Variability in the fecal egg count and the parasitic burden of hair sheep after grazing in nematode infected paddocks[1]

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ABSTRACT.- González-Garduño R., Mendoza-de Gives P. & Torres-Hernández G. 2013. **Variability in the fecal egg count and the parasitic burden of hair sheep after grazing in nematode infected paddocks.** *Pesquisa Veterinária Brasileira 33(4):469-475*. Universidad Autónoma Chapingo, Unidad Regional Universitaria Sursureste, P.O. Box 29 Teapa, 86800 Tabasco, México. E-mail address: robgardu@hotmail.com

This study aimed to evaluate the variability in the fecal egg count (FEC) and the parasitic burden of naive hair sheep after grazing in nematode infected paddocks. The research was carried out in Tabasco, Mexico, during two periods (August and December). In each period 32 lambs were grazed for one month on African star grass (*Cynodon plectostachyus)* contaminated with gastrointestinal parasitic nematodes. FEC, packed cell volume (PCV) and body weight (BW) were recorded. Gastrointestinal worms were recovered at necropsy. Data were analyzed with the MIXED procedure of SAS using a model of repeated measurements over time. A higher number of *Haemonchus contortus* worms was found in December (2814±838) than in August (1166±305). The opposite occurred with *Cooperia curticei* (2167±393 and 3638±441, respectively). The FEC and correlation coefficient in respect to the worm burden were higher in December (6516 ± 1599, r=0.83, respectively) than in August (4364±771, r=0.44, respectively). A high variability in resistance-susceptibility to gastrointestinal nematodes (GIN) occurred in Katahdin × Pelibuey lambs after grazing.

INDEX TERMS: *Haemonchus contortus, Cooperia curticei, Oesophagostomum columbianum,* hair sheep, resistance-susceptibility.

INTRODUCTION

The frequent and continuous use of chemical anthelmintic drugs for deworming sheep flocks has resulted in the presence of lateral and multiple anthelmintic resistance in the parasites. This resistance leads to a concerning ineffectiveness of anthelmintics with large economic losses in sheep productivity (Coles et al. 2006, Papadopoulos 2008, Sargison 2011). Twenty-four countries have reported the presence of nematodes resistant to several anthelmintics (Jabbar et al. 2006). This situation has motivated workers around the world to search for alternative strategies to control these parasites (Torres-Acosta et al. 2012). Some strategies to reduce the parasitic burden in the animals have been proposed: selective deworming (Besier et al. 2010), vaccination (Arunkumar et al. 2012), medicinal plants (Sawleha et al. 2010), cooper oxide needles (Vatta et al. 2009) and biological control (Waller 2006).

Selection of sheep that are genetically resistant to gastrointestinal parasitic nematodes is another possible strategy that has been explored in recent decades (Eady et al. 2003, Hutchings et al. 2007, Stear et al. 2007). This alternative has been used in combination with other control methods, especially when there is the problem of anthelmintic drug resistance (Molento 2009). The issue of resistance has increased mainly for sheep farming in humid, warm climate conditions such as in Brazil (Thomaz-Soccol et al. 2004). In these warm, humid areas the proliferation of nematode larvae in the grass increases, resulting in the possibility of larvae completing their biological cycles, thereby creating a major problem in the grazing sheep industry (Torres-Acosta & Hoste 2008).

The genetic selection of sheep resistant to nematodes is based on phenotypic markers. For example, the elimi-

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nation of eggs per g of feces in wool sheep breeds has been investigated for many years in Australia and New Zealand (Windon 1996, Gray 1997). In Merino, there are lines of sheep selected for both increased and reduced genetic resistance (Woolaston et al. 1990). These lines achieve average counts of 2730 and 17400 epg, respectively, making it possible to reduce the use of anthelmintics in controlling nematodes in sheep with genetic resistance. The search for genes or Quantitative Trait Loci (QTL) related to resistance against parasites in sheep is considered a major aspect to be investigated (Beraldi et al. 2007) by workers in some countries, *i.e.* UK (Davies et al. 2006, Bishop & Morris 2007) and Kenya (Silva et al. 2012). Despite the development of modern molecular techniques, traditional productive aspects such as weight gain, packed cell volume (Vanimisetti et al. 2004) and egg faecal count have been used to diagnosis parasites (Cringoli et al. 2010).

Studies indicate that hair breeds are more resistant to parasitic nematodes than wool breeds. For instance, in a work published by Notter et al. (2003), authors recorded 4011 FEC average after four to eight weeks in wool lambs. Meanwhile, in hair lambs maintained under the same conditions, only 1135 FEC was found. On the other hand, Katahdin and St Croix have been identified as resistant to gastrointestinal parasitic nematodes with values of 5170 and 4217 FEC and Dorper lambs with 8602 FEC (Burke & Miller 2004). Also, one hair sheep breed from the Canary Islands (Canaria hair breed sheep) (González et al. 2008) and another from Mexico (Pelibuey) are also recorded as resistant against gastrointestinal nematodes (Morteo-Gómez et al. 2004). Other studies also indicated the presence of resistance in the following breeds: Florida (Amarante et al. 1999, Díaz et al. 2000), Gulf Coast native (Li et al. 2001), Blackbelly (Amount et al. 2003), Red Maasai (Baker et al. 2003) and Dorper (Vannimiseti et al. 2004).

The high variability observed between and within breeds allows for selection based on the nematode egg count despite the heritability and repeatability being close to 0.3 (Jackson 2002). Nevertheless, it should be considered as an alternative for controlling parasitic nematodes. The aim of the present study was to identify the variability between the fecal egg count and the parasitic burden of hair sheep after grazing in nematode infected grazing paddocks.

MATERIALS AND METHODS

Experimental site

This study was carried out in the Unidad Regional Universitaria Sursureste of the Universidad Autónoma Chapingo in the Municipality of Teapa, Tabasco, Mexico, at 60 meters above sea level and located 17° 31' 38" North latitude and 92° 55' 50" West longitude. The prevalent climate of this region is Af (m) wi'g, hot and humid with rainfall throughout the year (Fig.1). The average annual temperature is 25.8ºC and rainfall of 3976mm (CONAGUA, 2012).

Animals and management

The grassland was planted with African star grass (*Cynodon plectostachyus)* nine months before starting the experiment. Three months before the lambs began the grazing period, one group of 40 sheep was grazed on this prairie in order to contaminate it with GIN.

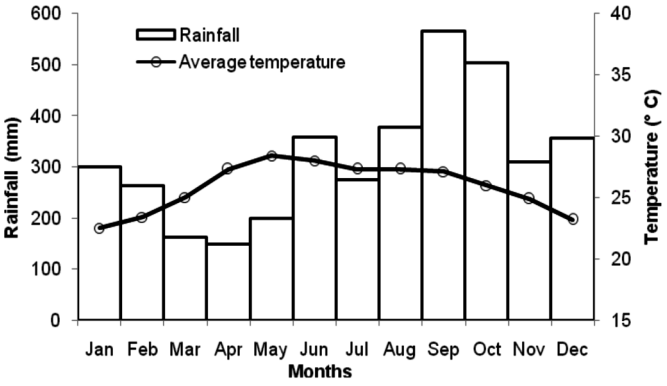

Fig.1. Rainfall and temperature averages from 1971 to 2000 recorded at the Meteorological Center, 27068 Puyacatengo, Tabasco, Mexico.

The study was divided into two periods. In each period, 32 Katahdin × Pelibuey three- month-old weaned male lambs were used. The lambs of each period had never been in contact with GIN because they were reared in house with ewes during the preweaning period. At weaning, they were negative to gastrointestinal nematodes; consequently, no acquired resistance against nematodes would be expected.

The first period was carried out in August (rainy season) and lambs were grazed for one month in a rotational system with 15 days grazing in each one of two 7500m^2 paddocks. During grazing, lambs were given 250g of a commercial feed with 14% crude protein. Also, they were provided with a mineral mixture and water *ad libitum*. After grazing, lambs were housed for 21 days to allow the larvae to evolve into adult worms. Lambs from this group were slaughtered to recover the nematode parasites.

After completing the first grazing period, an "extra" group of 38 infected sheep were grazed on the pastures for three months from September to November (rainy season) to continuously infect the prairie with gastrointestinal nematodes.

In the second period, another group of 32 grazing lambs with the same characteristics, origin and management, previously indicated, was introduced. This group grazed for a month (December) and was then housed for 21 days. The January group was slaughtered to recover the nematode parasites.

Individual body weight (BW) was recorded at the beginning and at days 35 and 42 of each period, and the average daily weight gain (DWG) was estimated. On the same dates, blood samples were taken from the jugular vein to measure the packed cell volume (PCV) by the micro-haematocrit technique (Benjamin 1991).

Parasitological method

During grazing, fecal samples were taken on days 14, 21, 35 and 42. During the housing period, samples were taken on days 21, 35 and 42. The McMaster technique was used to determine the FEC values (Thienpont et al. 1986). Fecal samples were pooled and then cultured to obtain nematode infective larvae for taxonomic identification (Niec 1968).

At the end of the housing time in each period, lambs were slaughtered according to the Mexican Official Standard NOM-033-ZOO-1995 for humanitarian slaughter of domestic animals and wildlife (SENASICA, 1996). After slaughter, the abomasum of each lamb was removed and dissected as follows: The organ was opened along the greater curvature and the content was collected in a plastic container and then the worms were recovered. At the same time, worms were recovered from the intestinal tract and also from distal part of the colon and rectum. At the same sampling time, 10% formalin was added to preserve the recovered parasi-

tes. The content of the abomasum, colon and rectum was washed with tap water and passed through a mesh (Mesh number 50, 0.297mm, Mont-steel) and content of small intestine was washed and meshed through a mesh number 400 (0.038mm, Mont-steel). Finally, the content and parasites retained in the mesh were collected in a 500 mL flask (Tarazona 1973). Recovered nematodes, including males and females from aliquots, were mounted on slides and observed under a microscope (10X and 40X) for the taxonomic identification (Vázquez 1989, Jacquiet et al. 1997).

Statistical analysis

Least square means and standard errors were estimated from repeated measure analysis of variance (SAS 1999). FEC was transformed to $\log_{10}$ (FEC + 25) to correct the heterogeneity of variance and to obtain a normal distribution approximation of data following the method of Gauly & Erhardt (2001).

The following model was used:

$$Y_{ijk} = \mu + \rho_i + \alpha_{j(i)} + \tau_{k(i)} + \varepsilon_{ijk}$$

Where: Y_{ijk} is the FEC, PCV or DWG, μ the mean, ρ_i the fixed effect of period, $\alpha_{j(i)}$ the random effect of j-esim animal in the i-esim period $\alpha_{j(i)} \sim N(0, \sigma^2_a)$. $\tau_{k(i)}$ Fixed effect of the time, $\varepsilon_{ijk} \sim N(0, \sigma^2_e)$ the residual error.

The results of counting the recovered worm species (*Haemonchus contortus, Cooperia curticei* and *Oesophagostomum colombianum*) and the total worm burdens were log + 25 transformed and analyzed using the same model, but removing the effect of time. One frequency table was performed to determine the dispersion of nematode counts in the group of lambs in each period, taking as class interval 1500 nematodes with the purpose of forming more than 6 classes. The correlation analyzes were performed using the CORR procedure among the study variables with SAS software (SAS 1999) and scatter plots and trend lines in Excel.

RESULTS

The total count of recovered parasites at necropsy was 4908 ± 4405 for all experiment. This count included a mixture of species conformed by *Haemonchus contortus, Cooperia curticei* and *Oesophagostomum columbianum* initially identified in the coprocultures and determined by morphologic measurements of nematode males (Table 1).

Only one lamb in each of the two periods had a very low worm burden (20 in the first period and 30 in the second). Apart from these animals, 10% of the lambs in the first period and 30% in the second period had the lowest parasite burden (1500 worms). On the other hand, 3% of the animals had more than 7500 worms in the first period while about 20% in the second period exceeded this number of nematodes (Table 2).

Table 1. Morphometry of the adult male nematode species found in the gastrointestinal tract of sheep

Specie	Location	N[a]	Body length (mm)	Spicules (µm) Right	Spicules (µm) Left
H. contortus	Abomasum	48	13.36 ± 1.7	39.8 ± 2.7[b]	21.0 ± 1.7[b]
O. columbianum	Intestine	10	13.5	800 ± 25.0	800 ± 25.0
C. curticei	Small intestine	20	[c]	153.0 ± 13.0	153.0 ±13.0

[a] N = Number of nematodes. [b] Measures of the hooks; the spicules measures were 407.6 ± 15.3.

[c] There was no measurement of the body because they were fixed in formalin and rolled.

Table 2. Relative frequency of worm burdens in the gastrointestinal tract of sheep grazing during two study periods

Classes Lower limit	Classes Upper limit	Classes mean value	Accumulate relative frequency (%) First period (September)	Accumulate relative frequency (%) Second period (January)
30	1515	772	9.7	31.0
1515	3000	2257	35.5	55.2
3000	4485	3742	45.2	65.5
4485	5969	5227	58.1	72.4
5969	7454	6712	80.6	75.9
7454	8939	8197	96.8	79.3
8939	10424	9682	100.0	86.2
10424[a]	16364	13394		89.7
16364	17848	17106		93.1
17848	19333	18591		100.0

[a] Four classes were grouped.

Similar averages in the worm burdens in both periods (4989 y 4832 worms, respectively) were observed. However, there were differences in the count of each species per period. There were more *H. contortus* in January (2814±838) than in September (1166±305). The opposite situation occurred with *C. curticei* (Table 3); the lambs slaughtered in September had 3638±441 nematodes of this species (p≤0.05) which was a higher number than the count worms found in lambs slaughtered in January (2167±393). In the case of *O. columbianum*, the lambs slaughtered in September had more worms (68±25) of this species compared to those recovered from the lambs sacrificed in January (9±4).

There were differences in fecal egg counts between periods (P≤0.01). Higher FEC values were found in the last period (December, 6516±1599 EPG) compared with the first (August, 4364±771). The highest FEC values were recorded at day 21 during the second period (Fig.2).

The correlation coefficient between the worm burdens and fecal egg counts in the first period (r= 0.44) was lower than in the second period (r=0.83). This coincided with the highest number of *H. contortus* found during the last period

Table 3. Average and standard error of worm burdens in Katahdin x Pelibuey sheep during two periods

Species and sex	First period (September) Worm count N*	Average	Std Error	Second period (January) Worm count N*	Average	Std Error
Haemonchus contortus						
Females	30	673[b]	185	29	1529[a]	446
Males	30	494[b]	127	29	1285[a]	395
Total	30	1166[b]	305	29	2814[a]	838
Cooperia curticei						
Females	30	1994[a]	219	29	1171[b]	207
Males	30	1643[a]	233	29	996[b]	187
Total	30	3638[a]	441	29	2167[b]	393
Oesophagostomum columbianum						
Females	30	38[a]	14	29	3[b]	1
Males	30	30[a]	11	29	6[b]	3
Total	30	68[a]	25	29	9[b]	4
Total	30	4832[a]	492	29	4989[a]	1053

[ab] Different letters in each row indicate statistical differences (p≤0.01). *N = Number of sheep.

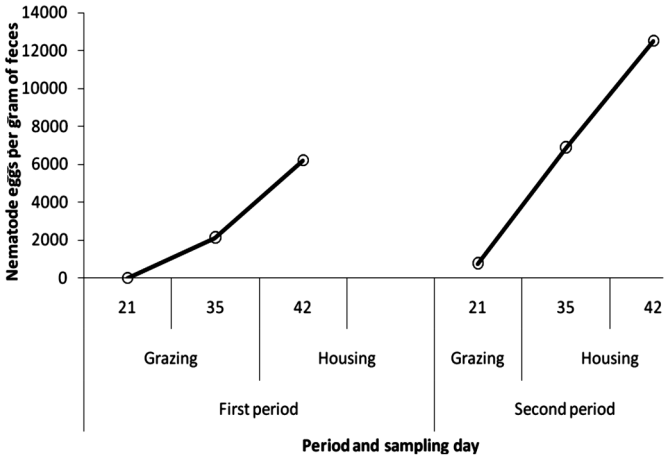

Fig.2. Fecal egg counts of gastrointestinal nematodes per period and sampling date in Kathadin x Pelibuey lambs.

and whose correlation coefficient was high (r=0.91) between the worm count of this species and fecal egg counts (Table 4). The fecal egg count and the worm burdens had a greater tendency after 42 days of infection (Table 5).

The PCV of lambs in the two periods decreased as the lambs became infected and the fall was more pronounced in the second period (December, Table 6). The largest reduction in PCV in the second period also coincided with the highest number of *H. contortus* and with high FEC. Therefore, the correlation coefficients between the PCV and the worm burden were negative in both periods (Table 7).

The high worm burdens coincided with the decrease in PCV values and showed a linear trend which had the best fit ($R^{2=60}$%) during the second period when there was a larger number of *H. contortus*. In the first period, PCV reduction was lower and less adjusted to the total count of worms in the gastrointestinal tract (R^2=60%).

The BW of the lambs fell in the initial samples and then increased (Table 6). In DWG differences were found between the two periods (p≤0.05). The DWG in the first period was lower than the second period and the most important changes were observed over time.

Table 4. Correlation coefficients of fecal egg counts and worm burdens of *Haemonchus contortus* and *Cooperia curticei* in the first period (above the diagonal) and second period (below the diagonal)

	Variable	Worm count	FEC	*H. contortus* worms	*C. curticei* worms
		First period			
Second period	Worm count	1	0.44*	0.44*	0.80**
	FEC[a]	0.83**	1	0.86**	-0.09[ns]
	H. contortus worms	0.94**	0.91**	1	-0.18[ns]
	C. curticei worms	0.68**	0.29[ns]	0.38*	1

[a] Fecal egg counts (eggs per gram of feces). * P≤0.05; ** P≤0.001; [ns] P≥0.05.

Table 5. Trend among fecal counts of nematode eggs (x) and the number of worms (Y) found in the gastrointestinal tract in mixed infections of *Haemonchus contortus* and *Cooperia curticei*

Days after infection	Worm count equation	R^2	Female worm count equation	R^2
First period[a]				
35	$Y = 1509\ x^{0.153}$	0.16	$Y = 868\ x^{0.15}$	0.16
42	$Y = 264.6\ x^{0.339}$	0.47	$Y = 161.7x^{0.329}$	0.45
Second period				
21	$Y = 199.3x^{0.437}$	0.44	$Y=120.7x^{0.422}$	0.42
35	$Y = -5*10^{-6}x^2+0.544x+2056$	0.58	$Y =-3*10^{-6}x^2+0.303x+1108$	0.58
42	$Y = -2*10^{-6}x^2+0.412x+1659$	0.75	$Y =-1*10^{-6}x^2+0.230x+891.1$	0.75

[a] On day 21 the FEC was 0.

Table 6. Percentage of packed cell volume and body weight per period in Katahdin x Pelibuey sheep

Day and period	N	Packed cell volume Average	Packed cell volume Std dev	Body weight Average	Body weight Std dev
First period					
1	31	28.8	2.7	19.4	4.0
35	31	28.6	3.9	19.0	4.1
42	30	26.7	3.6	20.2	4.2
Second period					
1	32	33.5	2.3	21.4	5.7
21	31	27.7	3.8		
35	30	23.9	4.8	21.1	5.2
42	30	25.6	5.9	22.2	5.5

Table 7. Correlation coefficients between the worm burden, fecal egg count (FEC), change in packed cell volume (PCV) and daily weight gain (DWG) of sheep in the first period (above the diagonal) and the second period (below the diagonal)

	Variable	Worm count	FEC	PCV change	DWG
		First period			
Second period	Worm count	1	0.44*	-0.44*	-0.38*
	FEC[a]	0.83**	1	-0.56**	-0.91**
	PCV change	-0.78**	-0.77**	1	0.55**
	DWG	-0.67**	-0.68**	0.81**	1

* P≤0.05; ** P≤0.001; [ns] P≥0.05.

DISCUSSION

The high coefficient of variation (93%) found in the worm burden indicates that a wide variability exists in the innate resistance-susceptibility of a contemporary group of hair sheep against gastrointestinal nematodes. The worm burdens observed in this study (30 to 19333) were similar to values found (from 7364 to about 19193) in grazing sheep (Uriarte et al. 2003). The wide variation among hosts has also been identified in *Teladorsargia circumcincta* parasitized sheep. This variability has also been widely recognized among and within breeds (Dominik 2005, Idris et al. 2012) and has been attributed to differences in the ingestion of infective larvae and the immune response (Stear et al. 2007).

During the first period, the correlation coefficient between FEC and worm burdens was smaller (r=0.44) than the one recorded in the second period (r=0.83). The correlation was the result of lower FEC recorded in August (4363) due to the lowest number of adult worms of *H. contortus* (1166) and high counts of *Cooperia curticei* (3638). In December the highest FEC (6516) coincided with the high number of specimens of *Haemonchus contortus* (2814) and the smallest number of *C. curticei* (2167). In a similar case, a high correlation between worm burdens and FEC (0.64 to 0.73) in Rhön sheep infected with *H. contortus* was recorded (Gauly et al. 2002). Also, a high correlation (0.85 to 0.91) between pre-slaughter FEC and total trichostron-

gyle burdens in Romney lambs has been identified (Bisset et al. 1996). It is noteworthy that in the case of a mixed infection (*H. contortus* and *C. curticei*) the correlation values were dependent on the abundance of *H. contortus* (r=0.86 in the first period and r=0.91 in the second period) rather than those of *C. curticei* (R= -0.09 in the first period and r=0.29 in the second period; not significant).

As noted in the study of Amarante (2000), with field infections the FEC does not reflect parasitic burdens. Other factors such as the abundance of each species and the oviposition rate become important. In the case of *H. contortus* the oviposition rate is very high, ranging between 5000-10000 eggs per day per female worm (Coyne et al. 1991, Romero & Boero, 2001). Whereas for the case of *C. curticei,* studies have reported a rate of 20 to 60 eggs and low values of correlation between the FEC and total worms (Amarante 2000).

It is also important to consider the immunological effect of lambs. In this regard, some studies have shown that lambs can not only effectively limit worm establishment, but can also suppress fecundity of those worms which do manage to establish (Bisset et al. 1996). The correlation observed between adult worm size and egg content leads to the hypothesis that egg production in *H. contortus* is limited by immune regulation of worm size and presumably growth. Mean worm size and fecundity declined as sheep received more prolonged trickle infections before necropsy, confirming that immune responses to adult worms are enhanced by ongoing larval challenge (Valderrabano et al. 2002, Rowe et al. 2008).

The relation between FEC and worm burden had a better fit after 35 days infection because the pre-patent period is about 15-28 days (Romero & Boero 2001) and on day 21 many animals had not excreted eggs. Therefore, the FEC does not correlate well with worm count, while on days 35 and 42 the curves are fitted over 50%. In the same manner as in the present study, the first egg counts did not correlate well following natural challenge in Romney lambs (Bisset et al. 1996).

A greater number of lambs with high worm burdens in the second period could be explained by the increase in infection of pastures once the number of grazing cycles had been increased. In the first period (September) lambs grazed on paddocks previously infected during three months by one flock. The lambs in the second period grazed on the same paddocks in which other flocks grazed for three more months; therefore, there were more cycles of infection in the pasture. So, more generations of parasites were contaminating such paddocks. Similar results have been recorded in Spain and they are considered as a cause of increased infection in Aragonesa sheep (Uriarte et al. 2003). However, environmental conditions could also affect the amount of parasites present in the pasture. During the months from September to December, the moisture increases in the region (Fig.1). Therefore, the environmental conditions lead to increase the nematode populations (Eysker et al. 2005).

The high frequency of *H. contortus* in the study area was reported in previous studies. This frequency was determined through fecal culture (González et al. 2003) and by using the same methodology. An abundance of *C. curticei* in this area was observed in both the rainy season and in winter (Vasquez et al. 2006). A previous study in Tabasco, Mexico, also confirms the higher prevalence of *H. contortus* in January compared with September, October and November. This study found abundance of *C. curticei* in July and August (González-Garduño et al. 2011).

In this study the negative relationship between the FEC and PCV was clear. This behavior is also reported in other breeds such as Dorset, Dorper and Kathadin by Vannimisetti et al. (2004), who found correlation coefficients of -0.46 between PCV and FEC. In other studies, PCV and FEC values were observed in Rhön sheep. A correlation range of r= -0.21 to -0.34 (Gauly & Erhardt 2001, Gauly et al. 2002), which was lower than those observed in the present study, was found. This can probably be explained because these authors evaluated only the PCV values. In the present study our information included the change in PCV levels.

As expected, weight gain, worm burdens and FEC are reversed with negative correlation coefficients (Table 7). This situation has already been stated in general terms in other breeds with smaller values (r= -0.25; Vanimisetti et al. 2004).

The low initial DWG was due to the drastic change of food system from confinement to grazing of all sheep. A similar situation occurred in the second period. The BW of the lambs fell in the initial samples and then increased perhaps as a result of management that was provided. For example, during the first month of grazing, lambs were supplemented with 250g of commercial food and were housed with *ad libitum* food the following 21 days.

These results showed that Katahdin x Pelibuey sheep had a high variation in resistance-susceptibility to gastrointestinal parasitic nematodes and at least 3% of the animals showed high resistance to nematode parasitic burden.

Fecal egg count may be a suitable indicator to estimate the number of *H. contortus* since the correlation coefficients were high. However, the relationship between FEC and the total number of *C. curticei* was not a good indicator for mixed infections.

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