



Research article

High energy levels in the diet reduce the parasitic effect of *Haemonchus contortus* in Pelibuey sheep

Yoel López-Leyva^a, Roberto González-Garduño^{b,*}, Maximino Huerta-Bravo^a, Rodolfo Ramírez-Valverde^a, Glafiro Torres-Hernández^c, Javier Arece-García^d, Ma. Eugenia López-Arellano^e

^a Posgrado en Producción Animal, Universidad Autónoma Chapingo, Estado de México, Mexico

^b Unidad Regional Universitaria Sursureste, Universidad Autónoma Chapingo, Teapa, Tabasco, Mexico

^c Colegio de Postgraduados, Campus Montecillo, Montecillo, Edo. de México, Mexico

^d Estación Experimental de Pastos y Forrajes "Indio Hatuey", Universidad de Matanzas, Cuba

^e Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad, INIFAP, Jiutepec, Mor., Mexico

ARTICLE INFO

Keywords:

Nematode parasites
Immuglobulins
Integral diet
Dietary protein
Dietary energy

ABSTRACT

Nutritional aspects modulate the parasitological and immune response in infected sheep. The aim of the present study was to evaluate the parasitological and humoral immune response of Pelibuey sheep experimentally infected with *Haemonchus contortus* that were fed diets with two different energy ($n = 12$) and protein ($n = 12$) levels. Twenty-four Pelibuey lambs infected with *H. contortus* were evaluated over 12 weeks. An additional six animals were considered as a control group. Fecal egg count (FEC) was determined, in addition to packed cell volume (PCV), total plasma protein (TPP), and immunoglobulin levels (IgA, IgG, and IgM) by indirect enzyme-linked immunosorbent assay (iELISA). Data were analyzed by repeated measures over time. The lambs that received a high-energy (HighE) diet had the lowest FEC ($P < 0.01$) regardless of whether they received a high (HighP) or low (LowP) level of protein. The effect of energy level was also observed over time: FEC values decreased and PCV and TPP values increased. Higher immunoglobulin levels were obtained for females ($P < 0.05$) than males yet, overall, the energy and protein levels of the diets did not affect the response of the immunoglobulins. Over time, however, an increase in IgG and IgM was observed, whereas the IgA level remained basal.

1. Introduction

Haemonchus contortus is a gastrointestinal nematode (GIN) that affects health of small ruminants. It is highly prevalent and pathogenic due to its hematophagous habit, causing gastroenteritis, anemia, weight loss, and even the death of susceptible animals (Burgunder et al., 2018; Torres-Acosta et al., 2012). It therefore causes economic losses to production systems that are exacerbated by the cost of the chemical products used for its control which, at the same time, often have low efficiency due to the increasing resistance of GIN to most anthelmintics (Callanan et al., 2018; Crook et al., 2016; Doyle et al., 2018). Several alternatives can be combined to compensate for the low efficiency of chemical products, including those that function by delaying or reducing the effects of anthelmintic resistance and improving the health conditions of animals (Arece-García et al., 2016; Campbell et al., 2017; Westers et al., 2017). One alternative, for example, is the development of natural resistance in

hosts through improving their immune response. In sheep, the production of antibodies such as IgA, IgG, and IgM in addition to eosinophilia, mastocytosis of the mucosa, and hyperplasia of globular cells forms part of the immune response (Mcrae et al., 2015).

Notably, nutritional aspects modulate the immune response. The researchers conclude that GIN resistance in hosts is enhanced by increasing the amount of protein in the diet rather than the amount of energy (Houdijk, 2012). However, ruminal fermentation modifies protein structure and quality, suggesting that supplementation with energy at the rumen level promotes the production of microbial protein. Also, microbial protein is considered to have a lower metabolizable value in the intestine, and the quality of amino acids is less compatible with immune proteins than dietary bypass proteins (Cériac et al., 2019). Therefore, it is necessary to continue to research the role of energy and protein supplementation in sheep's immune response to GIN infection. By this reason, the objective of the present study was to evaluate the

* Corresponding author.

E-mail address: rgonzalezg@chapingo.mx (R. González-Garduño).

parasitological and humoral immune response of confined Pelibuey sheep experimentally infected with *H. contortus* that were fed a diet based on fermented sugarcane with two different energy and protein levels.

2. Materials and methods

2.1. Location

The experiment was carried out in Salto de Agua municipality, Chiapas, Mexico, at 17° 34' N and 92° 29' W and 85 m above sea level. The regional climate is warm humid, with an annual average temperature of 26.6 °C and annual average rainfall of 3298 mm (CONAGUA, 2019).

2.2. Animal management

Thirty Pelibuey lambs (female = 14, and male = 16) five-month-old with an average initial live weight of 17 ± 5 kg were used. The main effects of two levels of protein (n = 12, female = 6, male = 6) and energy (n = 12, female = 5 male = 7) were evaluated in addition to a control group (n = 6, 3 female, 3 male) in grazing. One week before the start of the experiment, all lambs were dewormed with Oxfenil® (Oxfendazole) at a ratio of 4.5 mg kg⁻¹ of live weight (LW) to ensure that they were free of GIN.

All lambs were confined in individual cages, assigned to an experimental diet (Table 1), and the control group remained at pasture to compare the parasitological and immune parameter between confined vs grazing lambs. The diet offered to the lambs consisted of sugarcane processed by solid fermentation. The ground cane was placed in a firm and flat area under the roof, then the other ingredients were added and stirred and the mixture spread in a layer of 10 cm high and allowed to ferment aerobically for 24 h. At the end, it was ensiled in 200 L containers for conservation for 8–12 days (Elias et al., 1990). To the high-energy (HighE) treatments, 12.4 % of molasses was added to increase the amount of energy in the diet. The animals were fed twice a day, once in the morning and once in the afternoon, and had free access to water.

From the integral diet, the dry matter (DM) was determined after maintaining the sample 24 h in an oven at 60 °C. The crude protein (CP) was determined with an elemental analyser Flash 2000 (Thermo Scientific, Italy) and the ashes quantified by calcination at 550 °C in a muffle (Thermo Scientific, USA). The energy was calculated using tables of chemical composition of foods (NRC, 2007).

An adaptation period to the experimental diets was carried out (15 days). After the first week of the study, the lambs that receive experimental diets were infected biweekly four times (day 42) with third-stage *H. contortus* larvae (L3) at doses of 200 L3 kg⁻¹ LW (Figure 1). L3 were obtained by coproculture (Corticelli and Lai, 1963) from two donor

lambs previously infected with an oral dose of 300 L3 from a local strain of *H. contortus*. The grazing group was naturally infected in paddocks composed of *Brachiaria humidicola* and *Paspalum conjugatum* grasses. The lambs in the latter group grazed 9 h per day.

The live weight of the animals was recorded every fifteen days. Each week, fecal samples were taken directly from the rectum of the lambs and fecal egg count (FEC) was determined using the McMaster technique (Thienpont et al., 2003) with a minimum sensitivity of 50 eggs per gram (EPG) of feces. Blood samples were taken every 15 days using tubes (Vacutainer, Becton Dickinson, USA) with ethylene diamine tetraacetate (EDTA) as anticoagulant by puncturing the jugular vein. Packed cell volume (PCV) was determined by microhematocrit technique and total plasma protein (TPP) through refractometry (g/dL). Another sample with coagulation accelerator was taken for serum immunoglobulin determination; serum was obtained by centrifugation at 1100 g for 25 min and subsequently stored at -20 °C until analysis.

The immunoglobulin levels (IgA, IgG, and IgM) were determined by means of an indirect enzyme-linked immunosorbent assay (iELISA) using crude antigens (CAG) of *H. contortus* and *Trichostrongylus colubriformis* according to the procedure described by González Garduño et al. (2017). The optical density (OD) of each serum was obtained by subtracting from each well the values of the wells without serum, which represented the non-specific binding of the conjugate and the activity of the Igs were expressed as a percentage to a positive standard serum (RPS) to calibrate the values among the plates.

The advisory committee of the postgraduate program in animal science approved the experiment protocol. The procedures were performed in accordance with the Mexican Official Standard guideline 051-ZOO-1995 for production, care and use of experimental animals.

2.3. Statistical analysis

To maximize the number of repetitions per treatment, a factorial treatment design was used. The main effects were energy with two levels, high (n = 12) and low (n = 12), and protein with two levels, high (n = 12) and low (n = 12), in addition, the interactions were studied. To reduce variance and approximate the FEC to a normal distribution, the values were transformed to base 10 logarithm (Log₁₀ FEC+1). A multivariate linear mixed-effect regression model was used under a design of repeated measures over time with the autoregressive structure (AR1) by presenting lower AIC and BIC values. The fixed effect of gender was included in the model. The variables were analyzed through the SAS mixed procedure (SAS, 2017) following the statistical model (Eq. (1)):

$$Y_{ijklmn} = \mu + \mathcal{Z}_i + \epsilon_j + \rho_k + \epsilon^* \rho_{jk} + d_l + \gamma_m + d^* \epsilon_{jl} + d^* \rho_{kl} + d^* \epsilon^* \rho_{jkl} + \epsilon_{ijklmn} \tag{1}$$

where Y_{ijklmn} = the response of the variable. μ = the general mean. \mathcal{Z}_i = the gender of the animal (i = female, male). ϵ_j = the effect of the j-th energy level (j = high and low). ρ_k = the effect of the protein level (k = high and low). $\epsilon^* \rho_{jk}$ = the interaction of energy and protein. d_l = the effect of the l-th sampling (l = 1, 2, 3 ... 11, 12). γ_m = the random effect of the lamb. $d^* \epsilon_{jl}$; $d^* \rho_{kl}$; $d^* \epsilon^* \rho_{jkl}$ were the interactions, respectively; and ϵ_{ijklmn} = the error associated with repeated measurements. The separation of means was performed using the Lsmear procedure, and Pearson correlations using the SAS software.

3. Results

Table 2 shows the main effects of the studied variables.

The FEC gradually increased over time (P < 0.01), with values higher than 370 EPG at day 35. The energy level had a significant effect (P < 0.05) on the FEC. The lambs that received the high-energy diets (HighE) had the lowest FEC and the highest PCV and TPP values regardless of the protein level, whereas the protein level did not influence the FEC. In the grazing lambs (control group), the FEC were

Table 1. Ingredients of the diets used in feeding Pelibuey lambs.

Ingredients (%)	Low Energy		High Energy		Grazing Grz
	Low protein	High protein	Low protein	High protein	
Sorghum (%)	5	5	25	25	0
Soybean (%)	3.5	11.5	1.0	8.5	0
Sugarcane (%)	90	82	71.5	64.0	0
Mineral mix (%)	0.5	0.5	0.5	0.5	0.03
Urea (%)	1.0	1.0	1.0	1.0	0
Molasses (%)	0	0	1.0	1.0	0
Dry matter (%)	37	40	47	52	
Energy (Mcal/Kg)	2.6	2.6	3.0	3.01	
Crude protein (%)	9.4	12.5	8.5	11.3	
Carbon (%)	1.50	2.00	1.48	1.80	
Ashes (%)	5.49	5.89	5.84	5.27	

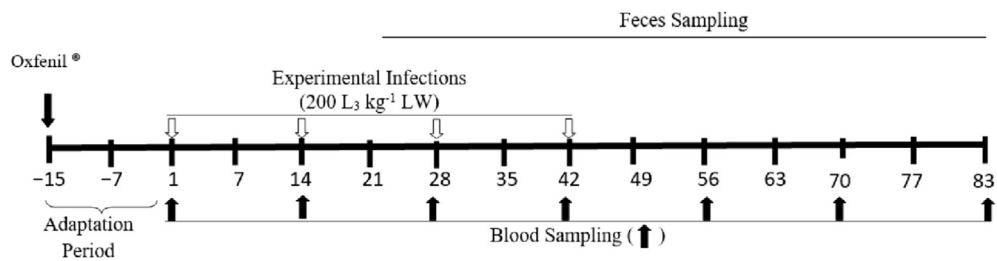


Figure 1. Experimental infections with gastrointestinal nematode larvae, blood and feces sampling in Pelibuey lambs. LW: Live weight.

higher than 1000 EPG and similar to those of the lambs that received low levels of energy.

The FEC of the lambs that received the high-energy diets ($P \leq 0.05$) were lower than those that received the low-energy diets (Figure 2) from day 56 post infection until the end of the study. After 42 days, an increase in PCV was observed for the animals that received the high-energy diets compared to the low-energy diets (Figure 3). The TPP (Figure 4) gradually decreased over time, especially in the low-energy treatments. This effect was evident from day 28 post infection, at which point the values fell below the threshold for healthy animals. On the other hand, the high-energy diets led to an increase in the TPP from days 42–70.

In the case of live weight, the lambs that received the low-energy diets had an equal or lesser weight than the grazing lambs. After 42 days, an increase in weight was observed in the lambs that received the high-energy diets (Figure 5).

With respect to IgA, no differences were observed among treatments ($P > 0.05$). The LowE-LowP group had a higher level ($P > 0.05$) of IgG compared to the grazing group (Grz) in response to both antigens used. IgM was only affected by the treatments ($P < 0.05$): The lambs that received the HighE-HighP diet had the lowest levels compared to the LowE-LowP and Grz groups (Table 3).

IgG gradually increased until day 42 and then sharply decreased by day 56 before slightly increasing until day 83 (Figure 6). On the other hand, IgM maintained high levels after day 42 (Figure 7), and IgA was found at levels lower than 20% throughout the study period.

4. Discussion

The high FEC observed over time in all lambs indicates the successful establishment of *H. contortus* in response to experimental infection. The

Table 2. Main effects and interaction of fecal egg count (FEC), live weight (LW), packed cell volume (PCV), and total plasma protein (TPP) with respect to gender, sampling days, and treatments.

	N	FEC		Live weight		PCV		TPP	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Gender		**		**		**		ns	
Female	14	731 ^b	102	20.8 ^b	0.47	26.1 ^a	0.5	5.7 ^a	0.1
Male	16	1597 ^a	255	27.8 ^a	0.65	24.3 ^b	0.5	5.9 ^a	0.08
Sampling day		**		**		**		**	
1	30	0 ^d	0	19.9 ^c	1.02	25.8 ^b	1.01	6.3 ^a	0.07
28	30	50 ^d	6	22.6 ^{bc}	1.13	28.7 ^a	0.5	5.9 ^{ab}	0.08
35	30	378 ^c	97	-	-	-	-	-	-
42	30	545 ^c	102	23.9 ^{ab}	1.19	25.6 ^{bc}	0.8	5.5 ^c	0.1
49	30	729 ^{bc}	131	-	-	-	-	-	-
56	30	860 ^{bc}	225	26.1 ^a	1.33	24.1 ^{bc}	0.7	5.8 ^b	0.1
63	30	1010 ^{bc}	377	26.7 ^a	1.43	-	-	-	-
70	30	1495 ^b	389	26.5 ^a	1.37	24.2 ^{bc}	0.7	6.1 ^{ab}	0.2
77	30	1545 ^b	299	-	-	-	-	-	-
83	30	2898 ^a	846	26.3 ^a	1.43	23.1 ^c	1.03	5.3 ^c	0.1
Energy		**		**		**		**	
High energy (HighE)	12	680 ^b	91	26.2 ^a	0.9	26.3 ^a	0.4	6.1 ^a	0.05
Low energy (LowE)	12	1653 ^a	259	22.4 ^b	0.6	24.2 ^b	0.6	5.5 ^b	0.1
Protein		ns		**		*		*	
High protein (HighP)	12	1265 ^a	279	25.4 ^a	0.7	26.0 ^a	0.6	5.9 ^a	0.08
Low protein (LowP)	12	1118 ^a	121	23.2 ^b	0.8	24.4 ^b	0.5	5.7 ^b	0.1
Interaction		*		ns		ns		ns	
LowE-LowP	6	1269 ^b	178	20.6 ^a	0.8	23.4	0.7	5.4	0.1
LowE-HighP	6	2127 ^a	529	24.5 ^a	0.7	25.1	0.9	5.7	0.1
HighE-LowP	6	936 ^{bc}	156	26.2 ^a	1.3	25.6	0.6	6.0	0.08
HighE-HighP	6	424 ^c	75	26.2 ^a	1.3	27.0	0.6	6.2	0.09
Grz	6	1621 ^{ab}	301	24.6 ^a	0.9	25.4	1.0	5.8	0.1
Standard deviation		1615		5.04		3.25		0.5	

N: Number of lambs. Different letters in each study factor in the same column are significantly different ($P \leq 0.05$). E: Energy. P: Protein. Grz: Grazing. SE: Standard error.

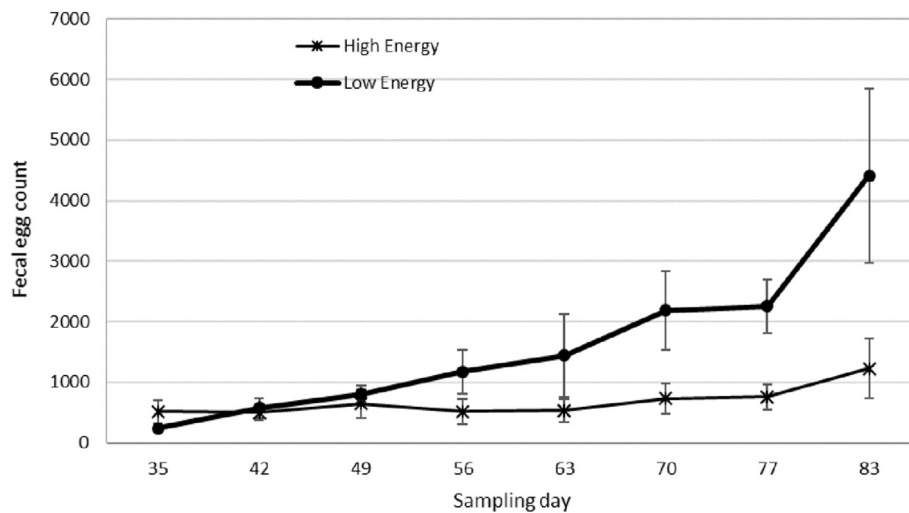


Figure 2. Effect of diet energy level on nematode fecal egg count in Pelibuey sheep during the study period.

gradual increase in the FEC after the prepatent period suggests an additive effect of the weekly infections and, therefore, an increase in the number of adult nematode females in oviposition (Arece-García et al., 2014).

Energy supply is also essential for the development of immunological mechanisms, as suggested by Colditz (2008). The effect of the energy level on growing lambs was shown herein given that the high-energy diet was associated with lower FEC regardless of the protein level. The results for the diet with the lowest level of crude protein (CP, 9%) confirmed that the main limiting element was energy. Even so, at this CP level, it was possible to maintain the immunological levels of the lambs, enabling a degree of GIN control. At the same time, parasitism increases the protein need for maintenance and repair of damaged tissues (Khan et al., 2012). Furthermore, in infected animals, protein utilization is affected, and lower weight gains are achieved (Greer et al., 2009). So, more energy is required to maintain a live weight increase similar to that of non-infected animals.

The lambs fed with the high-energy diets had high PCV values and the highest TPP levels compared to the lambs that received the low-energy diets. This finding coincides with other study that highlight the importance of high energy levels in the diet for defense against GIN (Colditz, 2008). However, some authors have also show the importance of proteins and adequate energy balance for correctly activating defense mechanisms (Torres-Acosta et al., 2012; Whitley et al., 2014). Ultimately, it appears that diets with both a high level of energy and protein have a positive impact on the immunological status of animals.

With respect to other studies, the PCV values found herein were higher compared to those found for sheep (18%) naturally infected with *H. contortus* in Iraq (Awad et al., 2016). As mentioned, the PCV values found herein were closely related with the energy and protein content of the diets and the time passed since initial infection (sampling days) due to the effects of parasitism. The highest PCV and TPP values were found in the lambs that received the high protein diets, although this was also observed for the high-energy diets above a certain threshold of health impairment (TPP = 6.0 g/dL and PCV = 20%). The low PCV and TPP values observed in the lambs that consumed the low-energy diets could be due to the establishment of parasites and damage caused by them at the gastrointestinal mucosal level. In addition, the decrease in these values after 42 days could indicate that the low-energy diets were not sufficient for tissue repair or for the complete establishment of defense mechanisms. Therefore, the infection continued its course, resulting in a decrease in both PCV and TPP over time.

Other study show that the hematophagous behavior and hemolytic activity of *H. contortus* affect the mucosa of the abomasum (Emery et al., 2016), leading to a considerable loss of protein. In addition, the synthesis

of TPP is related with the humoral immunological system, which was activated due to continuous infection with *H. contortus*. However, it may not have been possible to completely compensate for protein loss even with the high-energy diet evaluated herein.

The serum levels of the three immunoglobulins analyzed herein were previously found to differ in their response to primary experimental infection with GIN (González-Garduño et al., 2018). In the present study, the low IgA response to the *H. contortus* crude antigen appears to be independent of the energy and protein levels of the diets. Several studies have suggested that IgA has little value as a marker of *H. contortus* infection (Snoeck et al., 2006), although other studies have suggested it as a marker of susceptibility to GIN infections (Shaw et al., 2012). However, the IgA levels found herein were below the range (10%–15%)

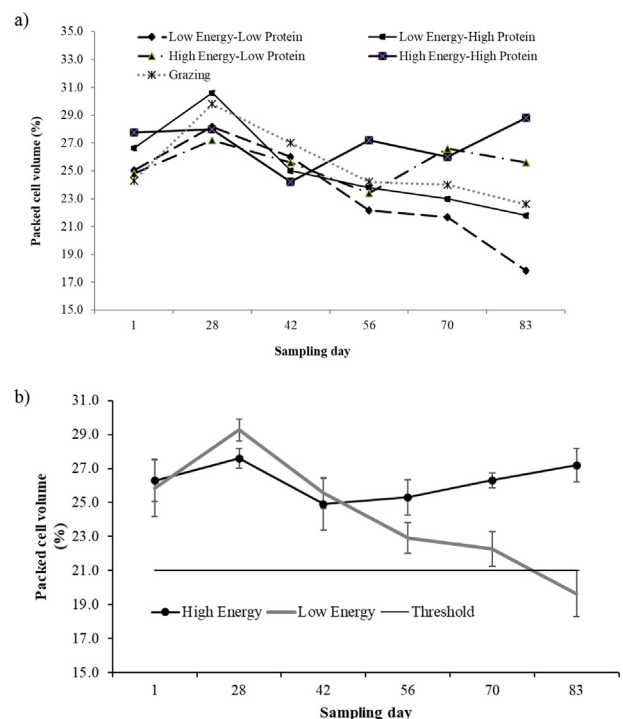


Figure 3. Packed cell volume (PCV) of Pelibuey lambs a) classified in four experimental groups according to diet energy and protein level including the grazing group. b) Principal effect of dietary energy level on PCV not including the grazing group.

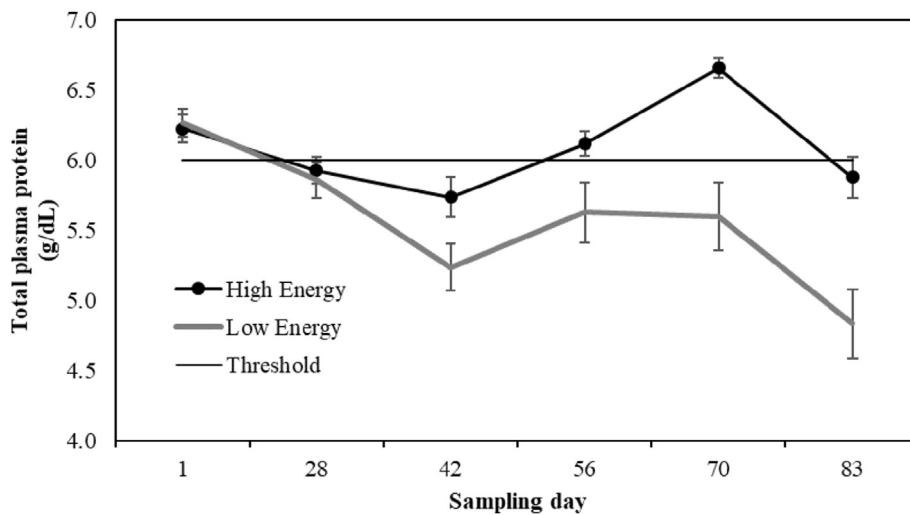


Figure 4. Effect of dietary energy level on total plasma protein in Pelibuey sheep during the study period.

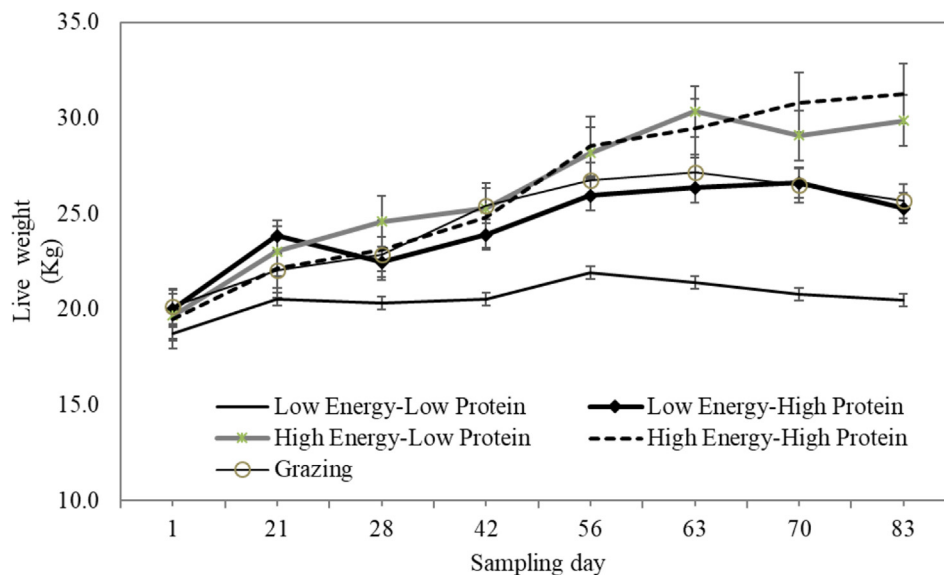


Figure 5. Effect of dietary energy level on the live weight of Pelibuey sheep during the study period.

indicated for growing lambs (González-Garduño et al., 2018). It may be that the IgA response is occurs locally rather than at the circulating level or in external secretions given that it has functions in saliva, milk, tears, bronchial mucous membranes, genital and urinary organs, and gastrointestinal tracts due to its direct protective role of mucosae, especially in

the abomasum and jejunum (McClure, 2008). In addition, IgA can be produced by plasma cells, in the differentiation of B cells by dendritic cells against parasite antigens, and in Peyer's patches at the intestinal level. Accordingly, its presence could be detected both locally and peripherally.

Table 3. Immunoglobulin percentages in Pelibuey sheep fed two energy and protein levels with respect to a positive control (%) and effect of animal gender.

Antibody	N	Treatment					Gender		SE
		LowE-LowP	LowE-HighP	HighE-LowP	HighE-HighP	Grz	Female	Male	
<i>Haemonchus contortus</i> antigen									
IgA	36	11.9 ^a	8.7 ^a	8.7 ^a	8.8 ^a	13.0 ^a	11.9 ^a	8.0 ^b	1.7
IgG	31	68.4 ^a	50.1 ^{ab}	46.7 ^{ab}	52.9 ^{ab}	35.9 ^b	37.1 ^b	65.6 ^a	7.2
IgM	35	72.2 ^a	67.3 ^{ab}	67.3 ^{ab}	57.4 ^b	75.6 ^a	71.0 ^a	63.4 ^a	4.2
<i>Trichostrongylus colubriformis</i> antigen									
IgA	32	7.0 ^{ab}	5.5 ^{ab}	3.5 ^b	5.5 ^{ab}	9.0 ^a	8.0 ^a	4.2 ^b	1.1
IgG	30	57.3 ^a	40.6 ^{ab}	45 ^{ab}	34.3 ^{ab}	28.0 ^b	33.6 ^a	49.5 ^a	7.1

SE: Standard error. N: Number of samples. Different letters in treatment in the same row are significantly different ($P \leq 0.05$). Different letters in gender in the same row are significantly different ($P \leq 0.05$).

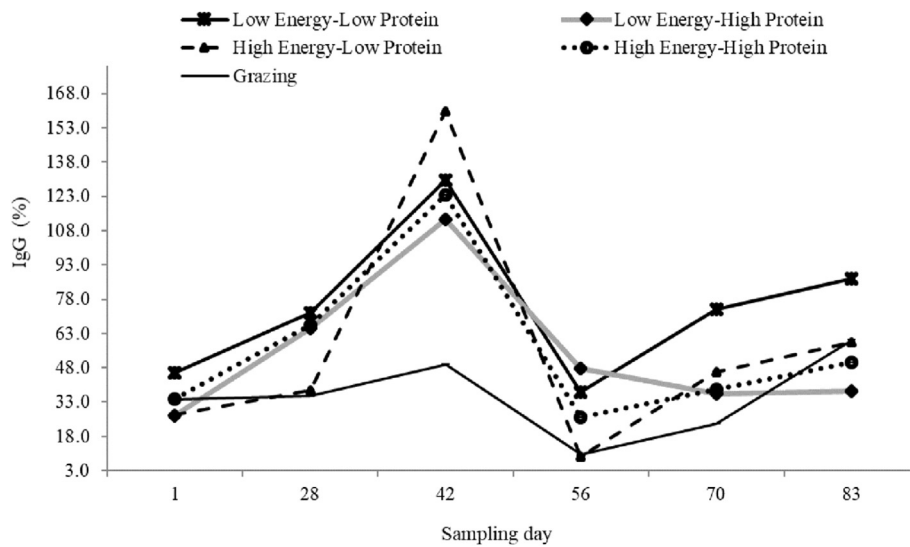


Figure 6. IgG level (%) in response to a crude *Haemonchus contortus* antigen with respect to a positive control (grazing) in Pelibuey sheep.

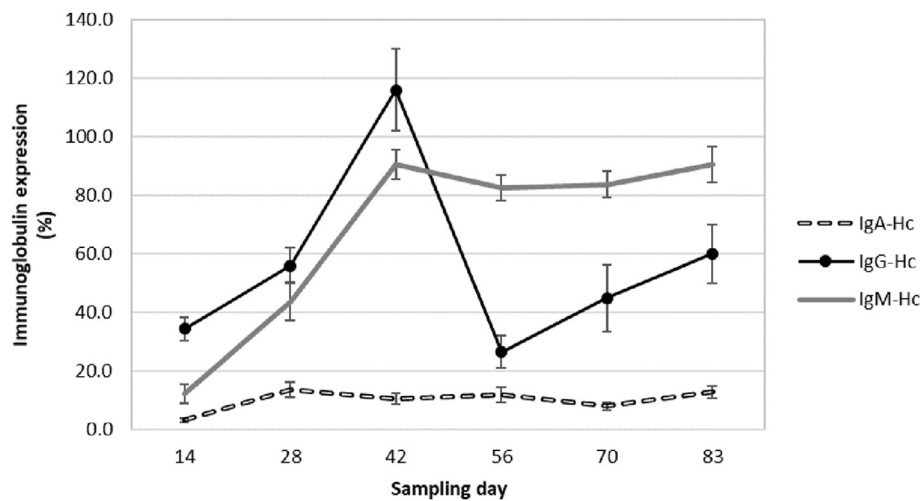


Figure 7. Effect of sampling days on the performance of IgA, IgG, and IgM in response to a crude *H. contortus* (Hc) antigen with respect to a positive control (%) in Pelibuey lambs.

Previously, IgM and IgG levels have been correlated with parasite burdens in immune sheep (Williams et al., 2010). Specifically, IgM has been described as the first immunoglobulin produced in the primary response to an antigen. Similarly, in the present study, its values gradually increased in response to weekly infections. Finally, it has been described along with IgA to play an important accessory role as a secretory immunoglobulin that acts on mucosal surfaces, and its presence in the gastrointestinal mucosa would be justified given the damage caused by *H. contortus* (Emery et al., 2016).

In the case of IgG, it has high affinity for specific antibody receptors (Fc receptors) and represents about 80% of the total immunoglobulins present in serum. It is considered one of the best markers for *H. contortus*-caused parasitism (Mcrae et al., 2015). The variability in the IgA, IgG, and IgM levels over time could be explained by the particular characteristics of each immunoglobulin, such as affinity to antigens and serum concentrations. For example, the increase in IgG and IgM from 14 days to 42 days post infection is a normal response to infection (Mcrae et al., 2015). Meanwhile, in the present study, IgM increased sharply until day 42, and this increase was maintained until day 83-post infection. In this case, the performance of IgM indicates that an acute response was maintained, possibly because animals were subjected to weekly infections until day 42. Therefore, IgM differentially responded to the

antigen replacements in comparison to the other isotypes. Other studies on lambs infected with *H. contortus* found an increase in IgA, IgG, and IgM levels over the course of 70 days (Schafer et al., 2015).

5. Conclusions

Lower FEC and higher PCV and TPP values were observed in the lambs fed with high-energy diets compared to the low-energy diets regardless of the protein level. The IgG and IgM levels increased over time post infection. On the other hand, the energy and protein levels in the diets did not affect the response of the evaluated immunoglobulins in the first infection in lambs.

Declarations

Author contribution statement

Yoel López-Leyva: Performed the experiments; Wrote the paper.
 Roberto González-Garduño, Glafiro Torres-Hernández, Javier Arece-García: Conceived and designed the experiments; Wrote the paper.
 Maximino Huerta-Bravo: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Rodolfo Ramírez-Valverde: Analyzed and interpreted the data; Wrote the paper.

Ma. Eugenia López-Arellano: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The author, Yoel López Leyva is a PhD student of the Program of Animal Production of the Universidad Autónoma Chapingo and thanks the CONACyT Program for the scholarship.

References

- Arece-García, J., López-Leyva, Y., González-Garduño, R., Torres-Hernández, G., 2014. Evaluation of strategic and selective anthelmintic treatments on Pelibuey ewes in Cuba. *Colomb. Ciencias Pecu.* 27, 273–281.
- Arece-García, J., López-Leyva, Y., González-Garduño, R., Torres-Hernández, G., Rojo-Rubio, R., Marie-Magdeleine, C., 2016. Effect of selective anthelmintic treatments on health and production parameters in Pelibuey ewes during lactation. *Trop. Anim. Health Prod.* 48.
- Awad, A.H., Ali, A.M., Hadree, D.H., 2016. Some haematological and biochemical parameters assessments in sheep infection by *Haemonchus contortus*. *Tikrit J. Pure Sci.* 21, 11–15.
- Burgunder, J., Petřelková, J.K., Modrý, D., Kato, A., Juránková, J., MacIntos, J.J.M., 2018. Fractal measures in activity patterns: do gastrointestinal parasites affect the complexity of sheep behaviour? *Appl. Anim. Behav. Sci.* 205, 44–53.
- Callanan, M.K., Habibi, S.A., Law, W.J., Nazareth, K., Komuniecki, R.L., Forrester, S.G., 2018. Investigating the function and possible biological role of an acetylcholine-gated chloride channel subunit (ACC-1) from the parasitic nematode *Haemonchus contortus*. *Int. J. Parasitol. Drugs Drug Resist.* 8, 526–533.
- Campbell, B.J., Pullin, A.N., Pairis-García, M.D., McCutcheon, J.S., Lowe, G.D., Campler, M.R., Fluharty, F.L., 2017. The effects of alternative weaning strategies on lamb health and performance. *Small Rumin. Res.* 156, 57–65.
- Cériac, S., Archimède, H., Feuillet, D., Félicité, Y., Giorgi, M., Bambou, J.C., 2019. Supplementation with rumen-protected proteins induces resistance to *Haemonchus contortus* in goats. *Sci. Rep.* 9, 1–10.
- Colditz, I.G., 2008. Six costs of immunity to gastrointestinal nematode infections. *Parasite Immunol.* 30, 63–70.
- CONAGUA, 2019. Servicio Meteorológico Nacional. Normales Climatológicas [WWW Document]. TXT. http://smn.cna.gob.mx/climatologia/normales/estacion/tab/NO_RMAL27068.
- Corticelli, B., Lai, M., 1963. Studies on the technique of culture of infective larvae of gastrointestinal strongyles of cattle. *Acta Med. Vet. Napoli* 9, 347–357.
- Crook, E.K., O'Brien, D.J., Howell, S.B., Storey, B.E., Whitley, N.C., Burke, J.M., Kaplan, R.M., 2016. Prevalence of anthelmintic resistance on sheep and goat farms in the mid-Atlantic region and comparison of in vivo and in vitro detection methods. *Small Rumin. Res.* 149, 89–96.
- Doyle, S.R., Laing, R., Bartley, D.J., Britton, C., Chaudhry, U., Gilleard, J.S., Holroyd, N., Mable, B.K., Maitland, K., Morrison, A.A., Tait, A., Tracey, A., Berriman, M., Devaney, E., Cotton, J.A., Sargison, N.D., 2018. A genome resequencing-based genetic map reveals the recombination landscape of an outbred parasitic nematode in the presence of polyploidy and polyandry. *Genome Biol. Evol.* 10, 396–409.
- Elías, A., Lezcano, O., Lezcano, P., Cordero, J., Quintana, L., 1990. Reseña descriptiva sobre el desarrollo de una tecnología de enriquecimiento proteico de la caña de azúcar mediante fermentación en estado sólido (saccharina). *Rev. Cubana Cienc. Agric.* 24, 1–12.
- Emery, D.L., Hunt, P.W., Le Jambre, L.F., 2016. *Haemonchus contortus*: the then and now, and where to from here? *Int. J. Parasitol.* 46, 755–769.
- González-Garduño, R., Mendoza-de Gives, P., López-Arellano, M.E., Aguilar-Marcelino, L., Torres-Hernández, G., Ojeda-Robertos, N.F., Torres-Acosta, J.F.J., 2018. Influence of the physiological stage of Blackbelly sheep on immunological behaviour against gastrointestinal nematodes. *Exp. Parasitol.* 193, 20–26.
- González Garduño, R., López Arellano, M.E., Conde Felipe, M.M., Mendoza de Gives, P., Aguilar Marcelino, L., Jaso Díaz, G., 2017. Immune and haematological parameters of Blackbelly ewes infected with gastrointestinal nematodes. *Colomb. Ciencias Pecu.* 30, 219–230.
- Greer, A.W., Sedcole, R.J., Jay, N.P., McAnulty, R.W., Green, R.S., Stankiewicz, M., Sykes, A.R., 2009. Protein supply influences the nutritional penalty associated with the development of immunity in lambs infected with *Trichostrongylus colubriformis*. *Animal* 3, 437–445.
- Houdijk, J.G.M., 2012. Differential effects of protein and energy scarcity on resistance to nematode parasites. *Small Rumin. Res.* 103, 41–49.
- Khan, F.A., Sahoo, A., Sonawane, G.G., Karim, S.A., Dhakad, S., Pareek, A.K., Tripathi, B.N., 2012. Effect of dietary protein on responses of lambs to repeated *Haemonchus contortus* infection. *Livest. Sci.* 150, 143–151.
- McClure, S.J., 2008. How minerals may influence the development and expression of immunity to endoparasites in livestock. *Parasite Immunol.* 30, 89–100.
- Mcrae, K.M., Stear, M.J., Good, B., Keane, O.M., 2015. The host immune response to gastrointestinal nematode infection in sheep. *Parasite Immunol.* 37, 605–613.
- NRC, 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. National Academy Press, Washington, DC.
- SAS, 2017. SAS/STAT User's Guide, Release 6. Ed. Cary, NC, USA.
- Schafer, A.S., Leal, M.L.R., Molento, M.B., Aires, A.R., Duarte, M.M.M.F., Carvalho, F.B., Tonin, A.A., Schmidt, L., Flores, E.M.M., Franca, R.T., Grandó, T.H., Minho, A., Krause, A., Antoniazzi, A.Q., Lopes, S.T.A., 2015. Immune response of lambs experimentally infected with *Haemonchus contortus* and parenterally treated with a combination of zinc and copper. *Small Rumin. Res.* 123, 183–188.
- Shaw, R.J., Morris, C.A., Wheeler, M., Tate, M., Sutherland, I.A., 2012. Salivary IgA: a suitable measure of immunity to gastrointestinal nematodes in sheep. *Vet. Parasitol.* 186, 109–117.
- Snoeck, V., Peters, I.R., Cox, E., 2006. The IgA system: a comparison of structure and function in different species. *Vet. Res.* 37, 455–467.
- Thienpont, D., Rochette, F., Vanparijs, O.F.J., 2003. Diagnosing Helminthiasis by Coprological Examination, Third. ed. Janssen Research Foundation, Beerse, Belgium.
- Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Hoste, H., Aguilar-Caballero, A.J., Cámara-Sarmiento, R., Alonso-Díaz, M.A., 2012. Nutritional manipulation of sheep and goats for the control of gastrointestinal nematodes under hot humid and subhumid tropical conditions. *Small Rumin. Res.* 103, 28–40.
- Westers, T., Jones-Bitton, A., Menzies, P., VanLeeuwen, J., Poljak, Z., Peregrine, A.S., 2017. Comparison of targeted selective and whole flock treatment of periparturient ewes for controlling *Haemonchus* sp. on sheep farms in Ontario, Canada. *Small Rumin. Res.* 150, 102–110.
- Whitley, N.C., Oh, S.-H., Lee, S.J., Schoenian, S., Kaplan, R.M., Storey, B., Terrill, T.H., Mobinid, S., Burke, J.M., Miller, J.E., Perdue, M.A., 2014. Impact of integrated gastrointestinal nematode management training for U.S. goat and sheep producers. *Vet. Parasitol.* 200, 271–275.
- Williams, A.R., Palmer, D.G., Williams, I.H., Vercoe, P.E., Emery, D.L., Karlsson, L.-J.E., 2010. Relationships between immune indicators of parasitic gastroenteritis, nematode burdens and faecal dry matter in sheep. *Anim. Prod. Sci.* 50, 219–227.