Immune and parasitic response to conjugated linoleic acid in the diet of pelibuey sheep infected with gastrointestinal nematodes

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ABSTRACT
The aim was to evaluate the immune and parasitic response of Pelibuey sheep infected with gastrointestinal nematodes (Haemonchus contortus, GIN) and supplemented with conjugated linoleic acid (CLA). Twenty-four Pelibuey male lambs were distributed into one of four groups of six animals, placed in individual pens, in a completely randomised design, allotted to four treatments as follows: T1) No larval challenge without CLA (control group); T2) Larval challenge without CLA; T3) Larval challenge and 1% CLA; and 4) Larval challenge and 3% CLA. All the lambs were on grazing conditions before the experimental period. The haematologic, immune, parasitic and productive responses were evaluated. Total leukocytes, lymphocytes and eosinophils were different between treatments (p ≤ .05), but not granulocytes (p > .05). Haemoglobin (HCT), and red blood cell count (RBC) decreased (p ≤ .0001), while mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) increased in infected lambs supplemented with CLA, and mean corpuscular volume (MCV) increased with respect to control lambs. Haemoglobin (HGB) was the only variable that was not different between treatments (P = .0113). The faecal egg count (FEC), plasma protein (PP), and immunoglobulin G (IgG) and immunoglobulin M (IgM) levels were not modified among treatments (p > .05). Daily weight gain was not different between treatments (p > .05). Supplementing Pelibuey lambs with conjugated linoleic acid did not modify their immune response, nor did the parasitic infection, but it did affect the blood parameters. The nutritional factor had an essential role in such response.

HIGHLIGHTS
• New alternatives in parasitic control in sheep
• Conjugated linoleic acid modulates the immune response in sheep
• Nutritional factor could be an excellent control mechanism against parasitic infections

Introduction
In sheep, gastrointestinal parasitic disease affects animal health, well-being, and productivity, decreasing the daily weight gain and milk production, and killing susceptible animals. Within the most important gastrointestinal nematodes (GIN) that affect the health of sheep are Haemonchus contortus, Trichostrongylus colubriformis and Cooperia curticiei (López-Ruvalcaba et al. 2013), being H. contortus the most harmful because when infecting causes anaemia (Ameen et al. 2010). Therefore, it is imperative to offer control alternatives, since the use of chemical products has been the main way to reduce the effects of parasites for a long time. Nevertheless, their constant use has caused high selection pressure in gastrointestinal nematodes, which have consequently developed anthelmintic resistance (Muchiut et al. 2018; Szulc et al. 2020). Currently, there are alternative control models, for example, the use of vaccines, selection for genetically resistant animals, biological control, grazing management, using plants with anthelmintic properties, and animal supplementation (Charlier et al. 2018; Mravčáková et al. 2020; Szulc et al. 2020). Some authors have associated this last aspect to immune expression (Houdijk 2012), as well as to resistance and resilience to GIN infections, which demonstrates the relationship between host nutrition and parasitic pressure.
and nematode infection (Hoste et al. 2016). Recently, there is increased interest in know the effect of supplementing sheep with conjugated linoleic acid (CLA), as it is deposited in meat and milk, making it beneficial for human consumption given its relation with human health (Chen and Park 2019; Bodkowski et al. 2020). Purba et al. (2020) states that the original diet in the feeding regimen can be supplemented with feeds or additional fats, such as forages, animal lipids and vegetable oils, to achieve increased CLA. It is important to consider that animal species respond differently to feeding strategies (Nudda et al. 2020). CLA has been reported to have an immune modulating effect (León-Sánchez et al. 2014) in animal models (mice, pigs) and humans, but no such reports exist for ruminants, where sheep have an important role in the Mexican livestock industry. The immune modulating effect of CLA is attributed to possible action mechanisms involving changes in eicosanoids, since CLA suppresses the biosynthetic pathway of arachidonic acid and modulates the activation of peroxisome proliferator activated receptors (PPAR) at the nuclear level (Yang et al. 2015), increasing the immune response, both cellular and humoral. In broiler chickens, CLA increases the proliferation of lymphocytes, mainly intestinal T CD8+ lymphocytes (Liu et al. 2017). Also, Sugano et al. (1998) reported that CLA in rats increases the production of IgA, IgG, and IgM immunoglobulins, but decreases IgE. This is an important aspect, since immunoglobulins act as a determined antigen. To this regard, the capacity of CLA to increase IgG in pigs challenged with lipopolysaccharides (LPS) (Moraes et al. 2012), restore the immune response in mice infected with Plasmodium berghei (Kumar et al. 2011), and decrease the parasitic load of Giardia lamblia due to the increase of antigen-presenting cells (Montalvo et al. 2018) has been highlighted. Under this scenario, it is hypothesised that CLA supplementation in sheep can decrease the faecal egg count (FEC) of GIN by increasing the immune response. Therefore, the aim of this study was to evaluate the immune and parasitic response of Pibuey sheep infected with gastrointestinal nematodes supplemented with conjugated linoleic acid.

Materials and methods

Location

The experiment was carried out in a private ranch located in Salto de Agua, Chiapas, Mexico (17° 34’ NL; 92° 29’ WL; 85 metres above sea level). The climate in the region is sub-humid warm with rainfall year-round, mean annual temperature and rainfall of 26.6°C and 3289.1 mm, respectively.

Animals, experimental design, and treatments

The animal care and management procedures were conducted according to the guidelines established by the Mexican federal law of animal health (DOF 25-07-2007).

Twenty-four Pibuey male lambs were used, with initial live-weight of 20.0 ± 3.9 kg and six months old on average at the beginning of the experiment. All the lambs were on grazing conditions before the experimental period, where they received natural infection with GIN. Once they were involved into the experiment, they were dewormed (Ripercol L 12%, Zoetis, 7.5 mg/kg⁻¹ LW) at the beginning of the study. The lambs were distributed into four groups of six lambs each, balanced as closely as possible between them for live-weight (T1) 20.0 kg; T2) 19.10 kg; T3) 19.82 kg; T4) 19.80 kg). The lambs were kept in total confinement in individual wooden pens with cement floor for 15 days of adaptation and 49 days of experimentation. The groups of lambs were randomly assigned to the dietary treatments. The four experimental groups were treated as follows: T1) No larval challenge without CLA (control group); T2) Larval challenge without CLA; T3) Larval challenge and 1% CLA; and 4) Larval challenge and 3% CLA. The three challenged groups were each exposed to oral doses of infective gastrointestinal nematodes larvae (91% Haemonchus contortus and 9% Trichostrongylus sp), at 100 L3 larvae/kg of LW on days 7, 14, 21, and 28 after the experiment began.

Experimental feeding

During the experimental period, the four groups of lambs were fed with a base diet (Table 1) formulated according to the recommendations of the National Research Council (NRC 2007). The CLA supplement was a mixture of microencapsulated isomers that supplied 6 g cis-9, trans-11 and 6 g trans-10, cis-12 CLA (Lutrell Pure®, BASF, Germany). Each lamb received 2 kg/day of the total diet, half in the morning, and half in the afternoon, 08:30 and 17:30 h. The CLA was mixed with the concentrate for each corresponding treatment, in order to ensure its total consumption. Once the animals consumed the concentrated feed, the corresponding amount of forage was supplied, individually, according to the treatment.
Dietary analysis for the treatment groups was performed in the Animal Nutrition Laboratory of the Livestock Program of the Colegio de Postgraduados, Montecillo Campus, in the State of Mexico. During the experimental period, two samples were taken from each of the experimental diets each time food was made, mixing them at the end to obtain a single sample per diet. The feed samples were ground in a Willey mill (Arthur H. Thomas, Philadelphia, PA, USA) with a 1 mm mesh. The analyses included dry matter, crude protein, ash, and ether extract (AOAC 2005), neutral detergent fibre and acid detergent fibre (Van Soest et al. 1991).

The degradability of the diets was performed in vitro using a Daysi® ANKOM® model D200 incubator (ANKOM Technologies). For each experimental diet, a 0.5 g sample was placed in an ANKOM® F57 5 x 4 cm bag with a pore size of 25 mm, made of polyester/polyethylene with filaments extruded in a three-dimensional matrix (ANKOM Technologies, Macedon, NY, USA). Incubations times were 0, 3, 6, 12, and 24 h, using ruminal liquid from a fistulated Jersey cow (500 kg LW average).

### Measured variables

#### Immune response

Two blood samples of 3 mL were taken per animal from the jugular vein once a week, in the morning, fasting. There were twelve blood samples per treatment a week, which were collected in Vacutainer® test tubes (Becton Dickinson, Franklin Lakes NJ, USA), half of them (six) with ethylene-diamine-tetraacetic acid (EDTA) for white and red cell counts, and the other half (six) with a coagulation accelerant (silicon coating) to retrieve the blood serum.

From the white series, total leukocytes, lymphocytes, granulocytes, and eosinophils were determined using a hematomal analyser (MEDONIC CA-620) (Boule Medical AB, Sweden), with the exception of eosinophils, which were determined through blood dilution in a Carpentier colourant (1:20). From this dilution, 10 μL were used in a haemocytometer (Neubauer, Brilliant line, USA), to count the total eosinophils using an optical microscope with 10x magnification.

From the red series, haematocrit (HCT) through the micro-haematocrit technique and total plasma protein (PP) measured with a refractometer (Atago, Japan), were determined. Also red blood cell count (RBC), mean corpuscular volume (MCV), haemoglobin (HGB), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), were obtained with the hematological analyser (MEDONIC CA-620).

#### Determination of IgG and IgM

Samples with coagulation accelerant were centrifuged at 3000 g for 20 minutes and the serum aspirated and frozen at −20°C until use in the indirect enzyme-linked immunosorbent assay (ELISA) for serum IgG and IgM levels determination. Somatic crude worm antigen of *Haemonchus contortus* was used, according to González-Garduño et al. (2017).

#### Faecal egg count

Twenty-four faecal samples were taken, one per animal, directly from the rectum of the lambs once a week using plastic bags identified by lamb and group. The faecal egg count (FEC) of GIN was determined through the McMaster technique with a sensibility of 50 egg per gram of faeces (Cringoli et al. 2004).

#### Productive response

Additionally, dry matter intake (DMI) and daily weight gain (DWG) were measured individually. The DMI was obtained from the difference between food offered and rejected, daily. The DWG was obtained from the difference between final live-weight and initial live-weight divided by the feeding days. The lambs were weighed weekly in the morning before feeding was offered.
Statistical analysis

The data were analysed under a completely randomised design with measurements repeated over time. Before analysing the data, an analysis of normality and homogeneity of variance was performed with the proc UNIVARIATE. To approximate the FEC to a normal distribution, the values were transformed to logarithm (Log FEC + 1) (SAS 2004). Different covariance structures were tested for each study variable and it was found that the autoregressive structure (AR1) was the one that was adjusted to each model when presenting lower AIC (Akaiké Information Criterion) and BIC (Bayesian Information Criterion) values. The information was analysed using the following statistical model:

\[ Y_{ijkl} = \mu + \alpha_i + d_j + \gamma_k + \delta_{ij} + e_{ijkl} \]

where \( Y_{ijkl} \) = the response of the variable (haematologic, immune, parasitic and productive), \( \mu \) = the general mean, \( \alpha_i \) = fixed effect of the treatment \( (i = \text{control, } 0\% \text{ CLA, } 1\% \text{ CLA and } 3\% \text{ CLA}) \), \( d_j \) = the fixed effect of the j-th sampling \( (l = 1, 2, 3 \ldots 7) \), \( \gamma_k \) = the random effect of the lamb, \( \delta_{ij} \) were the interactions between the treatment and the sampling, and \( e_{ijkl} \) = the error associated with repeated measurements. The separation of means was performed using the Lsmeans procedure (SAS 2004).

Results

Immune response

The cellular components of the white series of the immune system are shown in Table 2. Total leukocytes, lymphocytes and eosinophils were different between treatments \( (p < .05) \), but not granulocytes \( (p > .05) \). Leukocytes were different between 0 and 1% CLA treatment in the infected lambs. The eosinophils of the infected groups were greater than the non-infected group.

Haematologic parameters

The haematologic parameters show higher values of HCT and RBC in the not infected lamb compared to the infected lambs \( (p < .0001) \). Meanwhile, the RBC and MCV showed no differences between treatments of infected lambs, although there was a difference with respect to the not-infected lambs without CLA supplementation. On the other hand, MCH and MCHC were higher when supplementing the lambs with 1% and 3% CLA. HGB was similar between treatments (Table 3).

Parasitic response

The FEC and immunoglobulins means are shown in Table 4. There were no differences between treatments \( (p > .05) \) in FEC, total plasma protein, or the levels of immunoglobulins (IgG and IgM). Neither were differences found between treatments for FEC \( (p > .05) \) in time, despite the fact that the FEC increased in lambs infected and supplemented with CLA on day 35 post-infection (Figure 3). It is important to highlight that with the anthelmintic treatment the FEC was not reduced to zero until day 21, so anthelmintic

| Table 2. Means of peripheral cellular components of the white series in Pelibuey sheep fed with conjugated linoleic acid in the diet. |
|---------------------------------------------------------------|
| Variables          | No-infected | Infected |
|                    | 0% CLA | 0% CLA | 1% CLA | 3% CLA | *MSD | \( p \) Value |
| Leukocytes \( \times 10^3 \) × µL | 8.72ab | 9.75a | 8.12b | 9.06ab | 2.717 | .032 |
| Lymphocytes \( \times 10^3 \) × µL | 5.66ab | 6.24a | 5.25b | 5.62ab | 1.801 | .049 |
| Granulocytes \( \times 10^3 \) × µL | 0.77  | 0.82  | 0.58  | 0.82  | 0.469 | .079 |
| Eosinophils \( \times 10^3 \) × µL | 0.13b | 0.28a | 0.12b | 0.24ab | 0.254 | .004 |

CLA, Conjugated linoleic acid; *MSD, Mean standard deviation; \( \text{ab} \)Means with different letters within a row are significantly different \( (p < .05) \).
Six blood samples per treatment were used, weekly, for statistical analysis.
resistance is suspected for the product used. The subsequent increases in the FEC were due to the experimental infection.

**Productive response**

The means of DMI and DWG are shown in Table 5. The DMI was different between treatments ($p \leq .05$), mainly when lambs were supplemented with 3% CLA, increasing from day 14 (Figure 4), superior with respect to the other treatments, being constant throughout the experiment. The DWG was not different between treatments (Table 5; Figure 4) ($p > .05$).

### Figure 1.

Means of the cellular components in Pelibuey lambs supplemented with conjugated linoleic acid (CLA) and experimentally infected with gastrointestinal nematodes (NGI): (A) leukocytes, (B) lymphocytes, (C) granulocytes, and (D) eosinophils.

### Table 3.

Means of peripheral cellular components of the red series in Pelibuey sheep fed with conjugated linoleic acid in the diet.

| Variables | Treatment | CLA 0% | CLA 1% | CLA 3% | *MSD | $p$ Value |
|-----------|-----------|--------|--------|--------|------|----------|
| HCT (%)   | No-infected | 29.63a | 27.26ab | 24.83b | 4.577 | <.0001  |
|           | Infected | 25.51b | 25.51b | 25.51b |      |          |
| RBC (10$^6$ x μL) | No-infected | 10.14a | 8.91b  | 7.97b  | 1.814 | <.0001  |
|           | Infected | 8.28b  | 8.28b  | 8.28b  |      |          |
| MCV (fl)  | No-infected | 29.30b | 31.16a | 31.41a | 2.701 | .0006   |
|           | Infected | 30.96a | 30.96a | 30.96a |      |          |
| HGB (g/dL) | No-infected | 10.92  | 10.46  | 10.30  | 1.444 | .113    |
|           | Infected | 10.94  | 10.94  | 10.94  |      |          |
| MCH (pg)  | No-infected | 10.95c | 12.15bc | 13.49ab | 2.635 | <.0001  |
|           | Infected | 13.90a | 13.90a | 13.90a |      |          |
| MCHC (g/dL) | No-infected | 37.37a | 39.08bc | 43.19ab | 7.974 | <.0001  |
|           | Infected | 44.91a | 44.91a | 44.91a |      |          |

CLA: Conjugated linoleic acid; HCT: Haematocrit; RBC: Red blood cell; MCV: Mean corpuscular volume; HGB: Haemoglobin; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; *MSD: Mean standard deviation; Means with different letters within a row are significantly different ($p \leq .05$).

Six blood samples per treatment were used, weekly, for statistical analysis.

### Discussion

#### Cellular immune response

All peripheral white blood cellular components were within the normal range for sheep (Avellanet et al. 2007). The hypothesis was that CLA would increase immune parameters, since CLA, particularly the isomers cis-9, trans-11 and trans-10, cis-12, are high affinity-ligand and activators of the PPAR, according to Viladomiu et al. (2016), which is important in cell differentiation, increasing the immune response. However, in our study contradictory results were obtain in total leukocytes and the differences between...
treatments did not have a trend. The fact that in our study, the leucocytes and lymphocyte count did not increase as the CLA increased in the diet, suggests that there was no dose dependent effect as was expected. We observed that supplemented infected lambs, especially with 1% CLA, the leucocyte and lymphocyte presented the lowest values. This is due to lymphocytes, which represent around 60% of the total leukocytes (Egbe-Nwiyi et al. 2000). This is noticeable over time, where leukocytes and lymphocytes show the same performance in all groups of lambs, especially in those infected with 1% CLA, the total of leukocytes and lymphocytes decreased from day 28 post-infection. This situation has also been indicated by Moraes et al. (2012) who reported that in immune-suppressed pigs, supplemented with CLA, the total leukocyte count decreased, as did the lymphocyte count, because of lipopolysaccharide stress. Contrary to that reported by Wiegand et al. (2011) who found that CLA allows to increase the leukocyte count due to an increase in lymphocytes, since CLA suppresses the biosynthetic pathway of arachidonic acid, by competing for the same enzymes. This, consequently, leads to a decrease in the synthesis of eicosanoids (Yang et al. 2015) like prostaglandin E2 (PGE2), which is considered as a general immunosuppressant, inhibiting the production of interleukin 2 (IL2) (Loscher et al. 2005). All of this implies that PGE2 decreases due to CLA, increasing the proliferation of lymphocytes like TCD8⁺ and TCD4⁺, and lymphocytes B (Liu et al. 2017). Bassaganya-Riera et al. (2001) proved that CLA

Table 4. Faecal egg count and levels of immunoglobulins of Pelibuey sheep supplemented with conjugated linoleic acid in their diet.

| Variables | No-infected | Infected |
|-----------|-------------|----------|
|            | 0% CLA     | 1% CLA   | 3% CLA   |
| FEC       | 58.33       | 163.89   | 140.28   | 116.67   | 257.913 | .436 |
| PP (g dL⁻¹) | 6.14       | 6.11     | 6.02     | 6.01     | 0.534   | .188 |
| IgG       | 0.74        | 0.78     | 0.53     | 0.47     | 0.519   | .103 |
| IgM       | 0.29        | 0.21     | 0.25     | 0.16     | 0.208   | .208 |

CLA: Conjugated linoleic acid; FEC: Faecal egg count; PP: Plasma protein; IgG: Immunoglobulin G; IgM: Immunoglobulin M; *MSD: Mean standard deviation.

Six faecal samples per treatment were used, weekly, for statistical analysis.

Figure 2. Hematological parameters of Pelibuey lambs supplemented with conjugated linoleic acid (CLA) and experimentally infected with gastrointestinal nematodes (NGI): (A) haematocrit (HCT), (B) red blood cell count (RBC), (C) mean corpuscular volume (MCV), and (D) mean corpuscular haemoglobin (MCH).
causes an increase in the leukocyte count, mainly of CD8+ lymphocyte subsets in pigs challenged with an infectious disease, whose cell increases were recorded until day 42, a phenomenon that we did not find in the Pelibuey lambs in our research, who in the same period of time had similar leukocyte counts between treatments. Therefore, the leukocyte decrease in lambs infected and supplemented with 1% CLA, observed at day 28, was due to the first experimental infection with NGI, but not due to the effect of CLA.

The eosinophil count does not show a defined trend among treatments, which is opposite to that found by Jaudszus et al. (2008), who reported that the cis-9, trans-11 isomer reduces the IL-5 levels, that stimulate the proliferation and differentiation of eosinophils, which are important as they are the first line of defense against GIN, stopping the establishment of infecting larvae (McRae et al. 2015). In our study, even supplementing with 3% CLA generated no differences in the eosinophil count with respect to infected lambs.

Figure 3. Mean faecal nematode egg counts (FEC) before and after experimental infections with gastrointestinal nematodes (NGI) in Pelibuey lambs supplemented with conjugated linoleic acid (CLA).

Table 5. Dry matter intake and daily weight gain of Pelibuey sheep supplemented with conjugated linoleic acid in their diet.

| Variables          | Treatment       | 0% CLA | 0% CLA | 1% CLA | 3% CLA | *MSD  | p Value |
|--------------------|----------------|--------|--------|--------|--------|-------|--------|
| DMI (kg d⁻¹)       | No-infected    | 1.31abc| 1.30B  | 1.28ab | 1.39a  | 0.123 | .008   |
| DWG (g d⁻¹)        | Infected       | 204.68 | 206.89 | 206.77 | 217.83 | 0.074 | .922   |

CLA: Conjugated linoleic acid; DMI: Dry matter intake; DWG: Daily weight gain; *MSD: Mean standard deviation; abcMeans with different letters within a row are significantly different (p ≤ .05).
Six animals per treatment were used, weekly, for statistical analysis.

Figure 4. Means of (A) dry matter intake (DMI) and (B) daily weight gain (DWG), before and after infection with gastrointestinal nematodes in Pelibuey lambs supplemented with conjugated linoleic acid (CLA).
without CLA, as assumed, based on those findings by Bassaganya-Riera et al. (2001). Therefore, the increase in eosinophils in lambs supplemented with 0% and 3% CLA at day 28 post-infection is attributed to the first parasitic infection, although there was no correlation of eosinophils with hematological variables and FEC, as demonstrated Yacob et al. (2009).

Haematologic parameters

The hematological differences between the groups of animals were due to infection, since the HCT and RBC were lower in infected lambs supplemented with 1% and 3% CLA, indicating that the supplementation with CLA does not improve the hematological parameters of the animal. Similar results were reported by Moraes et al. (2012), who found that HCT, RBC, MCV, and MCH in pigs were not modified by CLA supplementation, but did so when being immunosuppressed with a lipopolysaccharide (LPS), causing the blood counts to decrease. Similar results to our study have been reported for HCT and MCV in laying hens, where it is argued that HCT decreases (Koronowicz et al. 2016). Moreover, Ostrowska et al. (2004) mentioned that CLA modifies hematological responses in pigs, although they attribute the immunological stress of the animals as being responsible for the differences of their results with other studies. In our case, the modification of the hematological parameters was due to infection with GIN, which is supported by the marked difference between no infected and infected animals, the latter being the ones that presented the lowest percentage of HCT and RBC, and higher MCV, MCH and MCHC from day 7 post-infection, similar to that reported by Ngetich et al. (2019). This hematological response was the effect of Haemonchus contortus, which represented 91% of the GIN used in the experimental infection in the lambs, and the haematophagous parasite, that causes constant blood loss in the intestinal mucosa, thus decreasing the concentration of red blood cells (HCT). It has been proven that grazing Blackbelly lambs infected with H. contortus and with FEC of 2950 show lower HCT compared to those not-infected lambs (González-Garduño et al. 2018). In the same way, Bordoloi et al. (2012) found decline on hematological parameters in Sahabadi ewes when they were infected with 700 infective larvae/kg LW and FEC of 2000 at day 28. This is similar to the findings in Pelibuey lambs in our experiment supplemented with 1% CLA, which presented 24% HCT, which is near the lower limit (<20%) to cause animal death (González-Garduño et al. 2014b).

It is important to point out that the decrease in HCT and RBC did not affect the HGB content, a contradictory aspect, since haemoglobin is part of erythrocytes, essential for oxygen transport (Anderson et al. 2018). This result is due to the fact that in the different degrees of anaemia, the red blood cells are of different sizes, therefore the HGB content may or may not vary. In our study, Haemonchus contortus was causing constant bleeding in the lambs, decreasing the RBC and consequently the formation of reticuloocytes, that was not possible to measure, but the increase in MCV, maintaining the HGB without modification, as demonstrated by Jiménez-Penago et al. (2021) for this same study.

The higher MCH and MCHC values found during the experimentation time in the infected lambs in our experiment indicate the presence of anaemia, which independently of the CLA level is attributed to the haematophagous effect of H. contortus. Thus, we suggest constantly monitoring the blood variables to identify animals infected by Haemonchus contortus. Arece et al. (2015) consider that there is an inverse relationship between the hematological variables when MCH and MCHC increase from the effect of H. contortus, since there is a decrease in RBC and HCT as seen in the Pelibuey lambs in our study. These results lead us to assume that a certain amount of L3 larvae embedded into the abomasum reaching adulthood, without being reproductive, due to the low FEC.

Parasitic response

The total FEC was equal between treatments, with an average count under 200. This value is lower than the expected average, given the experimental infection with 9800 L3 larvae per animal. With this infection, we expected a mean excretion of 14000 FEC in infected lambs (Ojeda-Robertos et al. 2017), independently of CLA supplementation. Despite there being no differences in the total FEC, all groups of lambs excreted eggs during the period of no infection, which could be a consequence of anthelmintic resistance of the adult parasites lodged in the abomasum before the experiment began, as reported by González-Garduño et al. (2014a). During the post-infection period, the groups of infected lambs presented slight parasitic infection, observing <400 FEC at 28 and 35 days, causing cellular and hematological modifications, although not as expected, but enough to limit the effect of CLA. To this regard, Cruz-Tamayo et al. (2020), reported that resistant Pelibuey lambs to gastrointestinal nematodes showed a FEC peak at four weeks, while the
susceptible and intermediate lambs showed a FEC peak at the sixth week, reflecting a late response. In a previous study, González-Garduño et al. (2019) showed that the FEC peaks in Pelibuey sheep were affected by the dose used in the GIN artificial infection, being at 35 days when the animals had resistance against GIN, while other groups showed the FEC peak on days 42 and 56. It is possible that the slight parasitic infection found in our study was due to some ingredient in the diet, perhaps forage, contained some secondary metabolites such as polyphenols, which have been proven to have an antiparasitic effect (Hoste et al. 2016). Although Mravčáková et al. (2020) and Szulc et al. (2020) found no effect on any basic ruminal fermentation parameters that could intervene in the parasitic population by adding herbal mixtures to the diets of GIN parasite infected lambs.

Regarding IgG and IgM, they did not vary with the addition of CLA to the diet of Pelibuey lambs. The values remained similar in all treatments, which is contrary to what was expected, since CLA has been reported to increase the production of immunoglobulins in mice (Yamasaki et al. 2003), chickens (Liu et al. 2017), and immunologically challenged pigs (Moraes et al. 2012). It is interesting to mention that Ramirez-Santana et al. (2009) found that CLA supplementation in pregnant and lactating rats contributes to the development of the immune system of the offspring. This indicates that it improves innate immunity, which is important in young animals since they have low immunity to parasitic infections. Although CLA has been reported to decrease IgG production by up to 50% (Ostrowska et al. 2004), we expected a higher production of immunoglobulins (Bassaganya-Riera et al. 2003). Despite CLA having no effect on the IgG and IgM levels, the groups of infected lambs showed no reaction to being infected with Haemonchus contortus, since the eosinophil, leukocyte, and immunoglobulin counts would have increased naturally respective to the group of no infected lambs (Lacroux et al. 2006).

It is interesting to highlight that even ruminants naturally produce CLA (Grininari et al. 2000; Kumar and Ranganathan 2017), Pelibuey lambs performance was not affected by the addition of CLA to their diet, as expected, even supplementing with 3% CLA, compared with non ruminants and chickens. It is possible that these results were due to the good nutrition of the animals, which increased the natural resistance, limiting a high larval implantation, and therefore it could have suppressed some possible effect of CLA. Indeed, it is an aim of our research group to continue to improve experimental conditions in order to clarify the results here presented.

**Productive response**

Animals supplemented with 1% CLA decreased DMI, compared to those of the 3% CLA treatment, who showed the highest DMI, and we assume this alleviates the low DMI caused by GIN infection (Méndez-Ortiz et al., 2019). In previous studies carried out in different species, supplementing with CLA did not present any effect on DMI, as reported by Wynn et al. (2006), Schiavon et al. (2010) and Granados-Rivera et al. (2017), in sheep, fattening cattle and lactating cows, respectively. In contrast, Pinelli-Saavedra et al. (2019) and Pormalekshahi et al. (2020) found a decrease in dry matter consumption when supplementing with CLA to pigs and goat kids, respectively. Given these results, similar results were expected for DWG of lambs, due to the ability of CLA to regulate energy metabolism and nutrient partition; however, we did not find any differences between treatments. While Pinelli-Saavedra et al. (2019) and Pormalekshahi et al. (2020) reported an increase in DWG due to the CLA effect, in pigs and goat kids.

It is pertinent to emphasise that possibly the effects of the CLA on the response productivity of the Pelibuey lambs in the present study were not appreciated due to the nutritional factor, since the diet offered covered the nutritional requirements of the animals. In addition, we consider that some ingredients may be modifying the concentration of total CLA provided by the diet. For example, corn, soybean and forage used in the diet of experimental animals contain acid linoleic (Nudda et al. 2020), precursor of CLA in the rumen, conditioning the individual synthesis of CLA at different level. This leads us to suppose that the concentration of CLA in the phospholipids of the membrane was modified, and in turn the cellular action. Even lambs infected with gastrointestinal nematodes showed similar productivity than dewormed lambs. In this sense, the diet improved the resistance and resilience of the lambs, so much so that there was no reduction in consumption characteristic of parasitosis.

**Conclusion**

The results indicate that supplementing Pelibuey lambs with conjugated linoleic acid did not modify their immune response, nor did the parasitic infection, but it did affect the blood parameters. It is assumed
that the nutritional factor had an essential role in such response, since the nutritional needs of the experimental animals were covered, thus positioning the nutritional factor as an excellent control mechanism against parasitic infections. It is possible that this nutritional aspect masked the effects of conjugated linoleic acid in the sheep of the present study, therefore future research should focus on its interactive effect with other components of the diet.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are available from the corresponding author, [OHM], upon reasonable request.

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