Original Article

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Humoral Immune Response in Pelibuey Ewes to *Haemonchus contortus* Antigen During the Peripartum Period

María Guadalupe PULIDO-LÓPEZ[1], Omar HERNÁNDEZ-MENDO[1], Roberto GONZÁLEZ-GARDUÑO[2], Glafiro TORRES-HERNÁNDEZ[1], Jaime GALLEGOS-SÁNCHEZ[1]

[1]College of Postgraduates, Montecillo Campus, Montecillo, State of Mexico, Mexico

[2]South-Southeast Regional University Unit, Chapingo Autonomous University, Teapa, Tabasco, Mexico

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Abstract

The anthelmintic resistance in endo-parasites forces the search for different alternatives to control gastrointestinal nematodes. One choice is promoting the natural resistance of the host to self-regulate gastrointestinal nematodes infections, as a sustainable method. The aim of this study was to analyze the performance of immunoglobulins A, G, and M in Pelibuey ewes infected with gastrointestinal nematodes during the peripartum period. Hematological and fecal egg count of *H. contortus* were recorded. In addition, blood serum obtained from 31 three-year-old Pelibuey ewes in the peripartum stage was used to detect antibodies against crude antigen of nematodes by indirect enzyme linked immunosorbent assay. Data from Pelibuey ewes were grouped by physiological stage (pregnancy and lactation) and divided into early (weeks 1–6), mid (weeks 7–14), and late (weeks 15–22) pregnancy. Lactation was also divided into early (weeks 1–4 postpartum), mid (weeks 5–9), and late (weeks 10–13) lactation. Data were analyzed as repeated measures over time. Fecal egg counts of gastrointestinal nematodes showed differences between pregnancy and lactation ($p < .03$), with lower fecal egg count during pregnancy (279 ± 86 eggs per gram of feces) than during lactation (489 ± 46 eggs per gram of feces). The susceptible ewes showed values greater than 1054 ± 1099 eggs per gram of feces, while the resistant ones showed fecal egg count on average of 81 ± 152 eggs per gram of feces. The relative expression of IgG and IgM was significantly higher during pregnancy (51.39% and 46.15%, respectively) compared to lactation (38.25% and 38.49%, respectively). The IgA response in resistant ewes had a significantly higher expression (32.7%) compared to the intermediate (19.6%) and susceptible (20.5%) groups. IgA stands out for its direct relationship with parasite inhibition, evidenced by negative correlations with eggs per gram of feces. Immunoglobulins (A, G, and M) have a relevant role in the humoral immune response during the peripartum period.

Keywords: Antibodies, EPG, FEC, immunoglobulins, nematodes

What is already known on this topic?

- *There is a peripartum rise in fecal egg count in hair and wool ewes.*
- *Eosinophil and immunoglobulin counts decrease in the peripartum period.*
- *Sheep breeds differ in their genetic resistance to gastrointestinal nematodes.*

What this study adds on this topic?

- *The relationships between IgA, IgG, and IgM immunoglobulins in Pelibuey ewes classified according to their genetic resistance to* Haemonchus contortus *during the peripartum period are highlighted. The behavior of immunoglobulins during the lactation period is also studied.*

Introduction

Sheep production in Mexico faces multiple technological, social, and health challenges that have caused the population to remain dormant for over 50 years. Of all the factors, health problems have been among those that have affected production, and gastrointestinal nematodes (GIN) are those that most limit productivity (Hou et al., 2023). Among the GIN, the genera *Haemonchus*, *Trichostrongylus*, *Cooperia*, and *Oesophagostomum* stand out (Lalramhluna et al., 2020), that mainly infect grazing sheep because animals are in constant contact with infective larval forms in the grass. The infected sheep may show signs of anemia, hypoproteinemia, depression, loss of body condition, reduced milk production, weight loss, decreased appetite; furthermore, animals have elevated cortisol levels (Hollema et al., 2018) and even death of susceptible sheep when infections are very high (Galyon et al., 2020). The parasitic problem is exacerbated by the low effectiveness of the chemicals used to control GIN, attributed to the constant use of anthelmintics which have generated anthelmintic resistance (AR). Due to the importance of the AR problem, there is a need to look for different alternatives to reduce it, for example, the use of extracts and essential oils that have been traditionally used in herbal medicine (Štrbac et al., 2022), manipulation of nutrition to increase animal immunity, selective deworming (Calvete et al., 2020), vaccines, copper needles, rotational grazing, and genetic resistance (Adduci et al., 2022). The latter develops naturally when sheep can self-regulate GIN infections, as a sustainable alternative (Zvinorova et al., 2016). Several studies have indicated that hair breeds, such as the Pelibuey, have demonstrated a great capacity to limit their GIN populations, possibly because of a strong immune response (Ojeda-Robertos et al., 2017). In determining genetic resistance to GIN in sheep, the number of eggs per gram of feces (EPG) has been widely used as a phenotypic variable, which indirectly represents the parasitic burden in the animal (González-Garduño

Corresponding Author: Roberto González-Garduño • **E-mail:** rgonzalezg@chapingo.mx

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et al., 2017). Immunity against GIN depends on immune factors, such as eosinophil populations in the mucosa of the gastrointestinal tract and specific immunoglobulins such as IgA and IgE, in addition to IgG and IgM circulating in blood. Other variables such as hematocrit (HCT) could help confirm host resistance against hematophagous GIN (Saddiqi et al., 2012). Additionally, in sheep, immunity is determined by the physiological stage (Zaragoza-Vera et al., 2023) due to the redirection of nutrients toward lactation (Beasley et al., 2010), reflected in the variation in the fecal egg count (FEC) during pregnancy and lactation, a process known as peripartum relaxation of immunity. During this process, IgA migrates from the intestinal mucosa to plasma and milk, temporarily reducing abomasal antibodies and favoring the establishment of larvae (Jeffcoate et al., 1992). Furthermore, progesterone participates in the modulation of the *H. contortus* cycle, since its decrease at the end of pregnancy favors the development of larvae into adults (Gutiérrez-Amézquita et al., 2017). Given this situation, it is important to understand the humoral immune response during peripartum. Therefore, the objective of this study was to analyze the performance of immunoglobulins A, G, and M in Pelibuey ewes naturally infected with GIN during the peripartum period.

Materials and Methods

Location

The samples of this study were obtained from January 2021 to April 2021 on a commercial farm located in the municipality of Salto de Agua, Chiapas, Mexico (17° 33' 20 N; 92° 20' 02" W; 20 masl). The climate is type Af (m) w"(i')g, warm humid with rain all year round, with an average annual temperature and precipitation of 26.6°C and 3289 mm, respectively (SMN, 2024).

Animal Handling

The study was conducted with the utmost regard for animal welfare, and all animal rights issues were appropriately observed. No animal suffered during the study.

Fecal egg count of GIN, and blood serum were obtained from 31 three-year-old Pelibuey ewes. These samples were taken from week 17 to week 22 of gestation and were subsequently taken weekly until week 11 of lactation. The number of samples varied during the study stages because weekly sampling was carried out, starting with sheep at different stages of gestation and therefore with different birth dates. Data obtained for each ewe around parturition were organized in an Excel database, thoroughly reviewed, and homogenized based on the birth date. Data were grouped by physiological stage (pregnancy and lactation). Pregnancy was divided into three phases: early pregnancy (weeks 1–6), midpregnancy (weeks 7–14), and late pregnancy (weeks 15–22). Lactation was also divided into three phases: early lactation (weeks 1–4 postpartum), mid-lactation (weeks 5–9), and late lactation (weeks 10–13).

The ewes were naturally infected with GIN during grazing in a rotation system of swirl grass (*Paspalum notatum*), bitter grass (*P. conjugatum*), star grass (*Cynodon plectostachyus*), and humidicola grass (*Urochloa humidicola*), with a stocking rate of 15 sheep per hectare. Reproductive management was based on an accelerated lambing model with three breeding seasons: March, July, and November.

Fecal Egg Count Determination

Fecal samples were collected during pregnancy and lactation stages. All fecal samples were processed through flotation by the McMaster technique to determine the number of EPG (Rodríguez-Vivas, 2015). At the end of the lactation stage, the average EPG was used to categorize ewes as resistant and susceptible, according to the methodology described by Morteo-Gomez et al. (2004). Specifically, resistant ewes (R) were those below the mean EPG minus three standard errors. Susceptible ewes (S) were those above the mean EPG plus three standard errors (upper segregation limit). Sheep with an EPG between these two thresholds were considered intermediate (I).

A coproculture was performed every fifteen days during pregnancy and lactation on the positive animals, and the infective larvae were recovered eight days later, which were identified morphologically using keys (van Wyk & Mayhew, 2013).

To obtain whole blood samples, a puncture was made in the jugular vein, and 3 mL of blood was extracted in vacutainer tubes with coagulation accelerator (Vacutainer; BD Biosciences, Franklin Lakes, NJ, USA). The tube was centrifuged at 3000 rpm for 20 minutes. The supernatant, containing the serum, was collected and stored at −20 °C.

The number of ewes in each phase was determined, and average HCT and EPG values, as well as immunoglobulin concentrations, were calculated.

Enzyme-Linked Immunosorbent Assay

Detection of antibodies against crude antigen of *Haemonchus contortus* by indirect enzyme linked immunosorbent assay (ELISA) was made. The extraction of the crude antigen was performed using the methodology described in a previous study (González-Garduño et al., 2017).

Crude *H. contortus* antigen was fixed to polystyrene plates (Nunc MaxiSorb, 96-well) for 12 hours at 4°C. The antigens were diluted to a concentration of 0.5, 2.5, and 1.5 µg/mL for IgG, IgA, and IgM, respectively (Table 1), in carbonate (30 mM)-bicarbonate (80 mM) buffer at pH 9.6, and 100 µL were added to each well; subsequently, three 5-minute washes were performed with PBST (PBS-Tween 20 at 0.05% at pH 7.2) with an automatic washing machine (Thermo Scientific Wellwash).

Then all the wells were blocked with a solution of 5% skim milk in PBST and left to incubate at 37ºC for 1 hour, and the plate was washed three times for 5 minutes with PBST. Once the washings were finished, 100 µL of the serum was applied to each well at a dilution of 1 : 100–1 : 300 (Table 1) in a solution of PBS-Tween 20 at 0.05% with sodium azide at 0.02% (w/v) and incubated for 1 hour at 37ºC. The plate was washed again three times for 5 minutes with PBST.

Table 1.
Dilution of the Components for Each Immunoglobulin

Immunoglobulin	IgG	IgA	IgM
Dilution of the conjugate	1 : 10000	1 : 5000	1 : 12000
Serum dilution	1 : 300	1 : 100	1 : 300
Antigen concentration	0.5	2.5	1.5

Subsequently, 100 µL of the conjugate was added per well (rabbit anti-sheep horseradish peroxidase IgG, IgA, IgM Bethyl Laboratories, Montgomery, AL, USA) diluted according to the immunoglobulin used (Table 1) in PBS-Tween 20 at 0.05%, incubating for 45 minutes at 37ºC.

The plate was washed again, and 50 µL of substrate was applied per well (tetramethyl benzidine (TMB) substrate, Sigma Aldrich, USA) for 15 minutes at room temperature.

Finally, the reaction was stopped by adding 50 µL per well of a 1M sulfuric acid solution, and finally the optical density was determined using an ELISA plate reader (680 Microplate Reader, Bio-Rad) at a wavelength of 450 nm.

The immunoglobulin (IgA, IgG, and IgM) OD values were corrected by subtracting the reading from the well without antigen (blank). The average value of the wells from negative animals was then subtracted from the OD, and finally the value was expressed relative to the positive control (PCt). The positive control consisted of identifying pregnant or lactating ewes with a high response of a specific immunoglobulin (IgA, IgG, or IgM) in previous samplings; therefore, they belonged to the same breed. These ewes also had high fecal counts of nematode eggs. Once their Igs levels were confirmed, the sera from several ewes were mixed to form PCt. The correction was performed for each plate, respectively.

The levels of immunoglobulins IgA, IgG, and IgM were compared between the stages of pregnancy (G) and lactation (L).

Statistical Analysis

All data were analyzed as repeated measures over time using SAS software version 9.2 (SAS, 2017). The EPG was log-transformed (EPG + 1) to correct for heterogeneity of variance and approximate a normal distribution. The following model was used:

$$Yijk = \mu + \rho i + \alpha j(i) + \tau k + \rho\tau ik + \xi ijk \quad (1)$$

Y_{ijk} = response variable (FEC, Immunoglobulins), µ = overall mean, ρ_i = fixed effect of physiological state (pregnancy and lactation), $\alpha_{j(i)}$ = effect of breed nested in the j-th physiological stage (pregnancy or lactation), ~N $(0,\sigma^{2nd})$, τ_k = fixed effect of time, $\rho\tau_{ik}$ = combined effect of physiological stage and time. $\varepsilon_{ijk=}$ Experimental error corresponding to treatment i in its repetition ijk. The comparison of means was performed using the Duncan test. Pearson correlation analysis was used to determine the relationship between immunity indicators (IgA, IgG, and IgM) and EPG.

Ethical Committee Approval

The initial project on phenotypic resistance in sheep was approved by the Bio-Ethics Committee of Autonomous University of Yucatán (Number: CB-CCBA-D-2017-001, Date:June 26, 2017) and subsequently ratified by those responsible for the thesis research project on February 2, 2026.

Results

Fecal Nematode Egg Count During Pregnancy and Lactation

Fecal counts of GIN eggs showed differences between pregnancy and lactation ($p < .03$), with lower FEC during pregnancy (279 ± 86 EPG) than during lactation (489 ± 46 EPG), confirming the effect of immunosuppression after parturition, when FEC increases. The intensity of infection during the peripartum period increases slightly about two weeks before parturition and subsequently a more pronounced increase occurs until reaching the peak at seven or eight weeks (Figure 1). After eight weeks of lactation, a reduction in FEC occurs.

When comparing the FEC according to the categorization of the groups by their resistance (R, I, and S), the ewes classified as susceptible reached values greater than 1054 ± 1099 EPG, while the intermediate category had values of 361 ± 413 EPG and the resistant ones showed FEC on average of 81 ± 152 EPG, with significant differences between the three groups ($p < .01$) (Table 2).

In fecal cultures, the highest prevalence was for *Haemonchus contortus* regardless of the physiological stage, with similar values between gestation and lactation. Other species, such as *Trichostrongylus colubriformis* and *Oesophagostomum columbianum*, were also found, but at a lower prevalence (Figure 2).

Ewes classified as resistant (R) had the lowest FEC in both pregnancy and lactation ($p < .01$). In contrast, intermediate (I) ewes had an increase in FEC during lactation, reaching values ranging from 231 EPG in pregnancy to 391 EPG in lactation. Meanwhile, susceptible (S) ewes showed the highest values in both stages, with a marked increase in lactation, reaching 1100 EPG (Table 3).

Humoral Response During Pregnancy and Lactation

Immunoglobulin A (IgA) did not show any differences between the physiological stages studied ($p > .05$). IgA expression compared to the PCt indicated similar values between pregnancy (25.89%) and lactation (25.28%). In the case of IgG ($p < .01$) and IgM ($p < .01$), the relative expression was significantly higher during pregnancy (51.39% and 46.15%, respectively) compared to lactation (38.25% and 38.49%, respectively) (Table 4).

Humoral Immune Response

Table 5 shows the immunoglobulin response in the three groups of sheep derived from the categorization by their resistance (R, I, and S). The IgA response in resistant ewes (R) had a significantly higher expression (32.7%) compared to the intermediate (19.6%) and susceptible (20.5%) groups, and in turn, these last two did not present differences between them ($p < .05$). No statistical difference was observed between the resistance groups with either IgG ($p > .05$) or IgM ($p > .05$).

In the interaction of the immunoglobulin response by physiological stage and resistance classification (Figure 3), it was noted that during pregnancy and lactation, resistant (R) ewes had the highest levels of IgA (30.3%), with differences from intermediate and susceptible ewes that showed values close to 20% PCt of expression. On the other hand, IgG levels during pregnancy and lactation showed no trend, presenting their highest values in intermediate ewes, while resistant and susceptible ewes had slightly lower levels. IgM maintained its values very similar for each category in pregnancy, but with values higher than those observed in lactation, which highlights the reduction in immunity in this last stage.

The performance of immunoglobulins over time showed that IgG and IgM levels were highest during pregnancy and decreased as lactation progressed. All three immunoglobulins (IgA, IgG, and IgM)

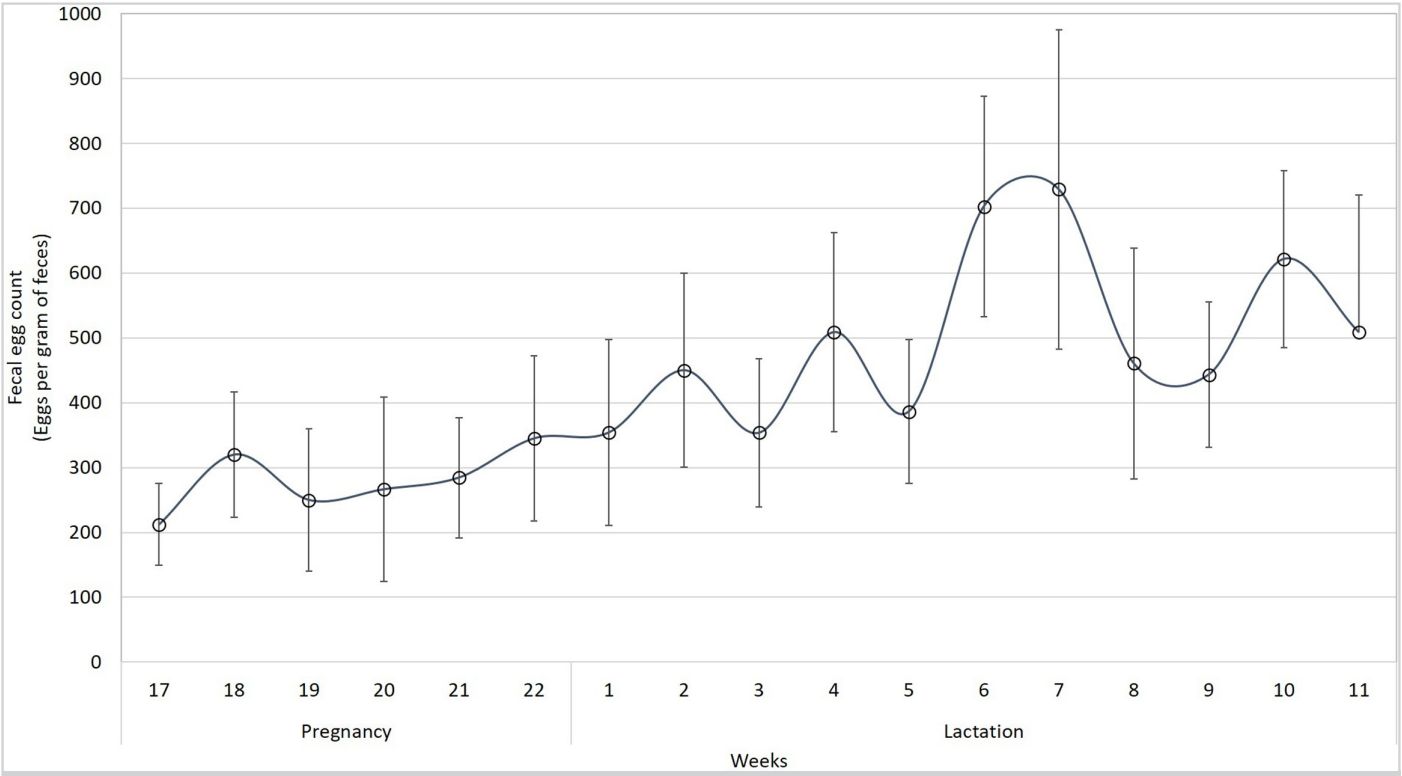

Figure 1.
Fecal Egg Counts of Gastrointestinal Nematodes by Week During Pregnancy and Lactation of Pelibuey Ewes.

tended to decrease toward mid- and late lactation (Figure 4), and only IgM increased slightly in late lactation.

Correlations

Correlation analysis between immunoglobulin levels and mean fecal nematode egg count across the EPG during pregnancy showed low and non-significant associations (Table 6). A moderate and highly significant negative correlation was observed between IgG and IgM ($r = -0.51$; $p < .001$), indicating an inverse relationship between the two immunoglobulins during pregnancy.

During lactation, correlation analysis showed low-magnitude associations between nematode FEC and immunoglobulins (Table 7). A low but significant negative correlation was found between EPG and IgA ($r = -0.18$; $p < .01$), as well as a low positive correlation with IgG ($r = 0.17$; $p < .01$). Among immunoglobulins, IgA did not show any correlation with IgG but did present a significant positive correlation with IgM ($r = 0.24$; $p < .01$). On the other hand, IgG and IgM were not significantly associated.

Table 2.
Mean Fecal Nematode Egg Count by Resistance Classification Category of Pelibuey Ewes

Category	Ewes (*n*)	Mean EPG	*N*	Standard Deviation	*F*	*p* > *F*
Susceptible	8	1054[a]	72	1099	48.09	<.0001
Intermedia	9	361[b]	71	413		
Resistant	14	81[c]	106	152		

Different letters (a,b,c.) are statistically different ($p < .05$).
Note: EPG = Eggs per gram of feces; N = Observation number; n = number of ewes.

Discussion

Fecal Egg Count Performance During Pregnancy and Lactation

Peripartum rise is a widely known event in sheep of many breeds, both wool and hair, in which an increase in the FEC occurs because of a reduction in the immunity of the ewes at the end of pregnancy and during lactation. One of the main causes of reduced immunity is attributed to the energy deficit experienced by the ewe due to the demands of raising the lamb (López-Leyva et al., 2020), and it is widely known that ewes with multiple lambs have a higher FEC (Aguirre-Serrano et al., 2020). However, this effect was not included in this study because all the ewes were on a similar feeding regime.

During this period, the level of immunoglobulins and eosinophils decreases significantly (González-Garduño et al., 2021). In the results, the FEC in pregnancy was lower than those of lactation, and over time the peak of the EPG was found in week seven of mid-lactation, which coincides with the process of immunosuppression (Beasley et al., 2010). This behavior has already been reported in other studies (Maza-Lopez et al., 2020), where there is a notable increase in FEC during lactation. Furthermore, it is an inheritable, repeatable, and reliable trait to measure the resistance of animals (Boareki et al., 2021). During this increase in FEC, low amounts of antibodies are observed, which causes greater susceptibility to infections, including pathogens such as *H. contortus* (Hamer et al., 2019), which causes

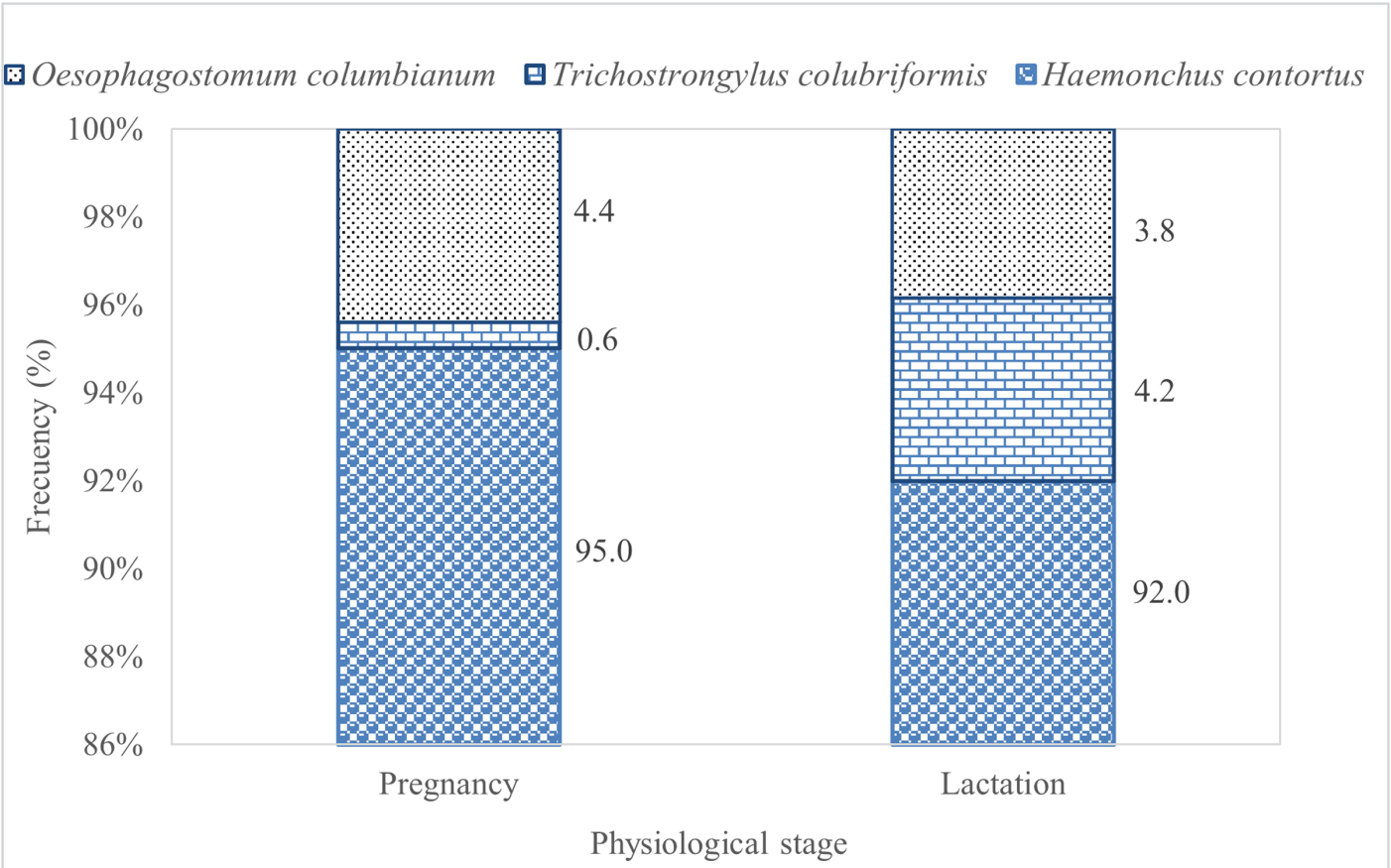

Figure 2.
Gastrointestinal Nematode Species Were Obtained from Fecal Culture of Grazing Ewes During the Pregnancy and Lactation Stages, Respectively.

anemia in sheep due to its hematophagous habits. Other authors show that this process of relaxation of the immune response is due to the peripartum stress that occurs even in supplemented ewes (Mena et al., 2025). Even given this effect, hair sheep show greater resistance against GIN compared to wool sheep, evidenced by lower FEC, better immune response (higher IgA, IgE, Th2 cytokine expression), and better body and hematological condition under infection (MacKinnon et al., 2015), which supports the performance of the Pelibuey breed in this study.

Humoral Response During Pregnancy and Lactation

In the present study, low IgA expressions were observed during pregnancy and lactation. The highest average values corresponded to approximately 26% PCt during lactation, with no differences observed during pregnancy. The low IgA level could be because this

Table 3.
Mean Fecal Egg Count of Pelibuey Sheep Nematodes According to Their Resistance Classification and Physiological Stage

Classification	Physiological stage	*N*	Mean	Standard Deviation
Resistant (n = 14)	Pregnancy	31	89^{c}	184
	Lactation	75	77^{c}	139
Intermediate (n = 9)	Pregnancy	13	231^{bc}	280
	Lactation	58	391^{b}	433
Susceptible (n = 8)	Pregnancy	12	825^{b}	667
	Lactation	60	1100^{a}	1165

Different letters at each stage indicate significant differences ($p < .05$). N = observation number; n = ewes number.

Table 4.
Comparison of the Relative Expression of Immunoglobulins A, G, and M with Respect to Positive Control During Pregnancy and Lactation in Pelibuey Sheep

Immunoglobulin	Stage	*N*	Mean (%)	*F*	Pr > *F*
IgA	Pregnancy	55	25.89^{a}	0.02	0.8815
	Lactation	170	25.28^{a}		
IgG	Pregnancy	55	51.39^{a}	21.1	<.0001
	Lactation	190	38.25^{b}		
IgM	Pregnancy	55	46.15^{a}	13.27	0.0004
	Lactation	176	38.49^{b}		

Different letters at each stage indicate significant differences ($p < .05$). N = observation number.

Table 5.
Comparison of Immunoglobulin Means by Resistance Categorization

Immunoglobulin	Categorization	*N*	Mean (%)	*F*	Pr > *F*
IgA	Resistant	96	32.7[a]	5.2	0.0064
	Intermedia	65	19.6[b]		
	Susceptible	64	20.5[b]		
IgG	Resistant	108	40.3[a]	0.55	0.5754
	Intermedia	66	43.7[a]		
	Susceptible	71	40.3[a]		
IgM	Resistant	64	42.0[a]	0.98	0.3763
	Intermedia	103	40.5[a]		
	Susceptible	64	38.2[a]		

Different letters for each immunoglobulin indicate significant differences ($p < .01$). N = observation number.

immunoglobulin is produced and exerts its action mainly locally in the mucosa, acting as a defense barrier (Hernández-Castellano et al., 2019). In another study, it was observed that salivary IgA responds with greater specificity to the larval surface antigen (CarLA) of trichostrongylid nematode species, confirming its specificity. This local production may be less affected in serological levels; however, there is a relationship between mucosal and blood serum levels for which its effect is not ruled out, and its low pronunciation in serum is understood (Aboshady et al., 2020).

IgG also showed significant differences, being higher during pregnancy and decreasing toward lactation. This event has been related to the decrease in immunity in the postpartum period and to the higher IgG content directed toward colostrum production, as part of a maternal immunological process for the provision of passive immunity to the lamb (González-Garduño et al., 2018).

IgM levels showed a significant decrease in lactation compared to pregnancy, coinciding with immunosuppression due to the peripartum period (David et al., 2020; González-Garduño et al., 2021). The reduction in immunity is widely known in many wool and hair sheep breeds, in which it has been shown that the maternal immune system at the end of pregnancy and during lactation is under greater physiological stress (Caroprese et al., 2010). The stress of parturition and increased metabolic demands during lactation result in increased production of reactive oxygen species, leading to reduced neutrophil function, antibody responses, and cytokine production by immune cells, compromising resistance to infections (González-Garduño et al., 2021).

Humoral Immune Response and Segregation

The higher IgA levels in resistant sheep suggest a possible association between increased IgA secretion or maintenance and natural resistance to GIN infection. These results indicate that, within the

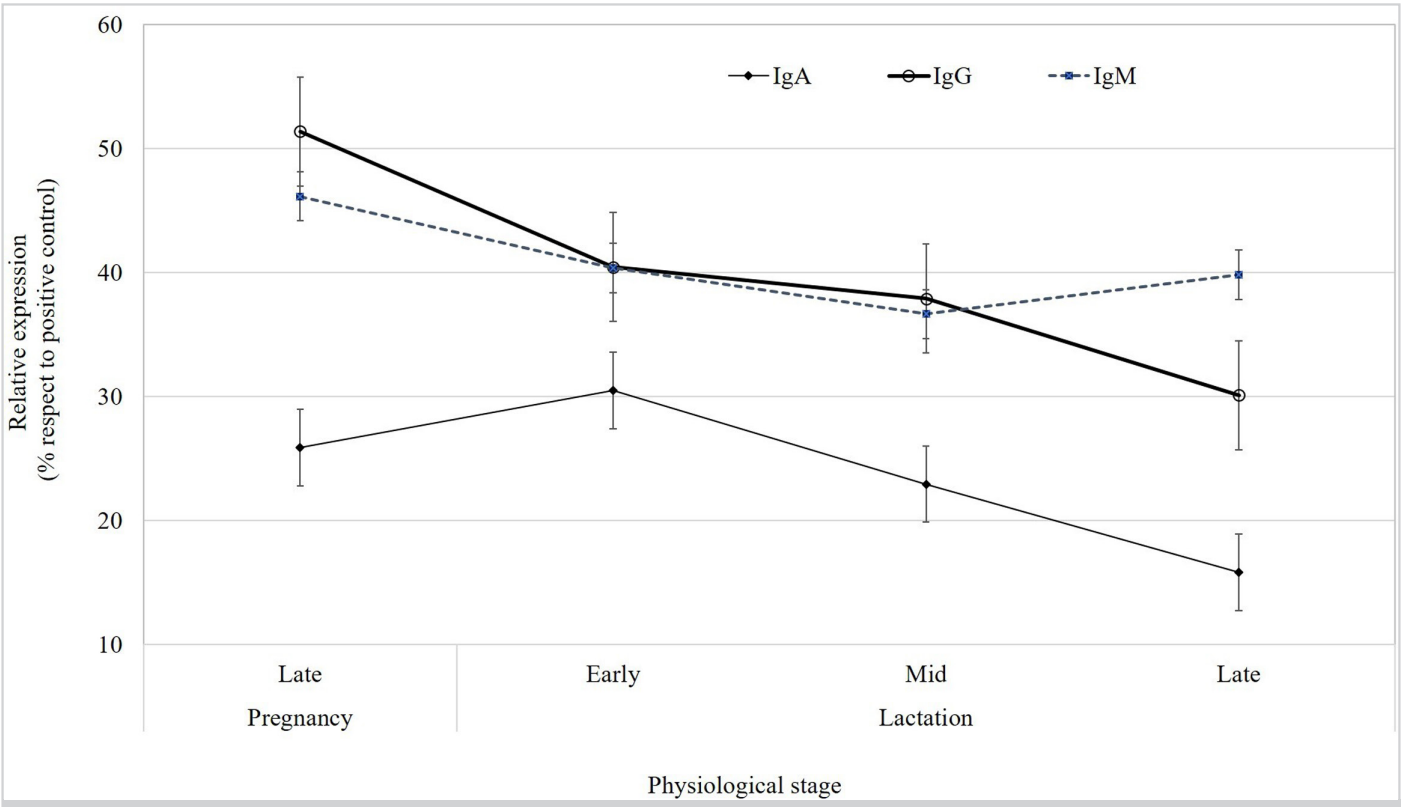

Figure 3.
Levels of Immunoglobulins A, G, and M in Pelibuey Ewes Classified by Resistance to Gastrointestinal Nematodes (Resistant, Intermediate, and Susceptible) During the Pregnancy and Lactation Stages Respectively.

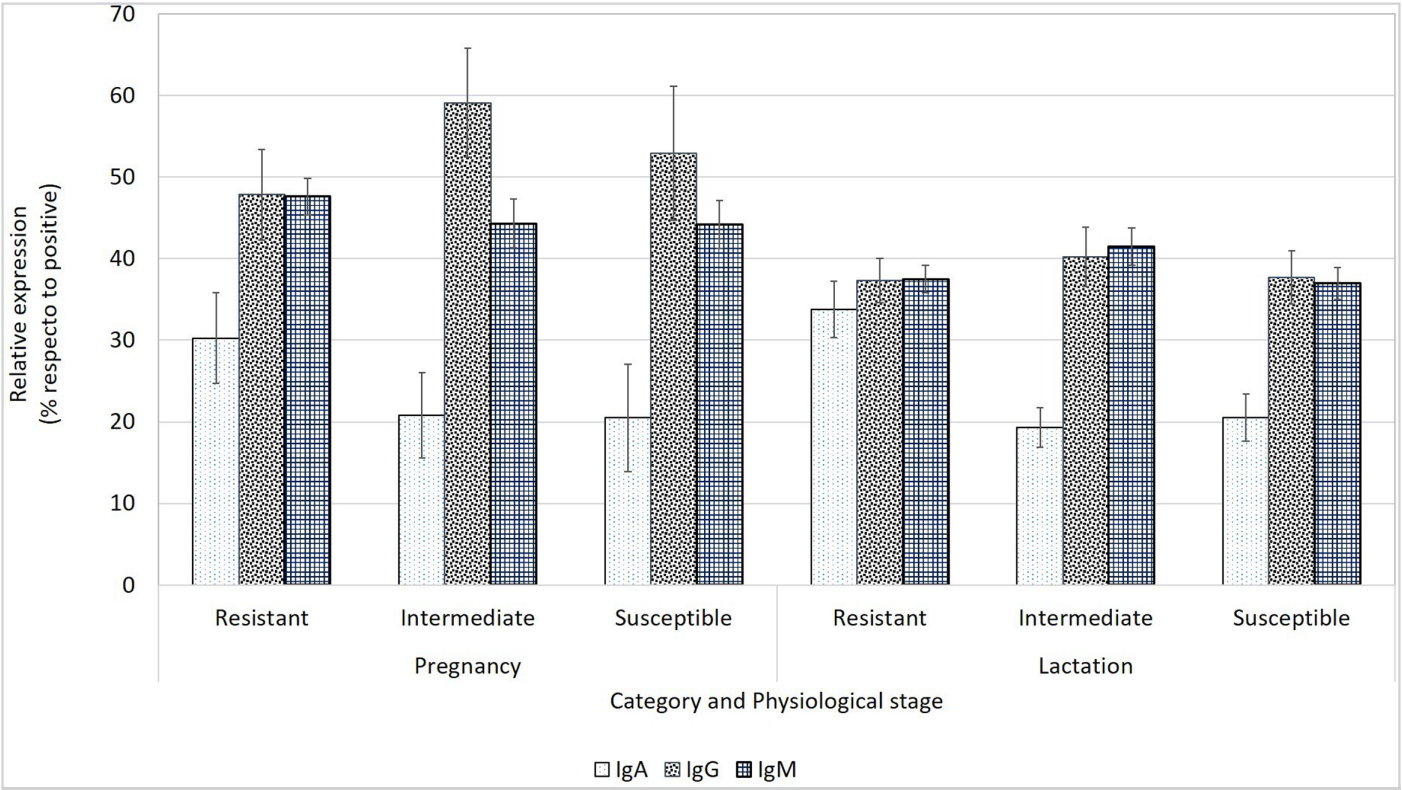

Figure 4.
Performance of the Levels of Immunoglobulins IgG, IgA, and IgM Over Time, Grouped By Weeks, Differentiated into Stages of Pregnancy (G), Early Lactation (IL), Mid-Lactation (LM), and Late Lactation (LT).

serological humoral profile evaluated, IgA is the immunoglobulin most associated with the GIN resistance category in Pelibuey sheep. Similar results were reported (Zaragoza-Vera et al., 2023), where IgA is shown to be the main immunoglobulin with a direct relationship between its levels and FEC. A study carried out on Corriedale and Rylington × Corriedale lambs from a resistant line to *H. contortus* showed similar performance to these results; IgA had higher levels in resistant lambs than in susceptible ones (Escribano et al., 2019). From a review, it was found consistent associations between higher levels of IgA and lower parasitological parameters (Ramos et al., 2022); mainly, this immunoglobulin can limit GIN infection through local mechanisms in the digestive tract, such as inhibition of larval development or interference with the parasite's digestive enzymes, and its level in mucosa is directly related to the amount in blood serum (Aboshady et al., 2020). It is important to state that the animal physiological stage has a particular role in the immunoglobulin performance (Zaragoza-Vera et al., 2023). For instance, during lactation, prolactin is produced, the hormone that acts as a hormone and as a cytokine, promotes the production and type of antibody (Borba et al., 2019) and it is attributed a suppressive effect on the immune system. On the other hand, the progesterone, by promoting immunological tolerance during pregnancy (Gutiérrez-Amézquita et al., 2017), while cortisol is produced with the stimulus of any stressful event (partum) and inhibits the proliferation of T cells and alters the function of immunoglobulins (Hollema et al., 2018), these hormones during the peripartum period directly influence the decline of the immune system.

Table 6.
Correlation of EPG and Immunoglobulins During the Pregnancy Stage

	EPG	IgA	IgG	IgM
EPG	1			
IgA	−0.037^{ns}	1		
IgG	0.11^{ns}	0.055^{ns}	1	
IgM	−0.19^{ns}	−0.013^{ns}	−0.51**	1

Correlations obtained from 55 observations.
Note: EPG = eggs per gram of feces.
**Highly significant $p < .01$, ns = not significant $p > .05$.

Table 7.
Correlations of EPG and Immunoglobulins During Lactation

	EPG	IgA	IgG	IgM
EPG	1			
IgA	−0.18*	1		
IgG	0.17*	−0.05^{ns}	1	
IgM	−0.12^{ns}	0.24**	−0.03^{ns}	1

Correlations derived from 170 observations.
Note: EPG = eggs per gram of feces.
*Significant $p < .05$ and, ns = not significant $p > .05$.
**Highly significant $p < .01$.

Because IgA has high specificity, the changes that occur at the local level could explain the fact that the levels of this immunoglobulin are not related to the physiological stage and maintain a constant level, while those of IgG and IgM show different patterns, due to the fact that these immunoglobulins have a widespread function in the response against helminths. In another study, no considerable changes in IgG levels were detected during the infection period for any of the GIN-resistant lines of lambs tested (Corriedale and Rylington x Corriedale), because those sheep showed a delayed immune response and do not present the peripartum effect as was the case in the present study (Escribano et al., 2019).

IgM and IgG decreased during lactation in all groups, but especially in the susceptible group, which could be the reason for the lower capacity of the immune system against parasitic infections, and consequently an increase in FEC during lactation due to the reduction in immunity, where the level of immunoglobulins and eosinophils is markedly reduced (González-Garduño et al., 2021).

The increase in late lactation of IgM may be due to the fact that immunoglobulin plays a central role in mammalian immunity by constituting the first isotype secreted during the primary humoral response, allowing it to act as an early defense against pathogens before the class switch to IgG or IgA occurs (Buchner et al., 2025), just the moment when lambs begin to have contact with other pathogenic microorganisms and occupy a primary defense system. Similar patterns have been described in the Suffolk breed, where IgM activity increases during lactation (Sha et al., 2024). Furthermore, the dynamics of the milk and digestive tract microbiota throughout lactation promote changes in this response; as weaning approaches, an increase in IgM is required to protect the offspring (Cao et al., 2024). Although classic comparative studies show decreases from colostrum to mature milk (Akhter et al., 2021), contrary to the study. Therefore, it would be convenient to evaluate the performance in milk and mucous membranes of lambs for future more specific studies.

Another aspect to consider is that the antigen used was a crude extract that cross-reacts with other nematode species, as observed in previous studies. In Blackbelly sheep, ELISA was used to confirm that different antigens were highly conserved and that the immune responses to *H. contortus* and *T. colubriformis* were similar (González Garduño et al., 2017). Therefore, the evaluation must consider that the response has a degree of no specificity and the immune response is also broad but reflects the level of immune development of the animal.

Correlations Between Eggs Per Gram of Feces and Immunoglobulins During Pregnancy and Lactation

The results suggest that parasitic resistance assessed by EPG did not present significant associations with immunoglobulin levels in pregnancy, although the negative interaction between IgA and the positive relation of IgM with EPG stands out as a possible regulatory mechanism of the immune response in lactating ewes.

The negative correlation between EPG and IgA has already been reported in other studies where there is an association between higher IgA levels and lower parasitological parameters (FEC) (Aboshady et al., 2020; DeWolf et al., 2025). The results were similar to those shown with high levels of IgA in saliva, serum, and abomasal mucosa that are associated with a lower parasitic burden in naturally infected lambs with *H. contortus* (Salgado et al., 2022). Furthermore, the FEC is used for the selection of resistant ewes against *H. contortus* in sheep, where lower counts reflect a greater efficacy of the immune response (Notter et al., 2017), as shown in the results. A study in goats demonstrated that inducing immunization with recombinant proteins such as rHcftt-2 produced higher serum IgA specific to *H. contortus*, in addition to IgG, which was associated with moderate reductions in egg excretions (Bu et al., 2020). Due to the correlation of IgA and the CarLA antigen, it is suggested to consider this immunoglobulin for the selection of animals resistant to NGI (Cunha et al., 2024).

Negative correlations between serum levels of IgG and IgM were significantly reduced, a performance that has been observed in other studies (Alam et al., 2020). It is also important to mention that there is the process of "Rclass switch combination (RSC)," which changes IgM to other immunoglobulins (Ig). During this process, the functional properties of the antibodies are modified, thus contributing to the organism's adaptive immune response (Liu et al., 2024), explaining the dynamics of change in immunoglobulins. The correlations, although weak, were significant during lactation, validating the selection of resistant animals during this stage (Zaragoza-Vera et al., 2023).

Conclusion

The results indicate that immunoglobulins can be used as indirect biomarkers associated with genetic resistance to GIN. Among them, IgA stands out for its direct relationship with parasite inhibition, evidenced by negative correlations with EPG. However, IgG and IgM also have a relevant role in the humoral immune response, especially during the peripartum period and in providing passive immunity to lambs, making their inclusion valuable in the evaluation of resistant animals. Overall, the incorporation of these immunoglobulins into genetic selection programs represents a complementary strategy for improving resistance to GIN and contributing to the sustainability of sheep production systems.

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