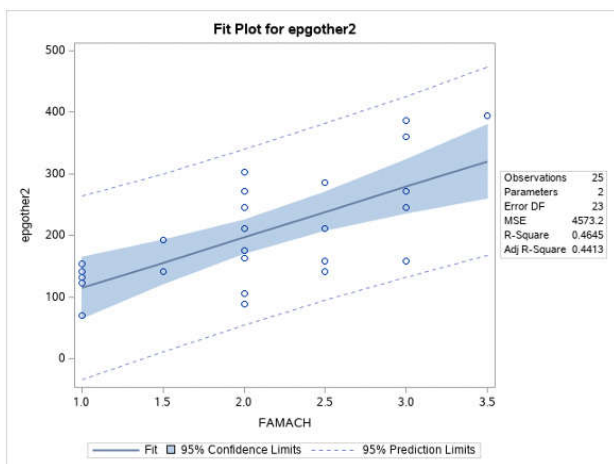
The regression analysis between traits within flocks resulted in coefficients (slopes) having different values for the same comparison, likely due to the very variable parasite loads among flocks and the limited numbers of animals per flock. In only a few flocks were the regression coefficients significant with an acceptable coefficient of

determination (R-square > 0.4). Plots of EPGO and PCV (as dependent variables) on FAMACHA (as independent variable) are shown in Figure 5. The regression equations were:

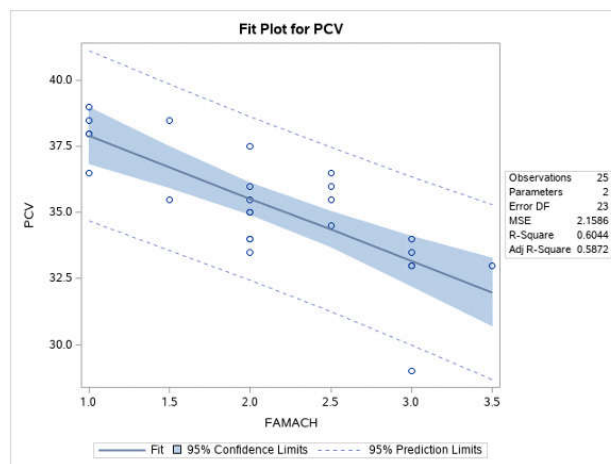
$$EPGO = 32.74 + 82.22 \text{ FAMACHA}$$

$$PCV = 40.28 - 2.37 \text{ FAMACHA}$$

EPGO



PCV



Source: Authors' own elaboration.

Figure 5: Fitted linear model (solid line) of EPGO (left) and PCV (right) as predicted by FAMACHA within flock No 5. Circles are the original observations, and the blue shaded area represent the Confidence Intervals (95 percent) of the model

4. Conclusions

In general, it can be said that there is diversity within and between flocks in terms of GIN species infections. Given that effects of body weight and sex were not significant, this diversity may primarily be due to management and climatic conditions. Genetic effects cannot be ruled out, however, as different parasite loads have been observed in lambs from a same flock. The diversity between individuals deserves further study, to understand the genetic basis of resistance or susceptibility to GIN in the breed investigated.

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