The effects of Diet and corticosteroid-induced immune suppression during infection by *Haemonchus contortus* in lambs

Nadino Carvalho a, José Henrique das Neves a, Carina Nazato b, Helder Louvandini b, Alessandro F.T. Amarante a,∗

a UNESP - Universidade Estadual Paulista, Departamento de Parasitologia, Instituto de Biociências, Caixa Postal 510, CEP 18618-000 Botucatu, SP, Brazil
b USP - Universidade de São Paulo, Centro de Energia Nuclear na Agricultura, Caixa Postal 96, CEP 13400-970 Piracicaba, SP, Brazil

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**A B S T R A C T**

To evaluate the effects of Diet and corticosteroid-induced immune suppression during infection by *Haemonchus contortus*, 28 lambs were allocated to one of four groups treated as follows: Group Basal Diet – Normal; Group Basal Diet – Immune-Suppressed; Group Supplemented Diet – Normal; and Group Supplemented Diet – Immune-Suppressed. The Basal Diet contained *Cynodon dactylon* (cv. coast cross) hay with 82 g crude protein (CP)/kg dry matter (DM), which was provided to the lambs in all groups ad libitum. In addition, animals on the Supplemented Diet received daily a commercial concentrate containing 171 g CP/kg DM, which was offered in an amount corresponding to 3% of the animal’s live weight. The Immune-Suppressed groups received treatments with the glucocorticoid methylprednisolone sodium succinate (1.33 mg/kg of body weight), administered weekly. All lambs received a single infection with 4000 *H. contortus* infective larvae (L3) and were euthanised 28 days post-infection. Differences in pH and in the short-chain fatty acid (SCFA) concentrations occurred in rumen as a result of the distinct Diets offered to lambs. Such changes, however, did not have any apparent effect on larvae exsheathment and/or larvae survival inside the rumen, with all groups presenting similar worm burdens. However, animals on the Supplemented Diet presented reductions in worm growth and faecal egg counts. There was a significant effect of the Diet on the IgG levels against total antigens of *H. contortus* L3 from 7 to 27 days post-infection, with supplemented animals showing higher overall mean values (P<0.05). The immunosuppressive treatments had no effect on worm burden despite the reduction in the numbers of inflammatory cells in the abomasal mucosa of the Immune-Suppressed groups. These groups showed longer worms and females with more eggs in comparison with their counterparts fed each Diet; however, only the length of males was significantly affected (P<0.05). In conclusion, the changes caused in the rumen contents by supplementation with concentrate did not impair *H. contortus* establishment.

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1. Introduction

*Haemonchus contortus* is a bloodsucking parasite that causes significant losses in sheep production worldwide. This parasite shows high prolificacy and high levels of genetic diversity, which can result in the quick appearance of populations with anthelmintic resistance (Gilleard, 2013). For example, an *H. contortus* population with resistance to the new anthelmintic monepantel appeared in sheep raised in Uruguay even after a relatively short period of its use in selective treatments (Mederos et al., 2014). Therefore, alternative methods for worm control are necessary to reduce the need for anthelmintics, and these methods include immunonutrition (Hoste and Torres-Acosta, 2011).

The third stage larvae (L3) with their double cuticles (sheath) are the infective form of *H. contortus*. The external sheath confers extra protection for the larvae in the environment, and it is lost as soon as the larvae come into contact with the rumen fluid, where larvae receive a stimulus to initiate exsheathment. This stimulus is triggered by the pH, which reflects a H₂CO₃/HCO₃⁻-buffer system in rumen (Hertzberg et al., 2002). A strong link between the rates of exsheathment and the biochemistry of the rumen environment, which may influence larvae viability and establishment, was demonstrated in Ostertagia ostertagi. The exsheathment efficiency was greater when the animal was on a grass Diet in comparison with a 71% grain Diet (DeRosa et al., 2005). In addition, incubation of larvae in the rumen for more than 12 h did not improve
Table 1
Average (± standard error) pH, acetate (mmol/l), propionate (mmol/l) and butyrate (mmol/l) in the ruminal liquid of the lambs 28 days after artificial infection with 4000L₃ of H. contortus. The treatments were Basal or Supplemented Diets and Normal or Immune-Suppressed as the immunological status in a 2 × 2 factorial design.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Basal Diet</th>
<th>Supplemented Diet</th>
<th>Effects (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (n = 7)</td>
<td>Immune-Suppressed (n = 7)</td>
<td>Diet Immunity Diet × Immunity</td>
</tr>
<tr>
<td>pH</td>
<td>6.59 ± 0.07&lt;br&gt;6.50 ± 0.05</td>
<td>5.83 ± 0.08&lt;br&gt;6.25 ± 0.14</td>
<td>P = 0.000</td>
</tr>
<tr>
<td>Acetate</td>
<td>54.73 ± 2.55&lt;br&gt;55.95 ± 1.50</td>
<td>54.06 ± 2.52&lt;br&gt;46.91 ± 2.04</td>
<td>NS&lt;br&gt;P = 0.037</td>
</tr>
<tr>
<td>Propionate</td>
<td>12.09 ± 0.92&lt;br&gt;11.10 ± 0.46</td>
<td>23.54 ± 1.61&lt;br&gt;15.86 ± 1.83</td>
<td>P = 0.000&lt;br&gt;P = 0.003</td>
</tr>
<tr>
<td>Butyrate</td>
<td>4.25 ± 0.30&lt;br&gt;4.24 ± 0.28</td>
<td>9.30 ± 0.52&lt;br&gt;7.34 ± 0.71</td>
<td>NS&lt;br&gt;P = 0.000</td>
</tr>
</tbody>
</table>

Different superscripts in the same line indicate a significant difference (Tukey's test; P < 0.05); NS: P > 0.05.

Table 2
Average (± standard error) of mast cells and eosinophils/mm² in the abomasal mucosa of the lambs 28 days after artificial infection with 4000L₃ of H. contortus. The treatments were Basal or Supplemented Diets and Normal or Immune-Suppressed as the immunological status in a 2 × 2 factorial design.

<table>
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<tr>
<th>Variables</th>
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<tbody>
<tr>
<td></td>
<td>Normal (n = 7)</td>
<td>Immune-Suppressed (n = 7)</td>
</tr>
<tr>
<td>Mast cells in fundic area</td>
<td>12.73 ± 2.04&lt;br&gt;5.61 ± 1.73</td>
<td>27.59 ± 4.82&lt;br&gt;6.21 ± 1.54</td>
</tr>
<tr>
<td>Mast cells in pyloric area</td>
<td>10.46 ± 2.61&lt;br&gt;4.70 ± 1.62</td>
<td>16.52 ± 2.51&lt;br&gt;6.21 ± 2.02</td>
</tr>
<tr>
<td>Eosinophils in fundic area</td>
<td>3.94 ± 0.83&lt;br&gt;2.12 ± 0.80</td>
<td>10.31 ± 2.84&lt;br&gt;1.36 ± 0.76</td>
</tr>
<tr>
<td>Eosinophils in pyloric area</td>
<td>11.82 ± 3.22&lt;br&gt;2.88 ± 0.72</td>
<td>24.25 ± 4.28&lt;br&gt;9.70 ± 2.55</td>
</tr>
</tbody>
</table>

Different superscripts in the same line indicate a significant difference (Tukey's test; P < 0.05); There was no significant Diet × Immunity interaction (P > 0.05). NS: P > 0.05.

exsheathment in infective ovine and bovine strongyloid larvae. On the contrary, the larval mortality rate rapidly increased between 12 and 24 h, indicating that the persistence of the larvae in the rumen for longer than 12 h is not beneficial for their survival (Hertzberg et al., 2002). It was also observed that the rumen function of sheep selected for increased resistance to Haemonchus was altered in response to H. contortus infection, with an increase in fluid outflow and turnover rate and a decrease in propionic acid concentration, suggesting that these changes might be a component of a greater host resistance (Doyle et al., 2011). Therefore, we developed a trial to test if changes in rumen pH and concentrations of short-chain fatty acid (SCFA) could cause impairments in larvae exsheathment and survival, with a consequent reduction in worm establishment in the abomasum. To test this hypothesis, one group of lambs was fed only with a grass diet, while another was supplemented with a large amount of concentrate. To minimize the influence of the immune response in worm establishment, half of the lambs in each group were Immune-Suppressed with prednisolone corticoid.

2. Materials and methods

This study was conducted according to the ethical principles for animal experimentation and was approved by the Ethics Committee on Animal Use (CEUA) protocol No 86/2012-CEUA/FMVZ. Twenty-eight three-month-old Suffolk male lambs were acquired just after weaning from a commercial farm and were maintained in individual pens with a concrete floor in the facilities for small ruminants at the University.

At arrival, the animals were shedding an average of 2100 (range 100–26400) strongyle eggs per gram of faeces (EPG) and 100 (range 0–500) Strongyloides papillosus EPG. Faecal cultures demonstrated infection by the following strongyles: Haemonchus spp. (74%), Trichostrongylus spp. (24%) and Oesophagostomum spp. (2%). Initially, lambs were drenched with mepenolate (2.5 mg/kg; Zolvix®, Novartis); however, due to the presence of Strongyloides papillosus eggs in faecal examination following this treatment, the animals were treated again with albendazole (10 mg/kg, Valbazen®, Pfizer) and levamisole (10 mg/kg, Ripercol®. Fort Dodge) orally for 10 consecutive days. Then, a series of faecal examinations were performed, which confirmed the elimination of infection by helminths.

The experimental animals remained indoors, and the concrete floor was washed every other day to minimise the risk of parasitic infection. The animals received decoquinate (0.5 mg/kg per day; Deccox® - Alpharma) according to the manufacturer’s recommendations throughout the trial period to prevent coccidiosis. The lambs were vaccinated against clostridiosis (Sintonax T Polivalente®, Merial S.A.) before the beginning of the experiment.

2.1. Experimental design and feeding

The experiment was conducted with a 2 × 2 factorial design; there were two Diets (Basal Diet or Supplemented Diet) and two immune categories (Normal or Immune-Suppressed).

The lambs were randomly allocated to one of four groups, balanced as much as possible for weight, and treated as follows: Group Basal Diet – Normal; Group Basal Diet – Immune-Suppressed; Group Supplemented Diet – Normal; and Group Supplemented Diet – Immune-Suppressed. The minimum and maximum pre-drench FEC recorded in each group were, respectively, 1100–10300; 1600–15000; 200–26400; and 100–5200 EPG.

The basal Diet contained Cynodon dactylon (cv. coast cross) hay with 82 g crude protein (CP)/kg dry matter (DM), which was provided to the lambs in all groups ad libitum. In addition, animals on the Supplemented Diet received daily a commercial concentrate (Suplementa Ovinos Campo, Presence) containing 171 g CP/kg DM, which was offered in an amount corresponding to 3% of the animal’s live weight. Animals in all groups had free access to tap water and mineral supplements (Presencéfios® ovinos, Presence). The adaptation of the groups on the Supplemented Diet started 21 days before the infection, when they started to receive a progressive increase in the amount of the concentrate offered.

The Immune-Suppressed groups received treatments with the glucocorticoid methylprednisolone sodium succinate (1.33 mg/kg of body weight; Solu-medrol®, Pfizer), administered intramuscularly weekly beginning 4 h before the experimental infection.

2.2. The production of infective larvae (L₃) of Haemonchus contortus

An H. contortus isolate with multiple anthelmintic resistance was used (Almeida et al., 2010). L₃ of this isolate had been stored in liquid nitrogen and were thawed to infect two worm-free lambs,
used as donors for the production of L3. The worm-free status of the donors was confirmed before the infection by a series of faecal examinations. During the trial, they were maintained indoors and had free access to tap water and grass hay (coast cross) purchased from a farm with no ruminants, avoiding risks of food contamination by nematode-infective larvae. Each donor lamb was artificially infected orally with 10,000 L2 of *H. contortus* in a single dose. Faecal cultures for the production of L3 were kept for one week in an incubator at 25 °C (Ueno and Gonçalves (1998)), and the infective larvae were harvested from cultures one day before the infection of the trial lambs.

2.3. Experimental infection and faecal and worm examination

Experimental lambs received a single infection orally with 4000 *H. contortus* L3 on day zero (day 0). Faecal egg counts (FECs) were performed at 21, 24 and 27 days post-infection, and lambs were sacrificed for worm counts 28 days after infection. FECs were determined by a modified McMaster method in which each egg counted represented 100 eggs per gram of faeces (Ueno and Gonçalves, 1998). All animals were euthanised 28 days post-infection for recovery and enumeration of parasites. Samples containing 10% of the abomasal contents were fixed in 5% formaldehyde. Worms were counted, sexed, and classified by their stage of development according to Ueno and Gonçalves (1998).

The body lengths of 10 male and 10 female *Haemonchus* worms, chosen at random per abomasum sample, were measured. Then, the number of eggs per female was determined by putting five female worms in a polyethylene tube with 950 μL of sodium hypochlorite solution, containing 0.25% active chlorine. After a few minutes, the worms were completely disintegrated, whereas the eggs were still intact and could be counted (Kloosterman et al., 1978). The tube was shaken in a vortex every two minutes until the disappearance of parasite fragments. At this moment, 50 μL of sodium thiosulphate solution 1% (anticlorine) was added to the tube to prevent degradation of the eggs. The total number of the eggs in the tube was estimated by counting eggs in 10 drops with 10 μL each, which allowed for the calculation of the number of eggs per female worm.

2.4. Haematology and body weight

Weekly, the body weight of the animals was recorded and blood samples (5 mL) were collected by jugular vein puncture into Vacutainer® tubes containing anti-coagulant (EDTA). The packed cell volume (PCV) was determined by microhematocrit centrifugation. Plasma samples were stored at −20 °C until immunoglobulin measurement (ELISA).

2.5. ELISA and histology

The plasma levels of IgG antibodies against total antigens of *H. contortus* L3 were estimated using the ELISA test. The production of antigens of *H. contortus* was previously described by Amarante et al. (2009), and the protocol used to measure the parasite-specific plasma IgG levels was according to Silva et al. (2012) with the following modifications: plates were coated with 2.5 μg/mL of antigen and peroxidase conjugate diluted 1:20,000. The results were expressed as the percentage of the optical density value (OD) of the positive standard serum and employed the following formula: % OD = [(OD mean of the tested serum – OD mean of blank)/(OD mean of the positive standard serum – OD mean of blank)] × 100 (Kanobana et al., 2001).

Tissue samples taken from the fundic and pyloric regions of the abomasum were fixed in 4% buffered formaldehyde for 48 h. Next, the samples were dehydrated with alcohol and embedded in paraffin wax. Sections (4 μm thick) were stained with toluidine blue 1% or haematoxylin and eosin (H&E). Mast cells were counted in the sections stained with toluidine blue, and eosinophils were counted in the sections stained with H&E. The cells were enumerated under a 10× eyepiece containing a calibrated graticule and 100× objective lens, viewing an area of 0.01 mm². Inflammatory cells were enumerated in 30 fields of the abomasal mucosa that were randomly selected per animal. The counts were expressed as the number of cells per mm² of mucosa.

2.6. Rumen content analysis

Immediately after the animal’s sacrifice, the rumen content was collected in 50 mL tubes and centrifuged at 1500 rpm for 15 min. The pH of the supernatant was measured, and a 1200 μL sample of the ruminal fluid was placed in a polyethylene tube, mixed with 300 μL of metafosfonic acid 25% and stored at −20 °C until processing.

Concentrations of short-chain fatty acid (SCFA) in the rumen were determined by gas chromatography according to descriptions by Palmquist and Conrad (1971) and Nocek et al. (1987), with adaptations. Following centrifugation of the ruminal fluid (15,000 g, 40 min, 4 °C), 800 μL of the supernatant was mixed with 100 μL of 2-ethyl-butyric acid (internal standard) and 200 μL of formic acid. A solution with known concentrations of each SCFA (acetic, propionic and butyric acids) was used for calibration of the equipment. One microlitre of the sample was injected into a gas chromatograph (Shimadzu GC-14A, Shimadzu Co., Kyoto, Japan) equipped with a glass column, 2 m × 2 mm, containing 10% SP-1200/1% H3PO4 on 80/100 Chromosorb WAW. The conditions were as follows: the column temperature started at 115 °C and rose continuously for 3.20 min, rising to 123 °C at 10 °C/min for 1.25 min and then to 126 °C at 10 °C/min for 5 min; carrier gas (He) at a constant flow rate of 25 mL/min; flame ionization detector temperature 260 °C; and injector temperature 200 °C.
Fig. 1. Levels of plasma IgG against third stage larvae (L3) antigens of Haemonchus contortus after infection with 4000 L3. Excepting day ‘0’, there was a significant effect of Diet on all sampling days (P < 0.05), with lower values in lambs on the Basal Diet. Bars represent standard error of the means.

Fig. 2. Mean number of eggs per gram of faeces (EPG) in lambs artificially infected with 4000 larvae of Haemonchus contortus. Diet had a significant effect, with lambs on the Basal Diet shedding significantly more eggs than lambs on the Supplemented Diet (P < 0.01). Bars represent standard error of the mean.

2.7. Statistical analysis

Data were analysed by one-way analysis of variance for the variables measured just once and analysis of variance with repeated measures for variables measured at several time points (FEC, body weight and PCV) using the Statistical Analysis System, version 9.2 (SAS Institute, Inc., Cary, NC, USA). Means were compared by Tukey’s test at the 5% significance level, and only significant interactions are reported in the results. FEC, eosinophils, mast cells and worm burden data were transformed to log10(x + 1) prior to analysis. Means are presented in the results as the arithmetic means (± standard error) of untransformed data. With regard to worm length and fecundity, the mean values determined per animal were used in the analysis of variance.

3. Results

The values of pH, acetate, propionate and butyrate in the ruminal liquid of the lambs are shown in Table 1. There was a significant Diet x Immunity interaction on pH and propionate. Supplemented Diet – Normal group means were significantly different from those of the other groups (Table 1). There were Diet-related effects on acetate and butyrate values that presented as higher and lower values, respectively, in animals on the Basal Diet (Table 1).

Immunological variables that include inflammatory cell counts in the abomasal mucosa and IgG levels against H. contortus L3 antigens are presented in Table 2 and Fig. 1, respectively. The immunosuppressive treatment did not prevent the rise in IgG levels against L3 antigens that peaked at 14 days post-infection (Fig. 1) in all groups with significant Time x Immunity and Time x Diet interactions (P < 0.01). There was a significant effect of the Diet on the IgG levels from 7 to 27 days post-infection, with supplemented animals showing higher overall mean values of IgG (P < 0.05). Paradoxically, the immunosuppressive treatment did not have a significant effect on the IgG level in any sampling (P > 0.05). Considering the group means, the Supplemented Diet – Normal group showed higher averages of IgG (P < 0.05) than the Basal Diet – Normal group at 21 and 27 days post-infection and higher averages than the Basal Diet – Immune-Suppressed group from 14 to 27 days post-infection.

The immunosuppressive treatment had a significant effect (P < 0.01) on the numbers of inflammatory cells in the abomasal mucosa (Table 2). In each Diet, the Immune-Suppressed lambs presented lower averages of inflammatory cell numbers in the abomasal mucosa than animals with intact immunity (Normal groups) (Table 2). A significant Diet effect was also observed in mast cell and eosinophil numbers in the fundic and in the piloric area of the abomasal mucosa, respectively (P < 0.05), with Supplemented animals displaying the highest values.

Diet had a significant effect on FEC on all days of evaluation (P < 0.01). Lambs fed with the Basal Diet shed on average 3.2 times more eggs in faeces than Supplemented animals. Conversely, there was no effect of the immunological status on egg output (P > 0.05). There was a significant Diet x Time interaction (P < 0.05). Mean FEC increased in all groups over time (Fig. 2); however, such increases were more pronounced in groups fed the Basal Diet. All groups presented similar numbers of adult males, adult females and total worm burdens (Table 3), without any significant influence of the Diet or the immunological status (P > 0.05). Only the numbers of juveniles was affected by the Diet (P < 0.01), with the highest values in animals Supplemented. In contrast, Diet had a significant effect (P < 0.05) on worm length: groups supplemented presented stunted worms and females with fewer eggs inside the uterus. With regard to the immunological status, Immune-Suppressed lambs showed longer worms and females with more eggs in comparison with their counterparts fed each Diet (Table 3); however, only the length of males was significantly affected (P < 0.05).

Lambs presented similar body weights at the beginning of the adaptation period that started 21 days (day -21) before the artificial infection (Fig. 3). Afterwards, due to differences in nutrition over the adaptation period (day -21 to day 0), the Supplemented animals gained more weight and showed higher body weights from day -7 until the end of the trial (P < 0.05). The immunological status had no influence on body weight gain (P > 0.05). Considering the period after the infection, there was a significant Diet x Immunity interaction (P < 0.05) with regard to daily body weight gain. Groups fed with the Basal Diet had the lowest body weight gain (0.102 kg/animal/day and 0.114 kg/animal/day in Normal and in Immune-Suppressed groups, respectively), while the Supplemented Diet – Normal group presented the highest average (0.268 kg/animal/day).

In relation to PCV, there was a significant Time x Diet interaction (P < 0.001). All groups presented progressive decline in PCV values; however, the reduction in mean PCV was more accentuated in groups fed with the Basal Diet (Fig. 4). Diet had a significant effect (P < 0.05) from 14 to 27 days post-infection, while the immunological status had no effect on PCV values (P > 0.05). At the end of the Trial, a few animals showed low values of PCV (between 21% and 24%): 3 lambs in the Basal Diet – Normal group; 2 in the Basal...
The values.

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Differences in groups parasite exsheathment (N.

Body weight of the lambs infected with 4000 larvae of Haemonchus contortus on day 0. Animals were adapted to the Diets from Day-21 to Day 0. There was a significant effect of Diet (P < 0.05) starting 7 days before the infection (Day-7) until the end of the study (Day 28). During this period, the Supplemented lambs were significantly heavier. Bars represent standard error of the mean.

Packed cell volume (%) of the lambs infected with 4000 larvae of Haemonchus contortus. There was a significant effect of Diet (P < 0.01) from 14 to 27 days after the infection. During this period, the Supplemented lambs showed higher overall mean values. Bars represent standard error of the mean.

Diet – Immunosuppressed group; and 2 in the Supplemented Diet – Normal group.

4. Discussion

Differences in pH and in the short-chain fatty acid concentrations occurred as a result of the distinct Diets offered to lambs. Such changes, however, did not have any apparent effect on larvae exsheathment and/or larvae survival inside the rumen, with all groups presenting similar worm burdens, i.e., all with similar parasite establishment in the abomasum. Therefore, previous observations about the influence of the rumen environment on the establishment of the nematodes (DeRosa et al., 2005; Doyle et al., 2011) were not confirmed in the present study.

There are several manifestations of resistance against gastrointestinal nematode infections, which include reductions in worm burden, due to elimination of incoming larvae and/or adult parasites, as well as impairments in parasite development and fecundity (Balic et al., 2000). In the present trial, such manifestations of resistance were clearly observed in animals on the Supplemented Diet, which presented reductions in worm growth and females with fewer eggs inside the uterus. As a consequence, they presented a significant reduction in mean FEC in comparison with lambs on the Basal Diet. Similar to our results, Wallace et al. (1995) reported no differences in the number of female worms recovered at necropsy from lambs fed on different Diets, but the worms from the protein-supplemented lambs (Diet with 17.3% CP) were shorter and contained fewer eggs than lambs fed Diets with 9.8% CP.

The immunosuppressive treatments caused a reduction in the numbers of inflammatory cells in the abomasal mucosa, but did not prevent a raise of antibody profiles. Such treatments had only a small effect on parasite growth and egg output, and they had no effect on worm burden. Similarly, no obvious effects of weekly treatment with prednisolone were observed on the abomasal establishment of H. contortus following single experimental infection in lambs (Sargison et al., 2011). There was also no difference in the recovery of Anthoclocyma ceylanicum adult worms from hamsters treated with cyclosporine A or prednisolone in comparison with a control group, despite half the recommended dose of both drugs being sufficient to inhibit the proliferation of more than 70% of hamster lymph node cells (Dias et al., 2013).

In contrast to our results, a similar protocol of treatments with prednisolone was sufficient to induce immune-suppression in lambs with increase in worm burden (Greer et al., 2005, 2008). Apparently, this corticoid treatment affects only the acquired immunity that develops over a prolonged period of exposure of the animals to a constant stimulus triggered by incoming infective larvae. This was the case for lambs after serial infections with Trichostrongylus colubritiformis; animals with intact immunity had on average 224 worms, while the animals immune-suppressed with prednisolone had 22,387 worms (Greer et al., 2005). Treatment with the immunosuppressive drug dexamethasone was associated with the depletion of globule leucocytes, mast cells and eosinophils from the small intestinal mucosa and abolished resistance to challenge infections in Romney sheep that were resistant to natural infection (Douch et al., 1986). Dexamethasone treatment also resulted in naturally resistant Native lambs becoming more susceptible to nematode infections (Peña et al., 2004).

Similar to our findings, Adams (1982) observed that treatment at various times with dexamethasone abolished acquired but not innate immunity to H. contortus and that acquired responses were not important in restraining the fecundity of adult worms during primary infection. Our results also indicate that the reduction in worm size and fecundity was due to improvements in the innate resistance of the Supplemented lambs. In addition, groups that received the Supplemented Diet clearly presented improved resilience against infection with higher values of PCV and body weight gain. The benefits of improved nutrition on the acquired resistance against nematode infections have been extensively documented (Shaw et al., 1995; Coop and Holmes, 1996; Datta et al., 1998; Bricarello et al., 2005; Louvandini et al., 2006; Khan et al., 2012), while low food intake and protein-energy deprivation impair the development and expression of host-protective immunity against haemonchosis (Roberts & Adams, 1990).

In conclusion, the changes caused in the rumen contents by supplementation with concentrate did not impair H. contortus establishment.

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