https://doi.org/ 10.32854/agrop.v14i2.1963

Analysis of the productive and immune response of lambs infected with gastrointestinal nematodes and fed with saccharin

González-Garduño, Roberto[1]; Silva Torres, Luis Matias[1]; Torres Hernández, Glafiro[2]; López Arellano, María Eugenia[3]; Flores Santiago, Ever del Jesus[1*]; Aguilar Caballero, Armando Jacinto[4]; Vargas Villamil, Luis Manuel[5]; Zaragoza Vera, Claudia[6]

[1]Universidad Autónoma Chapingo. Unidad Regional Universitaria Sursureste, Teapa, Tabasco, México. [2]Colegio de Postgraduados Campus Montecillo, Montecillo, Texcoco, Estado de México, México. [3]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Centro Nacional de Investigación Disciplinaria en Parasitología Veterinaria. Jiutepec, Morelos, México. [4]Universidad Autónoma de Yucatán. Facultad de Medicina Veterinaria y Zootecnia. Mérida, Yucatán, México. [5]Colegio de Postgraduados Campus Tabasco. Cárdenas, Tabasco, México. [6]Universidad Juárez Autónoma de Tabasco. Villahermosa, Tabasco, México.

***Corresponding author**: ever_flores18s@hotmail.com

ABSTRACT

Objective: Determine the productive and immune response of Blackbelly lambs infected with gastrointestinal nematodes (GIN) and fed with saccharin.

Design/ methodology/ approach: A total of 18 Blackbelly lambs, with an initial live weight (LW) of 13.9 + 3.2 kg, were randomly assigned to three different treatments (T): T1, anthelmintic treatment + basal diet (CTah); T2, basal diet without anthelmintic treatment (STah); and T3, grazing lambs without anthelmintic treatment (STPS). This experiment followed a completely randomized design with repeated measures over time; mean values were compared using Lsmeans. The parameters evaluated included live weight (LW), fecal egg count per gram (FEC), packed-cell volume (PCV), plasma protein (PP), white blood cell differential count (LEU), and IgA concentration by ELISA with *Haemonchus contortus* and *Trichostrongylus colubriformis antigens*.

Results: STah and CTah lambs showed higher FEC (885 ± 142) and LW (29.73 ± 5.06 kg). Grazing lambs (STPS) had lower PCV (26.4 ± 0.5%) compared to the STah and CTah lambs (27.4 to 28.4%) due to the high prevalence of *H. contortus*. The IgA concentration in grazing lambs ranged from 20.2 to 24.5% of the positive standard serum titer. The feedlot lambs (STah and CTah) showed values close to 5%.

Study limitations/implications: Due to anthelmintic resistance problems, it was impossible to maintain grazing lambs free of infection; therefore, this group was not included.

Findings/conclusions: Saccharin increases sheep resilience and achieves adequate weight gains in parasitized lambs.

Keywords: *Haemonchus contortus*, *Trichostrongylus colubriformis*, humoral immunity, cellular immunity.

Agroproductividad: *Vol. 14, Núm. 2, febrero. 2021. pp: 55-60.*
Recibido: *octubre, 2020.* **Aceptado:** *febrero, 2021.*

INTRODUCTION

Gastrointestinal nematodes (GIN) affect the health of grazing sheep and reduce flock productivity (Roeber *et al.*, 2013). Anthelmintics have been used as the only control strategy, but their continued use has decreased effectiveness and developed anthelmintic resistance (AR). Different control methods have been used to avoid AR (Bishop, 2012). In lambs, an alternative is feedlot fattening; this avoids parasitic effects and increases productivity. However, these lambs are susceptible to infection with AR GIN, even after receiving prophylactic treatment, with detrimental effects on their health.

Feeding strategies have been used to increase immunity and reduce parasitic effects (Torres-Acosta *et al.*, 2012). Sugarcane has stood out in ruminant feeding due to its high sugar concentration, which decreases feeding costs. Its processing as saccharin enriched with energy-protein compounds meets the nutritional requirements of sheep (Godínez *et al.*, 2017), improving productivity and promoting the immune system; thus, controlling the infection with established parasites. Therefore, this study aimed to determine the productive indices and the immune response of Blackbelly lambs infected with GIN and fed with saccharin.

MATERIALS AND METHODS

Location, animals, and feeding

This study was performed in 2016 in Salto de Agua, Chiapas (17° 34' N and 92° 29' W, at 85 masl). This region has a tropical climate with year-round rainfall, with annual rain precipitation of 3,346 mm and an annual mean temperature of 26.5 °C (CONAGUA, 2017). A total of 18 Blackbelly lambs, with an initial live weight (LW) of 13.9 ± 3.2 kg, were randomly assigned to three different treatments (T). In T1, lambs were dewormed every two months with albendazole and levamisole (10 and 7.5 mg kg^{-1} LW) and housed in individual cages with feeding and drinking troughs. Animals were fed an integral saccharin-based diet (CTah), formulated as indicated by Godínez-Juárez (2017). In T2 (STah), lambs did not receive anthelmintic treatment during the entire study. Animals were fed as those in T1 (Table 1). Lambs were subjected to a 15 d-adaptation period in which they were fed twice a day with amounts ranging from 3.0 to 3.5 kg in total. Rejection of at least 30% of the offered feed was allowed due to big sugarcane particles that the animals rejected. T3 included lambs fed by grazing of *Urochloa brizantha* cv. Humidicola CIAT-679 (Rendle) Schweickerdt, eight hours daily. These animals did not receive anthelmintic treatment (STPS). Lambs were locked up at night in a galley and provided with water and mineral salts.

Dry matter intake (DMI, kg d-1) was calculated daily by measuring the difference between the offered and rejected feed. Daily weight gain was also determined (DWG, kg d^{-1}), and feed conversion (FC) was calculated as the DMI/DWG ratio. Dry matter (DM), crude protein (CP), and true protein (TP) content were determined following the methodology indicated by the AOAC (AOAC, 2005).

Parasitological and hematological sampling

During the five-month study period, fecal samples were collected by rectal stimulation every 21 d; nine collections in total. Samples were collected in plastic bags and kept in ice until further processing. In the laboratory, samples were subjected to the McMaster technique, with a sensitivity of 50 FEC (Cringoli *et al.*, 2004). Fecal cultures were established to isolate the larvae and perform morphological identification (Van Wyk and Mayhew, 2013).

Table 1. Ingredients and chemical composition of the diet.

Content	Percentage	Bromatological composition		
		Chemical composition	Mean	Standard Deviation
sugar cane	74	Dry matter (%)	48.7	7.7
soybean paste	4	Crude protein (%)	14.3	2.4
ground sorghum	20	Ash (%)	2.8	0.7
Minerals	0.5	Organic matter (%)	97.2	0.8
Urea	1	Neutral Detergent Fiber (%)	28.9	6.7
Magnesium sulfate	0.5	Acid Detergent Fiber (%)	16.1	4.1
		Hemicellulose (%)	15.3	0.8

Additionally, two blood samples (3 mL) were collected from the jugular vein using Vacutainer needles. The first sample was collected in tubes containing an anticoagulant (BD Vacutainer); the second sample was collected in serum separator tubes, without anticoagulant (BD Vacutainer). PCV was determined by the microhematocrit technique (Weiss and Wardrop, 2010), and a refractometer was used to measure PP (g dL^{-1}).

IgA determination by ELISA

Serum samples were stored in Eppendorf tubes at −20 °C until IgA analysis. Following the methodology described by González-Garduño *et al*. (2017), IgA concentration was determined using an indirect ELISA with antigen obtained from the crude extract of *H. contortus* and *T. colubriformis* adult nematodes. The optical density (OD) of each serum was obtained by subtracting the blank, which represented the non-specific binding of the conjugate. The IgA activity was expressed as percentages of the positive standard serum, based on the equation indicated by Cardoso *et al*. (2013).

Statistical analysis

Obtained data were analyzed using the SAS MIXED procedure (SAS, 2017) for a completely randomized model with repeated measures over time. Log transformation of FEC was performed [Log (HPG+1)] to approximate the model to a normal distribution.

The statistical model used in this experiment was the following:

$$Y_{ijk} = \mu + \gamma_i + \zeta_j + \gamma^{*}\zeta_{ij} + \delta_k + \varepsilon_{ijk}$$

Where: Y_{ijk} = Response variable, μ = General mean, γ_i = Treatment effect (i=CTah, STah, STPS), ζj = Sampling day effect, $\gamma^{*}\zeta_{ij}$ = Treatment and sampling day effect, δ_k = Random effect of the animal, ε_{ijk} = Random error ~(0, σ2).

RESULTS AND DISCUSSION

Productive indices

DMI was higher ($p \leq 0.05$) in STah lambs compared to the CTah group (1.04±0.24 kg *vs* 0.94±0.28 kg). FC and DWG were the same in both groups. The DWG of feedlot lambs was 95 and 99 g, which is 102% higher than the DWG of grazing lambs (49 g) ($p \leq 0.05$; Table 2).

Godínez-Juárez *et al*. (2017) reported that a DMI of 1.2 kg d^{-1} results in DWG higher than 90 g in feedlot animals. STah and CTah lambs maintained similar DWGs and intakes. Contrary to that observed by Cardia *et al*. (2011), who reported that animals infected with *Trichostrongylus* spp. showed a 37% weight loss due to their lower nutrient absorption capacity (McRae *et al*., 2015). This was not observed in this study, probably because feedlot lambs' nutrient requirements were met through 14% CP diets, necessary for achieving DWG higher than 100 g (NRC, 2007; Table 1), even with parasitosis. Growth differences between the feedlot and grazing lambs were evident when comparing their DWG ($p \leq 0.05$); grazing animals had the lowest final weight of both groups. Although STah lambs showed higher FEC counts than the CTah group, their DWG were similar ($p > 0.05$), which indicates lambs develop some degree of resilience when fed properly (Torres-Acosta *et al*., 2012).

Parasitological and hematological variables

The FEC was lower ($p \leq 0.5$) in CTah lambs (146±31.0) than in grazing (502±71) and STah lambs (885±143; Table 3).

The PCV of feedlot lambs (STah and CTah) was higher (27.4 to 28.4 %) than that of grazing lambs ($p \leq 0.05$; 26.4±0.5 %); this may be related to the nutritional level. A previous study reported that the anemia induced by *H. contortus* was affected by nutrition (Cériac *et al*., 2017). The highest PP value ($p \leq 0.05$) was observed in grazing lambs (6.9±0.1 g dL^{-1}). STah (6.4±0.1 g dL^{-1}) and CTah (6.3±0.1 g dL^{-1}) had similar values ($p > 0.05$). Additionally, grazing lambs showed the highest ($p \leq 0.05$) IgA percentage with values ranging from 20.2 to 24.5% of the positive standard titer. STah and CTah lambs behaved similarly ($p < 0.05$; 2.9 to 4.0%) against

Table 2. Feed consumption, live weight, and feed conversion changes in Blackbelly lambs fed with saccharin.

Variable	Stabling		Grazing without anthelmintic GWT
	Without anthelmintic SWT	With anthelmintic STA	
Initial body weight (kg)	14.05±2.6^{a}	12.16±3.6^{a}	15.5±2.7^{a}
Final body weight (kg)	29.73±5.06^{a}	27.32±6.56^{a}	23.38±1.02^{b}
Change weight (kg)	15.68	15.16	7.88
Days	159	159	159
Food intake (kg d^{-1})	2.50±0.59^{a}	2.28±0.67^{b}	
Dry matter intake (kg d^{-1})	1.04±0.24^{a}	0.94±0.28^{b}	
Daily gain weight (kg d^{-1})	0.099^{a}	0.095^{a}	0.049^{b}
Food conversion	10.51	9.89	

Means with different literal in each row are significantly different ($P < 0.05$).

Table 3. Parasitological and hematological variables in Blackbelly lambs, with and without anthelmintic treatment.

Variable	Stabling		Grazing without anthelmintic GWT
	Stabling without anthelmintic SWT	Stabling with anthelmintic STA	
PCV (%)	27.4±0.6[a]	28.4±0.5[a]	26.4±0.5[b]
PP (g dL^{-1})	6.4±0.1[b]	6.3±0.1[b]	6.9±0.1[a]
EPG	885±813[a]	146±182[b]	502±493[a]
Log (EPG+1)	5.9±1.4 [a]	3.1±2.4[c]	5.1±2.2[b]
IgA (%) - *H. contortus*	3.7±0.5[b]	4.0±0.8[b]	20.2±5.3[a]
IgA (%) - *T. colubriformis*	2.9±0.5[b]	3.6±0.7[b]	24.6±8.2[a]

PCV. Packed cell volume. PP. Plasma protein, EPG. Eggs per gram of faeces. IgA (%) regarding to positive standard (RPS) using two crude antigens of adult nematodes. Averages with different literals in same rows are significantly different (P<0.05).

the *H. contortus* and *T. colubriformis* antigens (Torres-Acosta *et al*., 2012).

After larval identification, it was found that the most prevalent species was *H. contortus* (>33% of the identified larvae). *T. colubriformis*, *Cooperia curticei*, and *Oesophagostomum colombianum* were also abundant, and *Strongyloides papillosus* in low proportion (Figure 1).

The fecal egg count in STah increased 35 d after starting the confinement period. Subsequently, the FEC lowered and remained stable with similar values to those reported for grazing animals (Figure 2). Notably, STah animals had the highest FEC because parasites continue their life cycle.

The increase in FEC and its subsequent reduction in STah lambs has been reported in other studies as a long-term immunological effect. Infection levels were not high enough to a show statistical difference in the DWG of infected and non-infected animals (p≤0.05). However, there were differences in the immunological parameters when compared to grazing lambs. Therefore, it may be inferred that constant reinfection triggers the immune response since grazing lambs had higher IgA levels (Aguilar *et al*., 2011).

After infection, PCV values decreased in all experimental groups. However, these values returned to normal 35 days post-infection, especially in the STah group. The recovery in grazing lambs was slower (Figure 3).

PCV values were affected by the feeding practices; the lowest values were observed in grazing lambs infected with *H. contortus*. Moreover, due to the constant infection with GIN, the PP of grazing lambs was higher (p≤0.05) than in CTah and STah lambs; this was associated with the higher concentration of IgA observed in grazing lambs. After starting the study, IgA levels ranged from 15 to 30% of the positive standard titer. Later, these levels

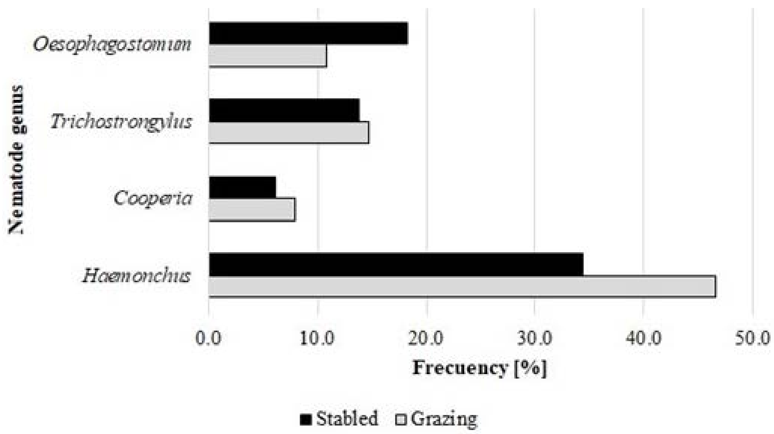

Figure 1. Gastrointestinal nematode genera found in Blackbelly lambs according to the production system.

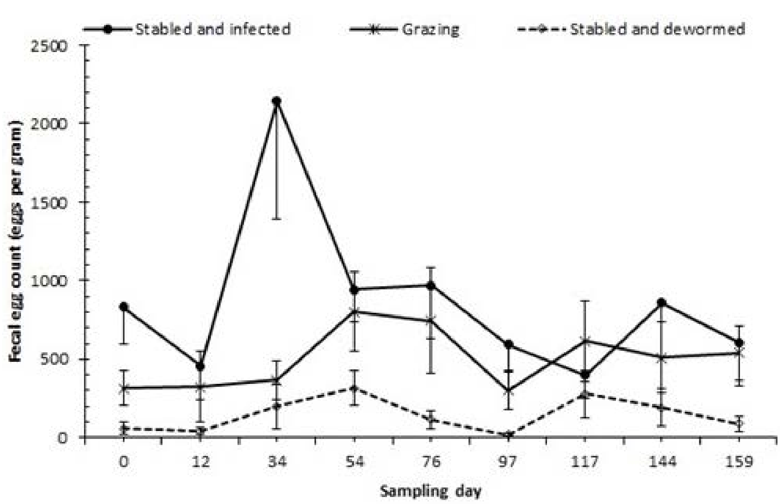

Figure 2. Fecal egg count per gram in Blackbelly feedlot (CTah and STah) and grazing lambs.

increased to 35%. Feedlot lambs (CTah and STah) showed very low levels of IgA (Figure 4). In grazing lambs, the host-parasite interaction after reinfection promoted higher levels of PP and IgA as an acquired immune response. Feedlot lambs (CTah and STah) had similarly low IgA levels (3.72±0.47 and 4.02±0.83% of the positive standard titer, respectively) against the *H. contortus* antigen.

Due to the lack of immunological stimulus, STah lambs could not maintain high levels of IgA. Strain and Stear (2001) reported that animals with properly supplemented diets had small nematodes and produced more IgA than lambs with non-supplemented diets. However, this did not occur in the present study; feedlot animals had no immunity-promoting reinfections (Santos *et al.*, 2014). Therefore, it is assumed that IgA production is stimulated by the infection degree (McRae *et al.*, 2015).

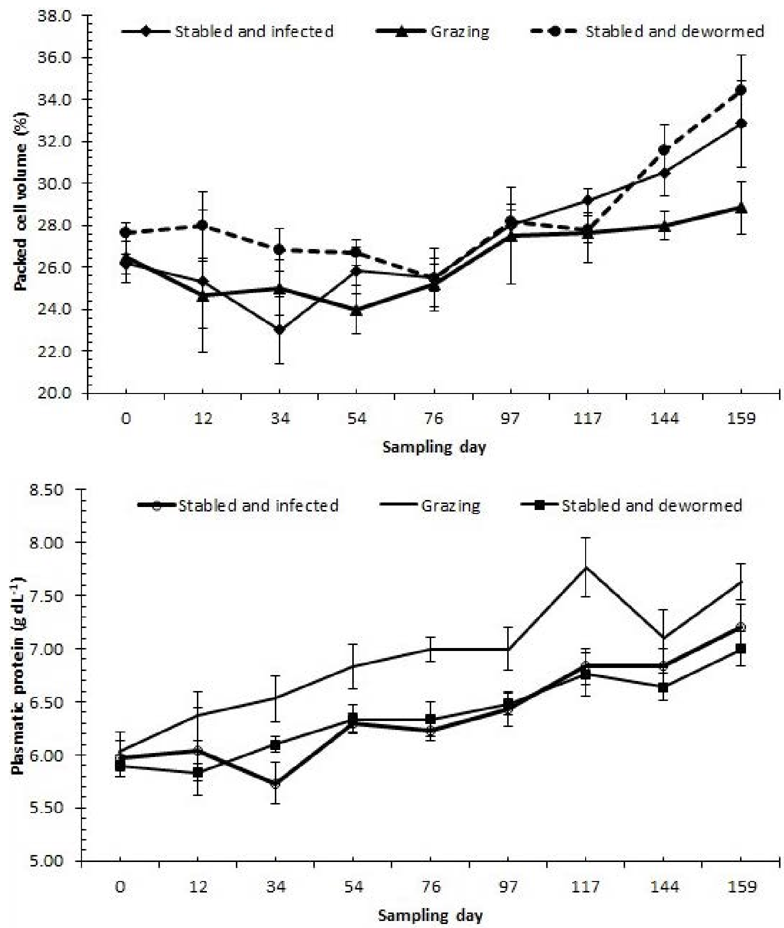

Figure 3. Packed-cell volume and plasma protein in Blackbelly lambs by sampling day.

CONCLUSIONS

Feedlot lambs with an established gastrointestinal nematode infection and without anthelmintic treatment showed higher fecal egg counts and lower IgA levels than grazing lambs; this implies that the reinfection continues during grazing and stimulates the development of the acquired immune response.

Growing lambs fed an integral saccharin-supplemented diet had higher packed-cell volumes and weight gains than grazing lambs; this allowed animals to express resilience by tolerating the established nematode infection and obtaining the highest productive parameters. However, their elevated fecal egg count indicates that animal resistance did not increase.

REFERENCES

Aguilar, C., Cámara, A.J., Torres A.S.R., & Sandoval C.C. (2011). El control de los nematodos gastrointestinales en caprinos: ¿dónde estamos? Bioagroc 4: 10-16.

AOAC. (2005). Official Methods of Analysis of AOAC International. 18th Ed., AOAC International, Gaithersburg, MD, USA.

Bishop, S.C. (2012). Possibilities to breed for resistance to nematode parasite infections in small ruminants in tropical production systems. Anial 6(5): 741-747.

Cardia, D.F., Rocha O.R.A., Tsunemi M.H., & Amarante A.F. (2011). Immune response and performance of growing Santa Ines lambs to artificial *Trichostrongylus colubriformis* infections. Vet Parasitol 182(2-4): 248-58.

Cardoso, C.P., Silva, B.F., Trinca, L.A., & Amarante, A.F. (2013). Resistance against gastrointestinal nematodes in Crioulo Lageano and crossbred Angus cattle in southern Brazil. Vet Parasitol 192: 183-191.

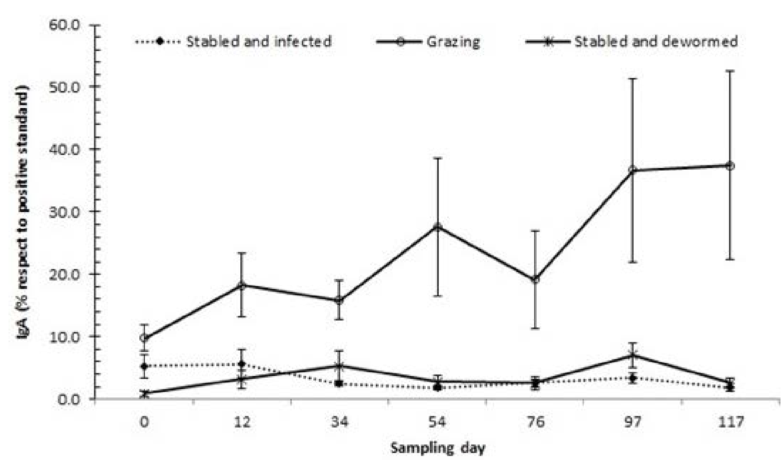

Figure 4. IgA production as a percentage of the positive control using a crude antigen obtained from *H. contortus* adult nematodes.

Cériac, S., Jayles, C., Arquet, R., Feuillet, D., Felicite, Y., Archimède, H., & Bambou, J. C. (2017). The nutritional status affects the complete blood count of goats experimentally infected with *Haemonchus contortus*. BMC Vet Res13(1), 1-10.

CONAGUA. (2019). Servicio Meteorológico Nacional. Disponible en: http://smn.cna.gob.mx/index.php?option=com_content&view=article&id=174:chiapas&catid=14:normales-por-estacion [Consulta: 09 de febrero del 2019].

Cringoli, G., Rinaldi, L., Veneziano, V., Capelli, G., & Scala, A., (2004). The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep. Vet Parasitol 123, 121-131.

Godínez-Juárez, B., Vargas-Villamil, L., González-Garduño, R., Saldivar-Cruz, J.M., Izquierdo, F., Hernández-Mendo, O., & Ramos-Juárez, J.A. (2017). Evaluación de la degradación, consumo voluntario y comportamiento productivo de ovinos alimentados con saccharina y maíz. Eco Rec Agrop 4(12): 431-441.

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. Rev Colomb Cien Pec 30(3): 2019-2030.

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(12): 605-13.

NRC. (2007). Nutrient Requirements of Small Ruminants. Sheep, Goats, Cervids and New World Camelids. Washington, DC. The National Academy Press.

Roeber, F., Jex, A.R., & Gasser, R.B. (2013). Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance-an Australian perspective. Parasite Vectors 6:153. doi.org/10.1186/1756-3305-6-153

Santos, M.C., Xavier, J.K., Amarante, M.R., Bassetto, C.C., & Amarante, A.F. (2014). Immune response to *Haemonchus contortus* and *Haemonchus placei* in sheep and its role on parasite specificity. Vet Parasitol 203: 127-138.

SAS (2017). SAS/STAT User's Guide, Release 6. ed. Cary, NC, USA.

Strain, J.A.S., & Stear, J. M. (2001). The influence of protein supplementation on the immune response to *Haemonchus contortus*. Parasite Immunol 23: 527-531.

Torres, A., J.F.J., Sandoval C., C.A., Hoste, H., Aguilar C., A.J., Cámara S., R. & Alonso D., 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.

Van Wyk, J.A., & Mayhew E. (2013). Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. The Onderstepoort J Vet Res 80: E1-E14).

Weiss, D., & Wardrop, J. (2010). Schalm's veterinary Hematology, 6Th ed, Schalm's Veterinary Hematology. Wiley-Blackwell, Iowa, USA.