UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

RESPOSTA IMUNOLÓGICA E DESEMPENHO DE CORDEIROS LACTENTES SANTA INÊS E ILE DE FRANCE INFECTADOS ARTIFICIALMENTE COM *Haemonchus contortus*

JOSÉ GABRIEL GONÇALVES LINS

Botucatu - SP
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“Leva-se tempo para erguer castelos”.

Aos meus maiores incentivadores: José Lins de Albuquerque e Joaquina Gonçalves Vieira Lins.

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BW - Body Weight

CP - Crude Protein

EDTA - Ácido Etilenodiamino Tetra-acético

EPG - Eggs Per Gram

FEC - Faecal Egg Count

g - Força Centrífuga

GIN - Gastrointestinal Nematodes

IF – Ile de France

IgA – Imunoglobulina A

IgE – Imunoglobulina E

IgG – Imunoglobulina G

IIF – Infected Ile de France

IL - Interleucina

INF- γ - Interferon-gama

ISI – Infected Santa Inês

L1 – Larva de primeiro estágio

L3 - Larva de terceiro estágio ou Larva Infectante

L4 - Larva de quarto estágio

L5 - Larva de quinto estágio

LGs - Leucócitos Globulares

NaCl – Cloreto de Sódio

ns - Not significant

OD - Optical Density

PAMPs - Padrões Moleculares Associados a Patógenos

PCV - Packed Cell Volume

PRRs - Receptores de Reconhecimento de Padrões

RBC - Red Blood Cell

SI - Santa Inês

STP - Serum Total Protein

Th1 - T helper 1

Th2 - T helper 2

TNF - Fator de Necrose Tumoral

WBC - White Blood Cell

SUMÁRIO

LINS, J.G.G. **Resposta imunológica e desempenho de cordeiros lactentes Santa Inês e Ile de France infectados artificialmente com *Haemonchus contortus.*** Botucatu, 2020. 89f. Dissertação (Mestrado) – Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, Universidade Estadual Paulista “Júlio de Mesquita Filho”.

RESUMO

Haemonchus contortus é o principal parasita gastrintestinal de ovinos criados em áreas tropicais e subtropicais. Este estudo objetivou avaliar a resposta imunológica de cordeiros lactentes das raças Santa Inês (SI) e Ile de France (IF) submetidos a infecções seriadas com *H. contortus*. Quatorze cordeiros SI e 12 cordeiros IF, foram distribuídos em quatro grupos: SI infectado (n=8), SI não infectado (n=6), IF infectado (n=8) e IF não infectado (n=4). Cordeiros dos grupos infectados foram submetidos a 27 infecções, realizadas a cada dois dias, do 14º até 68º dia de vida, com um total de 5400 larvas infectantes (L3) de *H. contortus* por animal. Aos 68 dias de vida, os cordeiros foram eutanaziados para recuperação de parasitas do abomaso, coleta de muco, tecido e linfonodos abomasais. Cordeiros SI apresentaram menores médias de ovos por grama de fezes em todos os momentos avaliados, e a partir dos 50 dias de idade, três cordeiros SI infectados deixaram de eliminar ovos nas fezes. Em média, a taxa de estabelecimento das L3 foi de 22,9% nos cordeiros IF e de 11,1% nos SI. O peso médio dos linfonodos abomasais de cordeiros SI infectados foi significativamente maior que dos demais grupos. Em comparação com os cordeiros IF infectados, os SI apresentaram número maior de eosinófilos, mastócitos e leucócitos globulares nos tecidos abomasais ($P<0,05$). Em conclusão, mecanismos envolvendo resposta celular local têm relação com a elevada resistência de cordeiros SI às infecções por *H. contortus* nas primeiras semanas de vida.

Palavras-chave: Helmintoses, Resposta Imune, Parasitas gastrintestinais, Ovinocultura

LINS, J.G.G. **Immune response and performance of Santa Inês and Ile de France suckling lambs artificially infected with *Haemonchus contortus***. Botucatu, 2020. 89f. Dissertação (Mestrado) – Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, Universidade Estadual Paulista "Júlio de Mesquita Filho".

ABSTRACT

Haemonchus contortus is the major gastrointestinal parasite of sheep raised in tropical and subtropical areas worldwide. This trial aimed to evaluate the immune response of Santa Inês (SI) and Ile de France (IF) suckling lambs serially infected with *Haemonchus contortus*. Fourteen SI lambs and 12 IF lambs were randomized in four groups: infected SI (n=8), non-infected SI (n=6), infected IF (n=8) and non-infected IF (n=4). Lambs of infected groups were submitted to 27 infections, conducted every two days, from 14 to 68 days of age, and each lamb received 5400 *H. contortus* infective larvae (L3). At 68 days of age, lambs were euthanized for recovering abomasal parasites, collection of mucus, tissue sample and abomasal lymph nodes. SI lambs had the lowest eggs per gram of faeces (EPG) means in all samplings, and from 50 days old, three SI lambs stopped shedding eggs on faces. L3 establishment rate average for IF lambs was 22.9% and 11.1% for SI lambs. Infected SI lambs had higher abomasal lymph node weight than the other groups. Compared to Infected IF lambs, infected SI presented the highest counts of eosinophils, mast cells and globule leukocytes in the abomasums tissues ($P<0.05$). Finally, mechanisms involving local cellular response are intended to confer resistance to SI lambs against *H. contortus* infections in the first weeks of life.

Keywords: Helminthiasis, Immune response, Gastrointestinal parasites, Sheep farming

CAPÍTULO I

1 INTRODUÇÃO

No cenário mundial, o Brasil ocupa o 18º lugar em número de ovinos, com um rebanho de aproximadamente 18 milhões de cabeças (IBGE, 2017). O sudeste detém 3,5% do efetivo nacional, sendo que mais de 57% deste rebanho, encontra-se no estado de São Paulo, com um número aproximado de 356,5 mil ovinos (IBGE, 2017).

Apesar do estado de São Paulo apresentar o maior rebanho e o segundo maior crescimento do número de ovinos dentre os demais estados da região sudeste (IBGE, 2016), assim como em outras regiões do país, os ovinocultores enfrentam alguns entraves quanto à produção de ovinos.

Ocorrências de doenças parasitárias são comuns, sendo estas, uma importante causa de mortalidade, principalmente em animais jovens (AMARANTE; AMARANTE, 2016). Em pequenos ruminantes, os parasitas mais importantes são *Haemonchus contortus, Trichostrongylus colubriformis, Cooperia* spp. e *Oesophagostomum columbianum*, sendo *H. contortus* o principal parasita gastrintestinal de ovinos em áreas tropicais e subtropicais (AMARANTE, 2014a). A profilaxia da haemoncose baseada no uso de produtos anti-helmínticos, tem se mostrado insustentável devido ao aparecimento de populações de nematódeos resistentes a todas as classes de medicamentos disponíveis para tratamento (ALMEIDA et al., 2010; ALBUQUERQUE et al., 2017).

A imunidade dos animais desempenha um papel muito importante na resistência à verminose, e fatores individuais como: a idade, a raça e a condição fisiológica interferem na resposta do hospedeiro contra os parasitas (AMARANTE, 2008). Desta maneira, a eficiência do controle da verminose pode ser aumentada por meio da identificação de raças mais resistentes e seleção de indivíduos ou rebanhos resistentes.

Diferenças entre as raças de ovinos em relação a sua susceptibilidade a infecções por nematódeos gastrintestinais foram relatadas por alguns autores (AMARANTE et al., 2004; ROCHA et al., 2005; AMARANTE et al., 2009; SHAKYA et al., 2009). Raças naturalizadas, também denominadas nativas, que tem prosperado em condições ambientais adversas, com manejo zootécnico rudimentar e sem histórico de tratamentos anti-helmínticos são mais resistentes

que raças importadas, de alta produtividade e selecionadas sob condições de manejo zootécnico (ALBA-HURTADO; MUÑOZ-GUZMÁN, 2013).

A Santa Inês (SI) é uma raça nativa originária do nordeste brasileiro e descendente de animais introduzidos no Brasil há alguns séculos (ROCHA et al., 2005). Como exemplo de raça exótica, pode-se citar a Ile de France (IF), raça francesa considerada susceptível a infecções por nematódeos gastrintestinais. Amarante et al. (2004) observaram que cordeiros da raça Santa Inês, após o desmame, apresentaram maior resistência a nematódeos gastrintestinais quando comparadas a cordeiros de raças europeias.

Existem vários estudos que demonstraram que ovinos SI são mais resistentes à infecção por *H. contortus* quando comparadas ovinos da raça Ile de France (AMARANTE et al., 2004; ROCHA et al., 2005; AMARANTE et al., 2009). Rocha et al. (2005), comparando a resposta imunológica pela contagem de eosinófilos sanguíneos em cordeiros SI e IF, observaram que cordeiros SI apresentaram maiores contagens de eosinófilos em todas as idades avaliadas. São escassos os trabalhos que avaliaram a resposta imunológica de cordeiros lactentes frente às infecções parasitárias por nematódeos gastrintestinais. Bahirathan et al. (1996) observaram que cordeiros Gulf Coast Native lactentes possuem a habilidade de resistir as infecções por *H. contortus*, enquanto que cordeiros Suffolk desenvolvem haemoncose e requerem tratamento com anti-helmíntico.

Em infecções por helmintos é desencadeada uma resposta imune do tipo 2 (Th2), responsável pela mobilização, ativação e recrutamento celular para o sitio de interação parasita-hospedeiro, a fim de promover a expulsão do parasita e controlar a infecção (INCLAN-RICO; SIRACUSA, 2018). Apesar dos vários estudos que avaliaram as diferenças entre raças ovinas resistentes e susceptíveis a infecção por *H. contortus* (BRICARELLO et al., 2005; SHAKYA et al., 2011; PATRA et al., 2016; JACOBS et al., 2016), os mecanismos imunológicos que conferem maior resistência aos animais ainda não foram completamente elucidados.

Deste modo, é auspiciosa a realização de estudos a fim de compreender e avaliar os parâmetros e variáveis que podem elucidar de uma melhor forma a diferença nos mecanismos imunológicos relacionados às respostas das raças resistentes e susceptíveis frente à infecção por *H.*

contortus. Para isso objetivou-se avaliar as variáveis hematológicas, parasitológicas, resposta celular local e imunoglobulinas envolvidas na resposta imune de cordeiros lactentes das raças Santa Inês e Ile de France infectados experimentalmente por *Haemonchus contortus*.

2 REVISÃO DE LITERATURA

2.1 Biologia e ciclo de vida da espécie *Haemonchus contortus*

A ampla distribuição geográfica deste parasita e a multirresistência aos anti-helmínticos disponíveis no mercado, faz de *H. contortus* uma das principais ameaças à sustentabilidade da ovinocultura (SACCAREAU et al., 2017).

H. contortus é um parasita com ciclo de vida direto e que apresenta duas fases de vida distintas (AMARANTE, 2014b). Na primeira fase, denominada fase de vida livre, os ovos são eliminados por parasitas adultos nas fezes de animais parasitados. No ambiente os ovos eclodem em L1 e desenvolvem-se até o estágio infectante (terceiro estágio, L3). As L3 possuem uma dupla cutícula remanescente da fase de segundo estágio (BALIC et al., 2000a). O processo de eclosão dos ovos até o desenvolvimento da L3 pode sofrer influência do ambiente e do microclima da pastagem (SANTOS et al., 2012), mas em temperatura constante de 25 ºC, as larvas tornam-se infectantes em aproximadamente sete dias (AMARANTE, 2014b).

Na segunda fase de vida, no hospedeiro, ocorre o período de desenvolvimento histotrófico e fase adulta (BALIC et al., 2000a). Após a ingestão da L3 de *H. contortus* pelo hospedeiro, a larva infectante sofre um desembainhamento (perda da dupla cutícula) normalmente no trato anterior ao nicho final, e ao chegar ao abomaso penetra a mucosa na fase histotrófica para o desenvolvimento em L4 (AMARANTE, 2014b), voltando ao lúmen do órgão. Balic et al. (2000a) observaram que da ingestão da L3 pelo hospedeiro até a mudança para larva de quarto estágio, leva entre 24 e 96 horas após a infecção. Após se diferenciarem em machos e fêmeas de quarto estágio final, as larvas de quinto estádio (L5) completam o desenvolvimento, amadurecem sexualmente, e, após a cópula, as fêmeas iniciam o período de oviposição (AMARANTE, 2014b).

Considerado um dos parasitas de ovinos mais prolíficos (SACCAREAU et al., 2017), a fêmea de *H. contortus* pode apresentar uma oviposição entre 5.000 a 15.000 ovos/dia (LE JAMBRE, 1995; EMERY et al., 2016).

2.2 Fisiopatogenia da infecção por *Haemonchus contortus*

As infecções por nematódeos gastrintestinais têm ocorrência significativa em ovinos, devido principalmente à sensibilidade desta espécie a esse endoparasita.

Haemonchus contortus é um parasita hematófago que habita o abomaso principalmente de pequenos ruminantes (AMARANTE, 2014b), sendo considerado o principal causador de mortes em ovinos e caprinos de todas as categorias (HERNÁNDEZ et al., 2016).

Cada parasita adulto causa uma perda no hospedeiro de aproximadamente 0,05 mL de sangue por dia, decorrente da ingestão e extravasamento de sangue das lesões (BOWMAN et al., 2003). Portanto, um animal que esteja pastejando uma área contaminada por *H. contortus*, facilmente pode albergar 500 parasitas, perdendo de 25 a 40 mL de sangue em um dia (MARQUARDT et al., 2000). Em infecções pesadas, o animal tende a apresentar uma diminuição das variáveis hematológicas, como a redução progressiva do volume globular, diminuição dos níveis de proteínas plasmáticas totais, diminuição da contagem de glóbulos vermelhos, desencadeando uma anemia severa e instauração da Haemonchose clínica (AMARANTE et al., 2004; AMARANTE, 2014b).

Desta forma, este parasita é considerado um dos principais problemas relacionados à sanidade de pequenos ruminantes, sendo associado à redução da produtividade e consequentemente elevadas perdas econômicas em todo o mundo (ALBA-HURTADO; MUÑOZ-GUZMÁN, 2013).

2.3 Resposta imunológica às infecções por nematódeos gastrintestinais

O desenvolvimento da resistência de ovinos a nematódeos gastrintestinais pode se manifestar pela diminuição da taxa de estabelecimento larval, pela expulsão de populações de nematódeos adultos, por mudanças na morfologia do parasita adulto, e diminuição da taxa de fecundidade de fêmeas (BALIC et al., 2000a).

Segundo Miller & Harohov (2006), durante as infecções por nematódeos gastrintestinais ocorrem processos imunológicos envolvendo respostas celulares e humorais. Entretanto, Mackinnon et al. (2015) observaram que houve variação de tempo na ativação da resposta imunológica para os

diferentes estágios do ciclo de vida do parasita. Em indivíduos imunocompetentes e expostos continuamente a larvas infectantes, a resistência ao estabelecimento de larvas desenvolve-se mais rápido que a expulsão de nematódeos adultos (BALIC et al., 2000a).

A resposta imune frente às infecções por nematódeos gastrintestinais é caracterizada por uma resposta do tipo 2 (GRENCIS, 2015) e envolve uma gama de células e moléculas que atuam em conjunto para proteger o hospedeiro contra parasitas nas superfícies mucosas (SOROBETEA et al., 2018). Segundo Jacobs et al. (2016), a intensidade da resposta inicial Th2 é o que diferencia raças resistentes das susceptíveis as infecções por nematódeos gastrintestinais.

Enquanto que a maiorias das células no sistema imune se diferenciam e se desenvolvem na medula óssea, as células T sofrem desenvolvimento adicional no timo (LAMB, 2012). Os linfócitos T são divididos de acordo com as citocinas secretadas, dentre os quais se pode destacar os Th1 (T helper 1) e Th2 (T helper 2). Entretanto antes de se diferenciarem em Th1 e Th2, os linfócitos passam de imaturos ou “naives”, para linfócitos Th0. Na transformação de linfócitos Th0 em Th1 e Th2, algumas interleucinas estão envolvidas, dentre elas a interleucina (IL) 12 que estimula a diferenciação em linfócitos Th1 e a IL-4 para os linfócitos Th2 (TIZARD, 2014).

A resposta imune de ovinos previamente infectados por *H. contortus* é caracterizada pela ativação específica de linfócitos T CD4+, por um aumento na taxa de recrutamento de linfócito para a mucosa do abomaso, e por mudanças qualitativas e quantitativas na ativação da população linfocitária nos nódulos linfáticos locais (BALIC et al., 2002).

Os linfócitos T CD4+, quando ativados, secretam citocinas importantes que promovem a produção e diferenciação dos linfócitos B e mastócitos. Linfócitos Th1 desempenham função contra agentes infecciosos (bactérias, vírus, protozoários, por exemplo), produzindo Interferon-gama (INF- **γ**), fator de necrose tumoral (TNF) e IL-2 (TIZARD, 2014). Enquanto que os linfócitos Th2 atuam em processos alérgicos e em infecções helmínticas, secretando IL-4, IL-5, IL-10 e IL-13, estimulando a produção de imunoglobulinas (IgA, IgE e IgG), e reações imunes mediadas por mastócitos e eosinófilos (LAMB, 2012; SOROBETEA et al., 2018).

Segundo Terefe et al. (2007), ovinos resistentes a *H. contortus* demonstraram uma maior produção de interleucinas relacionadas a resposta do tipo Th2, especialmente IL-4, IL-5 e IL-13, no abomaso e em células dos linfonodos abomasais.

Shakya et al. (2009), em estudo comparando a resposta imune do tipo Th2 em caprinos jovens, observaram que os níveis de IFN-y produzidos pelas células do linfonodo abomasal e do linfonodo mesentérico, apresentaram redução substancial quando comparada ao grupo controle não infectado com *H. contortus*, enquanto que as IL-5 aumentaram significativamente.

2.4 Recrutamento celular envolvido nas infecções helmínticas

O sistema imune inato é a primeira linha de defesa em infecções por nematódeos gastrintestinais (MCRAE et al., 2015). Os receptores de reconhecimento de padrões (PRRs) são importantes moléculas da imunidade inata, expressas por várias células dentre elas as células de superfície da mucosa, e envolvidas na detecção e reconhecimento de patógenos (ABBAS et al., 2012).

Em uma infecção por helmintos, tais moléculas identificam os padrões moleculares associados a patógenos (PAMPs) e os padrões celulares associados aos danos ou estresse celular, resultando na iniciação da resposta inflamatória sistêmica, pela indução na produção de citocinas (MAKEPEACE et al., 2012; MCRAE et al., 2015).

Após as larvas de nematódeos gastrintestinais chegarem ao local de predileção e passarem pela barreira do muco, as células epiteliais são as primeiras células do hospedeiro a entrar em contato com o parasita (SOROBETEA et al., 2018). Células epiteliais quimiossensoriais, também chamadas de tuft cells, são apontadas como importantes células no desenvolvimento e regulação da resposta imune Th2 do hospedeiro frente a infecções por helmintos (ZAPH et al., 2007; GERBE et al., 2012; HOWITT et al., 2016; ALBUQUERQUE et al., 2019).

Em relação à imunidade celular, os mastócitos e eosinófilos participam na resposta imune inata à infecção por nematódeos, devido principalmente ao seu papel na produção e liberação de uma variedade de citocínas e quimiocínas (COOPER; ELEFTHERIANOS, 2016). O aumento de eosinófilos,

mastócitos e leucócitos globulares no tecido estão geralmente associados à resistência a infecção parasitária (BALIC et al., 2000a).

2.4.1 Eosinófilos

Os eosinófilos são células polimorfonucleares com grânulos citoplasmáticos eosinofílicos (TIZARD, 2014). Juntamente com os mastócitos, são as primeiras células efetoras que estão associadas às infecções por nematódeos (BALIC et al., 2000b). A mobilização de eosinófilos para o sítio de localização do parasita pode ser induzida pela produção de IL-5 e quimiocinas pelos linfócitos Th2 e mastócitos degranulados (TIZARD, 2014). Os eosinófilos estão envolvidos no desenvolvimento e amplificação da resposta Th2, por meio da sua habilidade de produzir e secretar IL-4 (MAKEPEACE et al., 2012).

Como os helmintos são macropatógenos, para serem eliminados requerem que o sistema imunológico utilize outros mecanismos como por exemplo, a citotoxidade celular dependente de anticorpo (ADCC) mediada pela degraulação dos eosinófilos (MOTRAN et al., 2018), decorrente da opsonização de moléculas de IgE a superfície da larva (HOLT et al., 2015).

Os grânulos destas células possuem mediadores tóxicos e inflamatórios, que associados a mediadores lipídicos e outras partículas ligadas aos receptores de eosinófilos, desencadeiam uma oxidação eosinofílica (TIZARD, 2014). Dentre as proteínas citotóxicas contidas nos grânulos dos eosinófilos, pode-se citar a MBP 1 e 2 (proteína básica principal), peroxidase eosinofílica, proteína catiônica eosinofílica (ECP) e neurotoxina derivada de eosinófillos (EDN), que causam citólise do parasita quando liberadas no espaço extracelular do hospedeiro (MOTRAN et al., 2018).

Terefe et al. (2007) observaram em estudo realizado *in vitro*, que os eosinófilos mostraram-se capazes de lesarem larvas de *H. contortus*. Estas células desempenham importante papel na liberação de citocinas e mediadores contra os parasitas (GONZÁLEZ et al., 2011). Ainda, segundo Balic et al. (2002), em infecção por *H. contortus*, a infiltração de eosinófilos é induzida pela presença de larvas no tecido.

Os linfócitos T CD4+ são necessários à ativação e recrutamento de eosinófilos para a expressão de resistência genética. Contudo, o recrutamento

de eosinófilos no tecido abomasal é mais efetivo e rápido, em ovinos previamente infectados (BALIC et al., 2000b).

Uma eosinofilia significante é observada durante a infecção por nematódeo gastrintestinal (MEEUSEN et al., 2005). Além disso, é observado um aumento nos níveis de IL-5, citocina responsável pela geração e recrutamento de eosinófilos (ELSE; FINKELMAN, 1998).

2.4.2 Mastócitos e Leucócitos Globulares

Os mastócitos são células inflamatórias imunes inatas da linhagem mieloide (RYAN; OGHUMU, 2019), que possuem numerosos e pequenos grânulos intensamente basofílicos, de núcleo pouco evidente (HUNTLEY et al., 1984), encontradas principalmente no tecido conjuntivo e nas mucosas (TIZARD, 2014).

Segundo Rothwell (1989), mastócitos e leucócitos globulares (LGs) são encontrados em grande quantidade no trato gastrintestinal durante infecções parasitárias, especialmente em animais previamente infectados. Na resposta ao parasitismo, as moléculas de IgE aderidas aos mastócitos pelos receptores FcƐRI, ligam-se ao parasita, favorecendo o desencadeamento da degranulação destas células (TIZARD, 2014).

Os mastócitos desempenham um papel importante na modulação da resposta imune Th2 caracterizada pela produção e liberação de citocinas IL-4, IL-5 e IL-13 (GONZALEZ et al., 2018). Além disso, a degranulação dos mastócitos estimula o aumento da produção de mucina, contração da musculatura lisa do trato gastrintestinal associado ao aumento do peristaltismo e criação de um ambiente tóxico para os helmintos (MUKAI et al., 2016).

A degranulação dos mastócitos resulta em formação dos leucócitos globulares (LAMB, 2012). Em ruminantes, os LGs são derivados de mastócitos da mucosa, no entanto, eles contêm menos proteoglicano e monoamina que os mastócitos, sendo considerados mastócitos parcialmente degranulados (HUNTLEY et al., 1984).

Os LGs podem ser encontrados no epitélio de membranas mucosas de muitos vertebrados, e estão associados com reações imunoinflamatórias decorrentes de infecções parasitárias. Em outros processos infecciosos, o seu envolvimento é de menor importância (AKPAVIE; PIRIE, 1989).

Em relação a sua morfologia, os LGs são células com núcleo geralmente ovoide ou esférico e com membranas nucleares bem distintas. Quanto ao seu citoplasma, o mesmo apresenta-se retraído e com a presença de grânulos acidofílicos, os quais se apresentam com números e tamanhos variados (AKPAVIE; PIRIE, 1989).

Os LGs juntamente com os mastócitos, são responsáveis pela resposta rápida de rejeição de larvas encontradas nos tecidos (MEEUSEN et al., 2005; KEMP et al., 2009).

2.5 Imunoglobulinas

Infecções por nematódeos gastrintestinais são tipicamente acompanhadas pela elevação da concentração de imunoglobulinas e liberação de citocinas (BALIC et al., 2000b).

Os linfócitos Th2 ativados pela infecção por *H. contortus,* promoverão a ativação de linfócitos B que se diferenciarão em plasmócitos (MCRAE et al., 2015), e estes serão responsáveis pela produção de glicoproteínas, denominadas imunoglobulinas (TEREFE et al., 2007; TIZARD, 2014)).

Bowdridge et al. (2013) observaram que em resposta a infecção por *H. contortus*, ovinos adultos St. Croix apresentaram uma maior produção de IgA, sendo capazes de produzirem e manterem uma potente resposta humoral específica, quando comparadas aos animais lanados. A IgA é responsável por neutralizar as enzimas metabólicas e interfere na habilidade do parasita em se alimentar (VARELLAL; FORTTE, 2001).

Hernández et al. (2016) observaram que a IgA da mucosa do abomaso (mIgA) demonstrou ser importante regulador da resposta do hospedeiro em infecções por *Haemonchus* e *Teladorsagia*, onde mecanismos como anticorpos, citotoxicidade celular e inibição enzimática podem mediar a resistência.

Cordeiros da raça Santa Inês naturalmente infectados com nematódeos gastrintestinais apresentaram maiores níveis de IgG e IgA, quando comparados a cordeiros Ile de France. Contudo quando comparado os níveis de IgE no plasma sanguíneo, não foi observado diferenças entre as raças (ALBUQUERQUE et al., 2019).

2.6 Interleucinas

Segundo Sorobetea et al. (2018) uma vez que um nematódeo é detectado pelas células epiteliais ou outras células da superfície da mucosa, um sinal é transmitido às células do sistema imune inato, de modo que uma resposta inflamatória é iniciada, pela produção e liberação de interleucinas provenientes de células linfoides inatas do tipo 2, tais quais IL-4, IL-5, IL-9, IL-10 e IL-13.

No Quadro 1 estão apresentadas algumas das principais interleucinas da resposta Th2 envolvidas em infecções parasitárias.

Quadro 1 – Interleucinas envolvidas em infecções parasitárias.

Interleucina	Principal fonte	Funções principais	Referência
IL-4	Mastócitos, Linfócitos Th2 e Linfócitos B	Induzir a produção de IgE e aumento da expressão de receptores para IgE na superfície de eosinófilos.	Balic et al., 2000a; Lamb, 2012; Jacobs et al., 2016.
IL-5	Mastócitos e Linfócitos Th2	Auxiliar na diferenciação e recrutamento de eosinófilos, e na participação destes na expressão de receptores para o componente secretor da IgA secretora.	Balic et al., 2000a; Lamb, 2012; Mackinnon et al., 2015; Jacobs et al., 2016.
IL-9	Linfócitos Th2 e Th9	Atua principalmente como um fator de maturação de mastócitos, e pode ser necessária para o aumento da produção de IL-5 e IL-13.	Lamb, 2012; Licona-Limon et al., 2013;
IL-10	Macrófagos, Células Dendriticas, Linfócitos T e B	Sua principal função está relacionada à inibição de Th1 e de outras interleucinas, como por exemplo, IFN-**γ**, IL-2 e IL-12.	Lamb, 2012; Tizard, 2014.
IL-13	Linfócitos Th2	Atua mediando uma proteção contra alguns estágios larvais, além de induzir a produção de IgG, além de estar envolvida na diferenciação, recrutamento e degranulação de eosinófilos no tecido, produção de muco, e inibição da resposta Th1.	Zurawki; Vries, 1994; Else; Finkelman, 1998; Lamb, 2012; Sorobetea et al., 2018.

As interleucinas 4, 9 e 13 também estão envolvidas no estímulo a contração de células da musculatura lisa, aumentando o peristaltismo do trato gastrintestinal (SOROBETEA et al., 2018).

A IFN-γ está relacionada à infecção crônica, pois reduz a resistência a nematódeos gastrintestinais, e sua produção está associada à ativação de células Th1 (PATRA et al., 2016). Níveis elevados de IFN-γ comprometeriam a capacidade do hospedeiro de debelar uma infecção por nematódeo (PATRA et al., 2016).

Tem sido relatado que em comparação com ovinos resistentes, ovinos suscetíveis produzem relativamente mais IFN-γ e menos anticorpos séricos específicos contra parasitas; também apresentam menor número de eosinófilos sanguíneos na mucosa do abomaso (TEREFE et al., 2007).

Adotamos como hipótese que, cordeiros lactentes da raça Santa Inês infectados com *Haemonchus contortus* tendem a elaborar uma resposta imune mais rápida e eficaz quando comparados a cordeiros da raça Ile de France, sendo esta capaz de controlar a infecção por *H. contortus.*

3 OBJETIVOS

3.1 Objetivo geral

Avaliar as variáveis hematológicas, proteínas plasmáticas totais, variáveis parasitológicas, resposta celular local e imunoglobulinas envolvidas na resposta imune de cordeiros lactentes Santa Inês e Ile de France durante a infecção experimental por *H. contortus.*

3.2 Objetivos específicos

- Avaliar as alterações hematológicas decorrentes da infecção por *H. contortus*;
- Quantificar as células inflamatórias abomasais e relacioná-las com as variáveis parasitológicas de cada raça;
- Distinguir os mecanismos imunes inatos e adaptativos envolvidos na resistência de cordeiros a infecção por *H. contortus*.

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CAPÍTULO II – Immune response of Santa Inês and Ile de France suckling lambs artificially infected with *Haemonchus contortus*

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Immune response of Santa Inês and Ile de France suckling lambs artificially infected with *Haemonchus contortus*

Abstract

This trial aimed to evaluate the immune response of Santa Inês (SI) and Ile de France (IF) suckling lambs serially infected with *Haemonchus contortus*. Fourteen SI lambs and 12 IF lambs were randomized in four groups: infected SI (n=8), non-infected SI (n=6), infected IF (n=8) and non-infected IF (n=4). Lambs of infected groups were submitted to 27 infections, conducted every two days, from 14 to 68 days of age, and each lamb received a total of 5400 *H. contortus* infective larvae (L3). Blood and faeces samplings were collected every six days, and at 68 days of age, lambs were euthanized for recovering abomasal parasites, and collection of mucus, tissue sample and abomasal lymph nodes. SI lambs had the lowest eggs per gram of faeces (EPG) means in all samplings, and from 50 days old, three SI lambs stopped shedding eggs on faces. Those three SI lambs had L3 establishment rates of 0.37%, 0.02% and 0.78%. L3 establishment rate average for IF lambs was 22.9% and 11.1% for SI lambs. Infected SI lambs had higher abomasal lymph node weight than the other groups. Eosinophils, mast cells and globule leukocytes counts in the fundic and pyloric regions of the abomasums were significantly higher in infected SI lambs than infected IF lambs. There was no relation among immunoglobulin A and G anti-L3 and infection degree of animals. SI lambs in suckling period are more resistant than IF lambs, and that mechanisms involving local cellular response may be intended to confer resistance.

Keywords: Helminthiasis, Immune response, Gastrointestinal parasites, Sheep farming

1. Introduction

Occurrence of parasitic diseases in sheep are common, being an important cause of mortality, especially in young animals (Amarante and Amarante, 2016). *Haemonchus contortus* is the main gastrointestinal parasite of small ruminants in tropical and subtropical areas worldwide (Amarante, 2014),

and haemoncosis prophylaxis based on the use of anthelmintic products has been shown to be unsustainable due to the emergence of nematode populations resistant to all classes of drugs available for treatment (Almeida et al., 2010; Albuquerque et al., 2017).

Animal immunity plays an important role in resistance to worms, and individual factors such as age, breed and physiological condition may regulate host response against parasites (Amarante, 2014). In this way, identifying more resistant breeds and selecting resistant individuals or herds may increase the worm control efficiency.

Differences between sheep breeds regarding their susceptibility to infections by gastrointestinal nematodes have been reported (Amarante et al., 2004; Rocha et al., 2005; Amarante et al., 2009; Shakya et al., 2009). Santa Inês (SI) is a native breed from northeastern Brazil, considered more resistant to *H. contortus* infection when compared to Ile de France (IF) sheep (Amarante et al., 2004; Rocha et al., 2005; Amarante et al., 2009). Santa Inês lambs, after weaning, showed a higher resistance to gastrointestinal nematodes when compared to lambs of European breeds (Amarante et al., 2004; Albuquerque et al., 2019).

There are few studies that evaluated the immune response of suckling lambs against parasitic infections by gastrointestinal nematodes. Bahirathan et al. (1996) observed that Gulf Coast Native lambs have the ability to limit *H. contortus* infections by eliminating parasites, while Suffolk lambs develop haemonchosis and required anthelmintic treatment. Rocha et al. (2005) observed that SI lambs had higher eosinophil counts than IF suckling lambs.

In helminth infections, a type 2 (Th2) immune response is triggered, responsible for mobilization, activation and cellular recruitment to the parasite-host interaction site to promote parasite expulsion and control infection (Inclan-Rico and Syracuse, 2018). Despite the various studies that have been evaluating the differences between resistant and susceptible sheep to *H. contortus* infection (Bricarello et al., 2005; Shakya et al., 2011; Patra et al., 2016; Jacobs et al., 2016), the immunological mechanisms that confer greater resistance to the animals have not been completely elucidated yet.

Thus, in order to understand and evaluate the parameters and variables that can better elucidate the differences in immunological mechanisms related

to the responses of resistant and susceptible breeds to *H. contortus* infection, the objective of this study was to evaluate the hematological, parasitological, local cellular response and immunoglobulin variables involved in the immune response of Santa Inês and Ile de France suckling lambs experimentally infected with *H. contortus*.

2. Material and Methods

The experiment was conducted in the experimental area of the Parasitology Department of the Bioscience Institute, UNESP Botucatu Campus. All the procedures involving animals in this study were conducted in accordance with the local Ethics Committee on Animal Use (protocol number 0118/2018, FMVZ-UNESP).

2.1. The production of infective larvae (L3) of *Haemonchus contortus*

An *H. contortus* isolate susceptible to anthelmintics (Echevarria et al., 1991) stored in liquid nitrogen, was thawed to infect two donor lambs. The worm-free status of donors was confirmed by several faecal examinations. Each lamb was infected orally with 5500 *H. contortus* L3 in a single dose.

Donor lambs faeces were collected individually into collecting plastic bags twice a day, and then, each sample was used to perform faecal cultures, according to Ueno and Gonçalves (1998), for the production of L3 to be used in the infection of the experimental lambs.

During the trial, donor lambs were housed indoors, fed *ad libitum* with *Cynodon* spp. hay free of nematode-infective larvae. They also had free access to potable water and mineral salt (OvinoFós Tortuga®).

2.2. Experimental Design

Ile de France and Santa Inês pregnant ewes in the last third of gestation were acquired from two farms located in the rural area of São Manuel and Pardinho, respectively, both of them in São Paulo state, Brazil.

The Ile de France and Santa Inês female sheep after arriving in the experimental area, they were weighted, identified and then housed into four pens (eight sheep in each). Faecal samples were collected for EPG counting and faecal cultures.

After the naïve lambs were born, each ewe and its respective lamb were allocated into individual concrete floor pens with feeders and water fountains. Each pen was cleaned daily with water using a high-pressure pump.

Naïve Santa Inês (n=14) and Ile de France (n=12) lambs, 14 days old, were divided and allocated into four groups (Figure 1). The criterion for distribution of the lambs into the groups was the order of birth. The fist male or female born of each breed was allocated in the infected group; the second female or male born was allotted in the control group, and then consecutively until all groups be composed. It was used a 2 x 2 factorial design, with two Infection status (Infected and Control) and two sheep breeds (Santa Inês and Ile de France).

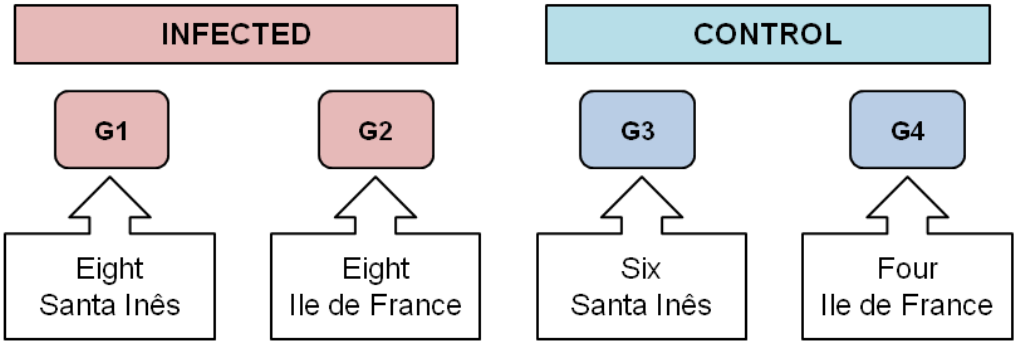

Figure 1. Experimental design factorial 2x2.

2.3. Food and sanitary management

Ewes and their lambs were fed with *Cynodon* spp. hay, free of nematode-infective larvae, and received a daily dietary supplement with 18% of crude protein (CP) (Nutrição Animal Coopermota®). Additionally, the animals had free access to tap water and mineral salt (OvinoFós Tortuga®). During all the study, it was offered 2.5% of dietary supplement based (on the body weight (BW) of the ewes (1.25% in the morning and 1.25% in the afternoon).

2.4. Experimental infection and euthanasia

Naïve lambs of the infected groups were infected from 14 days until 66 days old, with *H. contortus* L3 through a serial infection protocol with a progressive increase in L3 numbers. Control groups were kept worm-free during the whole experiment.

Experimental infections were carried out each two days, being divided into three steps, differing in the number of L3 administered at each stage. In total, it was conducted 27 direct artificial infections (Figure 2).

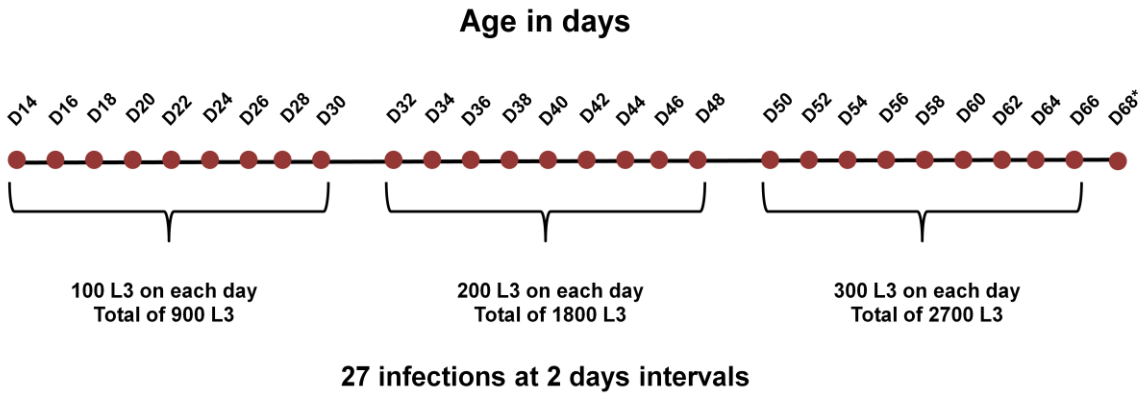

Figure 2. Timeline with the protocol of the experimental infections of the lambs with infective larvae (L3) of *Haemonchus contortus.* *All lambs were euthanized at 68 days of age.

L3 were administered directly into the lamb oral cavities. At the end of the infections, each lamb had received 5400 L3. Two days after the last infection (68 days old), lambs were euthanatized under sedation with an association of ketamine (4.5 mg/kg of BW, Cetamin®, Syntec) and xylazine, (0.05 mg/kg of BW, Calmiun®, Agener União), administered intravenously.

Immediately after sedation and confirmation of lack of awareness, it was performed exsanguination through jugular and carotid sections, causing death by acute hypovolaemia.

2.5. Sample collection and examination

Samples (faeces and blood) were collected each six days for laboratory analysis: the first on 14th day of life, and the last on 68th day of life, previously to the euthanasia process.

2.5.1. Faeces

Faecal samples were collected directly from the rectum of animals and conditioned in polyethylene bags previously identified and kept refrigerated until

the moment of processing. Faecal egg counts (FEC) determination was performed using a modified McMaster technique in which each worm egg counted represented 100 eggs per gram (EPG) of faeces (Ueno and Gonçalves, 1998). When EPG was negative, a more sensitive technique, Willis-Mollay simple flotation was performed (Willis, 1921).

Additionally, composite culture was performed on the last sampling to confirm that the lambs were not infected by other nematodes, and the L3s obtained from cultures of infected lambs were identified according to descriptions of Ueno and Gonçalves (1998).

2.5.2. Blood

Blood samples were collected by jugular vein puncture into plain tubes containing anticoagulant (Vacutainer® K2 EDTA 7.2mg, BD, Brazil). Haematological exams performed were packed cell volume (PCV), total plasma protein concentrations (TPP), red blood cell count (RBC), white blood cell count (WBC) and blood smear for differential leukocyte count (Schalm and Jain, 1986; Weiss and Wardrop, 2010; Weiser, 2012).

PCV was determined by microhematocrit centrifugation (5 min/ 3000 rpm), and total plasma protein concentrations (TPP) were estimated using a refractometer (Refractometer SPR-N, Atago).

RBC count was performed manually in a Neubauer chamber using a 1:201 of blood (20µL of blood and 4000µL of saline solution 0.9% NaCl). The dilution was mixed continuously for two minutes by mechanical shaker (Schalm and Jain, 1986).

WBC count was performed manually in a Neubauer chamber using a 1:20 dilution of blood (20µL of blood and 380µL of Turk`s solution) (Schalm and Jain, 1986).

For differential leukocyte count, a blood smear was stained using a Diff Quick stain (Panoptico Rápido, Laborclin, PR, Brazil). In addition, 100 leukocytes were differentiated and results were presented in absolute values (Schalm and Jain, 1986; Reagan et al., 2011).

Blood in the tubes with EDTA was centrifuged (15 min/ 2500 rpm at 4 ºC), to allow plasma. Subsequently, aliquots of samples were stored at -80 ºC

until immunoglobulin measurement by Enzyme-linked immunosorbent assay (ELISA).

2.5.3. Tissue sampling collection

After each lamb euthanasia, its abomasum was removed, opened along the greater curvature and the contents collected in a container for worm recovering.

Tissue samples of the abomasums (fundic and pyloric region) were collected (2cm x 2cm) in duplicate. Abomasal lymph nodes were also collected and weighed. Part of the tissue samples (including abomasal lymph nodes) were set into buffered formalin solution (4%) to be fixed for eight hours. Then formalin solution was removed and 70% alcohol was added. Tissue samples were kept at 4°C until be embedded in paraffin wax for routine histological procedures. Tissue sections of abomasums were cut to 5 µm thick and mounted on glass. Eosinophils and globule leukocytes were counted on Haematoxylin and Eosin (H&E) stained sections, whereas mast cells was counted on sections stained with 1% toluidine blue (Balic et al., 2000b). All cells were counted in thirty randomly selected fields of view per animal in a 0.01 mm^2 area at 1000× magnification (adapted from Balic et al., 2002). The counts were expressed as cells/mm^2 tissue surface.

2.5.4. Worm burden

After collecting abomasum contents into containers, the abomasums was soaked in 320 mL of saline solution at 37 °C for 6 h. A 10% aliquot of the contents and 100% of the digested material were collected and set in different containers, then they were frozen at -20 °C. The remaining 90% aliquots of contents were preserved frozen at -20 °C as backup. All nematodes presented in 10% aliquots and 100% of the digested material were counted and identified according to Ueno and Gonçalves (1998).

2.5.5. Plasma and mucus samples for Immunoglobulin determination

Plasma samples were stored at -80 °C until use in immunoglobulin G (IgG) measurements against antigens of *H. contortus* L3. The protocol applied to determine the parasite-specific serum IgG levels was previously described by

Silva et al. (2012). Some modifications were done: plates were coated with 2 µg of antigen/mL; each wash was done three times, rotating through 180° and re-washing three more times; the negative control (NC) sample used was from a worm free animal, as previously described by Santos et al. (2014); and positive control (PC) was a plasma sample from a naturally trichostrongylid infected lamb described by Albuquerque et al. (2019). The production of *H. contortus* L3 antigens was previously described by Amarante et al. (2009). Plasma samples were diluted in PBSGT (1:400) and conjugated was diluted at 1:40000. Results were presented in optical density (OD).

Mucus was taken from the abomasums mucosa to determine the levels of IgA antibody. After opening the organ, a piece (2cm x 2cm) was collected from the abomasum (fundic and pyloric region) and quickly stored at -20 °C. Mucus was collected by slightly scraping the mucosal surface with a glass slide. Scrapings were collected into a Falcon tubes which were kept on ice. Three mL of cold phosphate-buffered saline (PBS) supplemented with protease inhibitors (1 tablet in 50 mL ultrapure water; Complete Mini Solution1®, Roche, USA) were added to each sample (1:3 dilution). The tubes were manually shaken for 1 h at 4 °C and centrifuged (3000 *g*) for 30 min at 4 °C. Supernatant was spun down for 30 min at 4 °C and 15,000 *g*.

IgA levels in abomasal mucus were tested against *H. contortus* L3 as described by Silva et al. (2012), but with a few differences: the plates were coated with 2 µg of antigen/ml; each wash was done three times, rotating through 180° and re-washing three more times; mucus samples were diluted in PBSGT (1:20) and rabbit anti-sheep IgA peroxidase-conjugated antibody was diluted at 1:80000. The production of *H. contortus* L3 antigens was previously described by Amarante et al. (2009). The results were expressed as the sample percentage of OD minus blank OD (Kanobana et al., 2001).

2.6. Statistical analysis

Data were evaluated in a normality test (Shapiro-Wilk test) and when necessary they were transformed to log10(x + 1) prior to analysis. Furthermore, data were analyzed by analysis of variance through the General Linear Model (GLM) for the variables measured just once and for variables determined at several time points, using the Statistical Analysis System, version 9.2 (SAS

Institute, Inc., Cary, NC, USA). Means were compared by Tukey's test at a 5% significance level, and only significant interactions were reported in the results. Means are presented in the results as the arithmetic means (± standard error). Additionally, we assessed differences between the more and the less resistant infected Santa Inês suckling lambs by a univariate analysis using the t test at a 5% significance level.

3. Results

Lambs from control groups did not shed egg in faeces all over the experiment and L3 was not recovered from their faecal cultures. The *Haemonchus* EPG means of the infected groups are shown in Table 1. *H. contortus* eggs were detected for the first time 24 days after the first infection, when lambs were 38 days of age.

There was a significant time effect on the EPG counting (P = 0.0189). FEC mean increased in both infected groups over the time (Figure 3), however, such increase was more pronounced in the infected IF suckling lambs with significant difference between (P < 0.05) group means on the last two samplings.

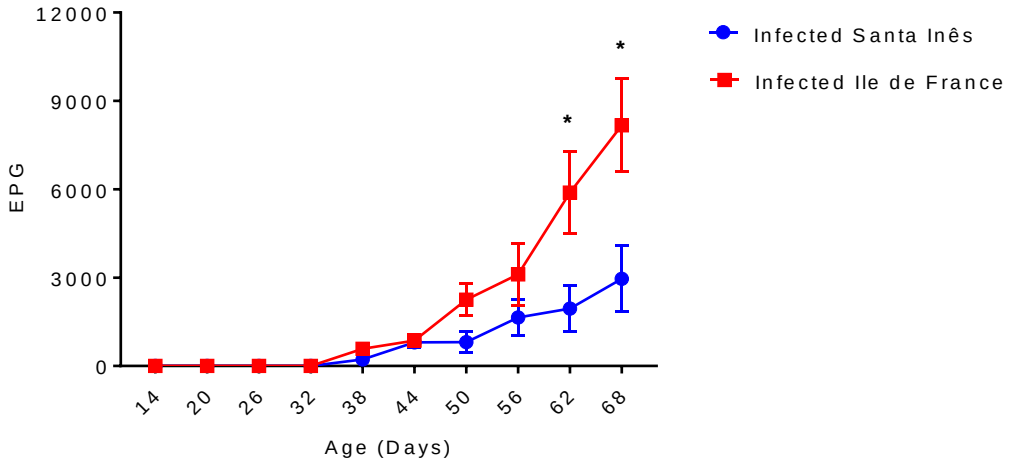

Figure 3. Eggs per gram of faeces (EPG) of the Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus*. Bar: standard error. * Means statistically different (P < 0.05).

Over the experiment, three lambs of the infected SI group (animals 1, 2 and 4) stopped shedding eggs (Figure 4). EPG counting of animal 1 was negative at 38 days old and on simple flotation at 56 days old. Animal 2 was negative on EPG and on simple flotation at 68 days old and animal 4 on EPG at 50 days old and negative on simple flotation at 62 days old.

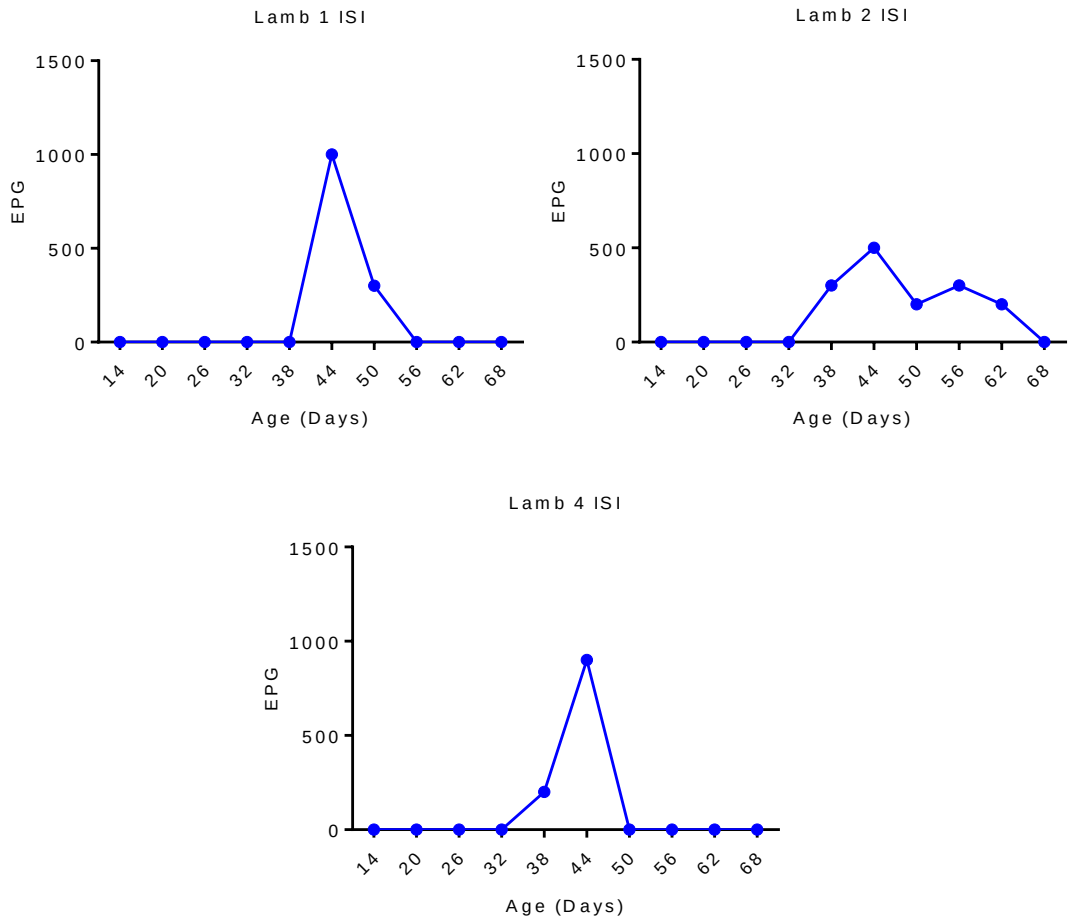

Figure 4. EPG counting of the most resistant Santa Inês suckling lambs (number 1, 2 and 4) experimentally infected with *Haemonchus contortus* L3.

All IIF lambs were shedding eggs at the end of the trial. However, lamb 23 was more resistant to *H. contortus* infection, when compared to the other IF suckling lambs. Such lamb presented patent period later, at 62 and 68 days old.

The identification of third stage larvae from cultures, confirmed the infection only by *H. contortus*.

Lambs from non-infected (control) groups had no worms in their abomasums contents at the end of the study. Ile de France lambs presented

higher number of *H. contortus* parasites than Santa Inês lambs, with regards to all stages of development (Table 2 and Figure 5). However, there was significant differences only in the female late L4 (P = 0.0421), female early L5 (P = 0.0067), male early L5 (P = 0.0286) and adult male (P = 0.0441) means. IF had higher total worm burden than SI (P = 0.0443).

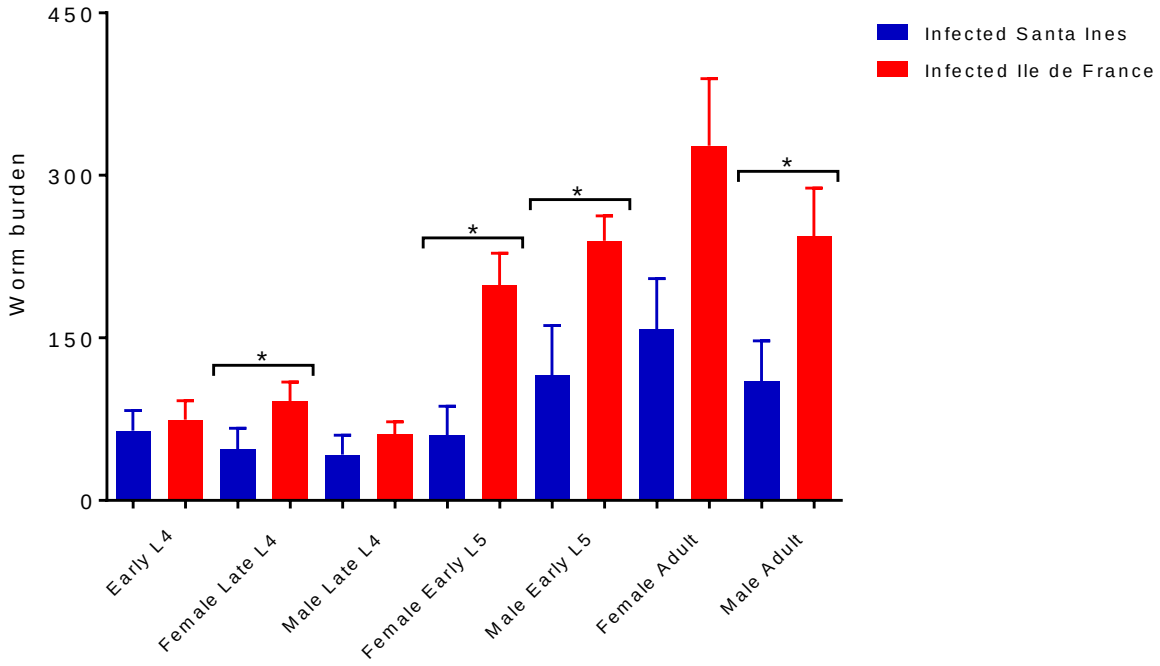

Figure 5. *Haemonchus contortus* averages (± standard error) of Ile de France and Santa Ines suckling lambs experimentally infected. * Means statistically different (P < 0.05).

Total Plasma Protein (TPP) means are presented in Table 3. It was not observed any statistical difference among experimental groups. Additionally, there was time effect (P = 0.0153) on the TPP level.

Over the experiment, TPP averages (Figure 6) remained stable and most of them below the normal reference (6.0 to 7.5 g/dL) range for sheep (Schalm and Jain, 1986).

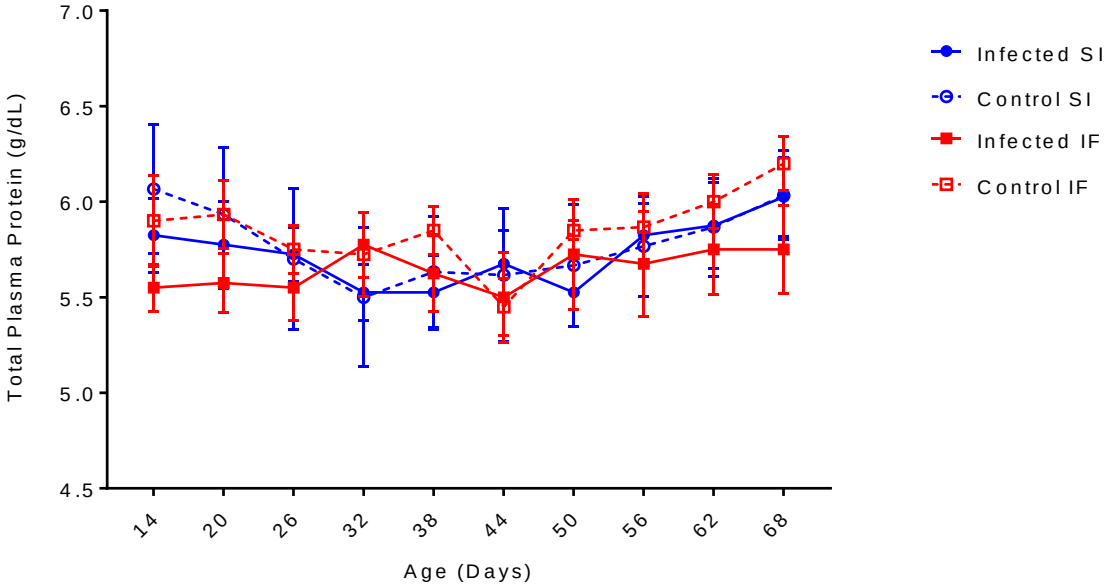

Figure 6. Total Plasma Protein averages (± standard error) of Ile de France (IF) and Santa Inês (SI) suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

There was time effect ($P < 0.0001$) on PCV values (Table 4). Breed had a significant effect on PCV values from day 14 to 44 days of age, with Santa Inês lambs showing the lowest PCV values (Figure 7).

In addition, there was an infection status effect ($P < 0.05$) at 56 days of age (42 days after first infection) with control lambs showing the highest averages.

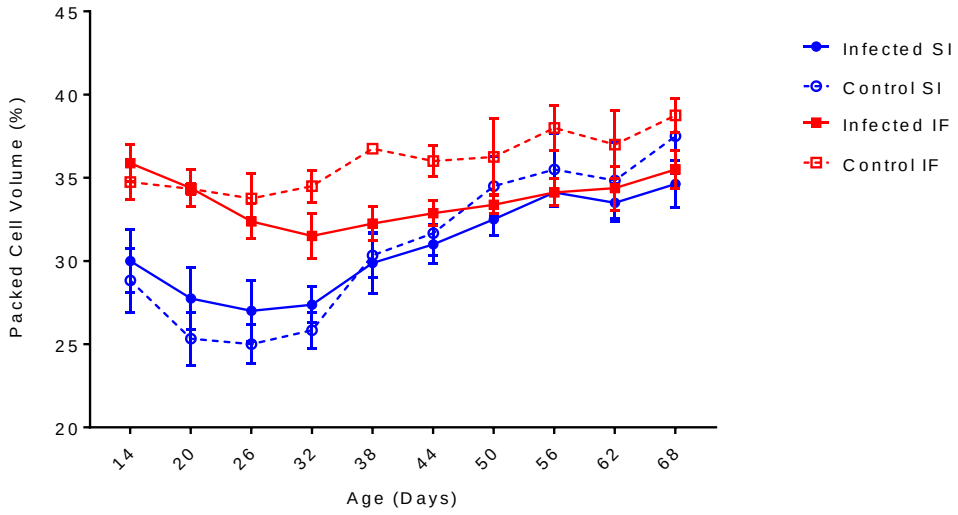

Figure 7. Packed Cell Volume averages (± standard error) of Ile de France (IF) and Santa Inês (SI) suckling lambs artificially infected with *Haemonchus*

contortus and non-infected control. There was breed effect (P<0.05) from 14 to 44 days of age and infection effect (P<0.05) at 56 days of age.

There was a breed effect on Red Blood Cell (RBC) counting at 20, 44, 50, 56, 62 and 68 days old, with Santa Inês lambs presenting the highest averages from 44 to 68 days of age. RBC trend was similar in both breeds, and the counting increased slightly as the suckling lambs got older (Figure 8). There was Time effect (P < 0.0001) and a significant Time x Breed interaction (P < 0.0001) on the RBC counting with SI lambs showing higher averages in the last four samplings.

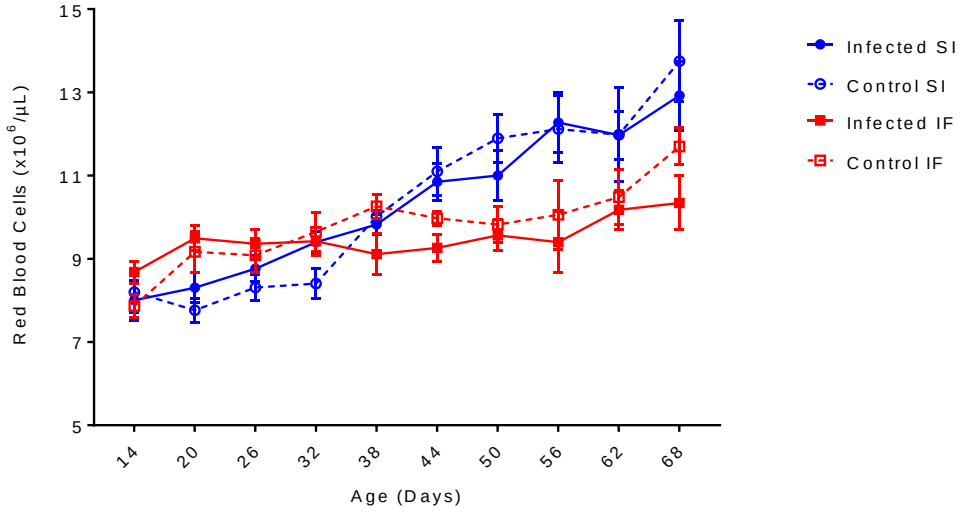

Figure 8. Red Blood Cell averages (± standard error) of Ile de France (IF) and Santa Inês (SI) suckling lambs artificially infected with *Haemonchus contortus* and non-infected control.

Lambs from ISI presented a higher number of RBC when compared to IIF lambs at 44 (P = 0.017) and 56 (P = 0.0346) days old. However RBC values of infected groups were within the normal reference (8.0 to 16.0 x 10^6/ µL) range for the species (Schalm and Jain, 1986).

White Blood Cell (WBC) counts of infected and non-infected (control) groups are shown in Figure 9. There was not any significant difference among the groups all over the experiment. Breed influence (P = 0.0182) occurred only at 32 days old (18 days after the first infection) where Ile de France lambs showing the highest counts. All values found for WBC were within the normal

reference range for the species. There was a significant Time effect (P < 0.0001), Time x Breed (P = 0.0479) and Time x Breed x Infection status (P = 0.0326) interactions on the WBC counting.

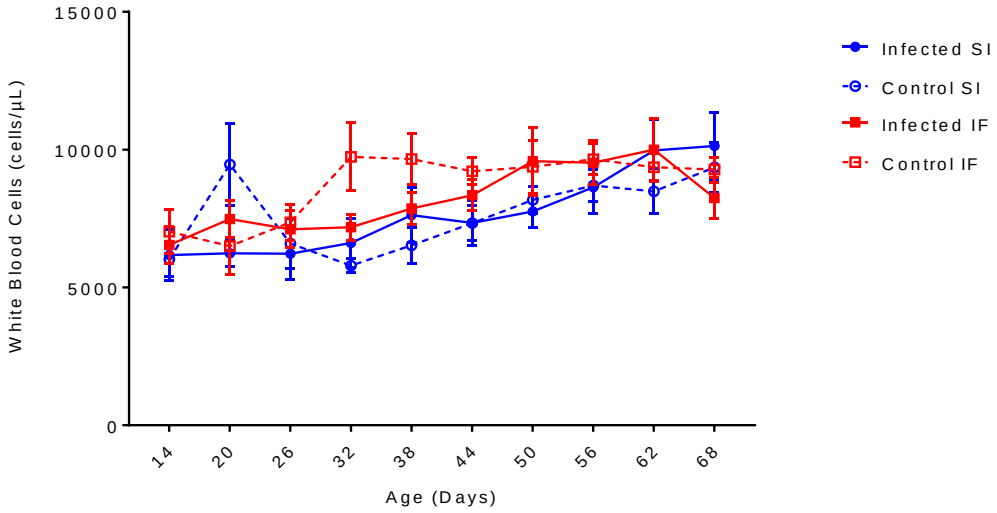

Figure 9. White Blood Cell averages (± standard error) of Ile de France (IF) and Santa Inês (SI) suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Differential leukocyte counting is presented in absolute numbers. With regards to eosinophils means, there was a breed effect (P = 0.0037) on eosinophils counting at 20 days old (6 days post-first infection), with a significant difference only between infected and non-infected lambs of Santa Inês breed (P = 0.0037). The same trend was observed at 56 days old, when lambs from ISI presented higher number of eosinophils than non-infected SI lambs (P = 0.0242). There was a significant Breed x Infection status interaction on eosinophils counting at 38, 50, 56 and 68 days old. Additionally, ISI lambs presented higher number (P = 0.0256) of eosinophils at 68 days old, when compared to IIF lambs.

Even though significant differences between the infected groups, with SI lambs presenting higher number of eosinophils when compared to IF lambs, all the values obtained were within the reference interval for the species (Figure 10).

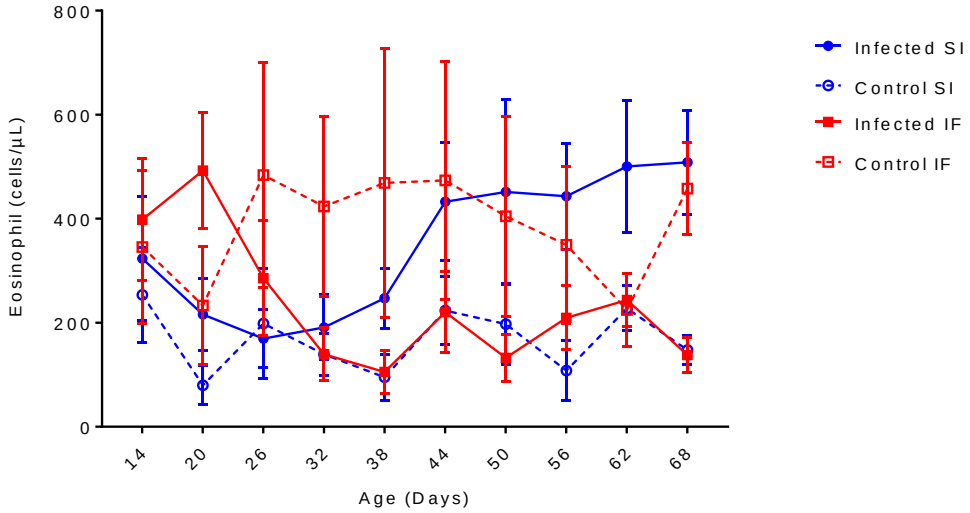

Figure 10. Eosinophil averages (± standard error) of Ile de France (IF) and Santa Inês (SI) suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

There was an infection status effect on neutrophils counting (*P* = 0.0481) at 20 days old (Figure 11). ISI lambs had higher number of neutrophils than non-infected SI lambs (*P* = 0.0376). There was a significant Time x Breed interaction (*P* = 0.0125) on the neutrophil counting.

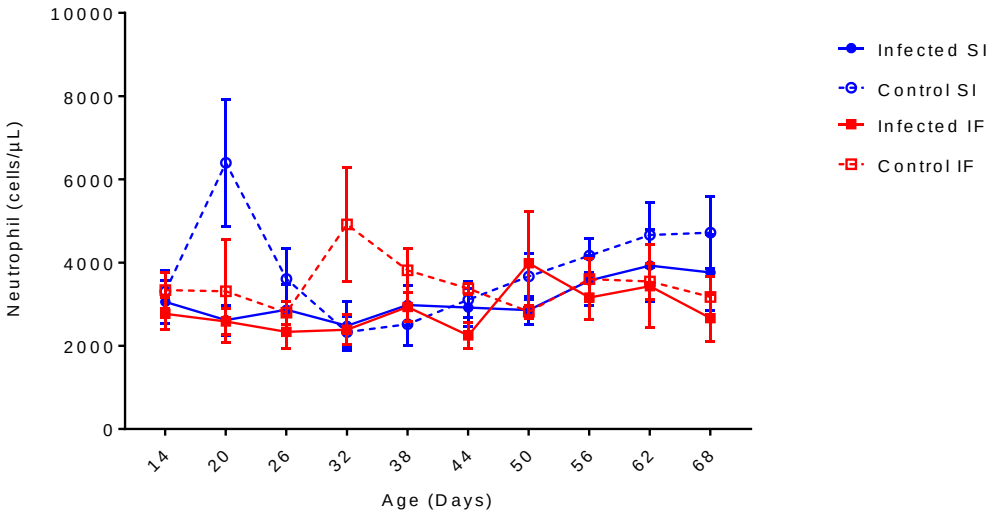

Figure 11. Neutrophil averages (± standard error) of Ile de France (IF) and Santa Inês (SI) suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Regarding to basophils counting (Figure 12), ISI lambs had higher values when compared to IIF, furthermore there was a significant difference between infected groups only at 20 days old (*P* = 0.0001). Such increased in basophils number coincided with the increase in eosinophils values at 20 days old as well.

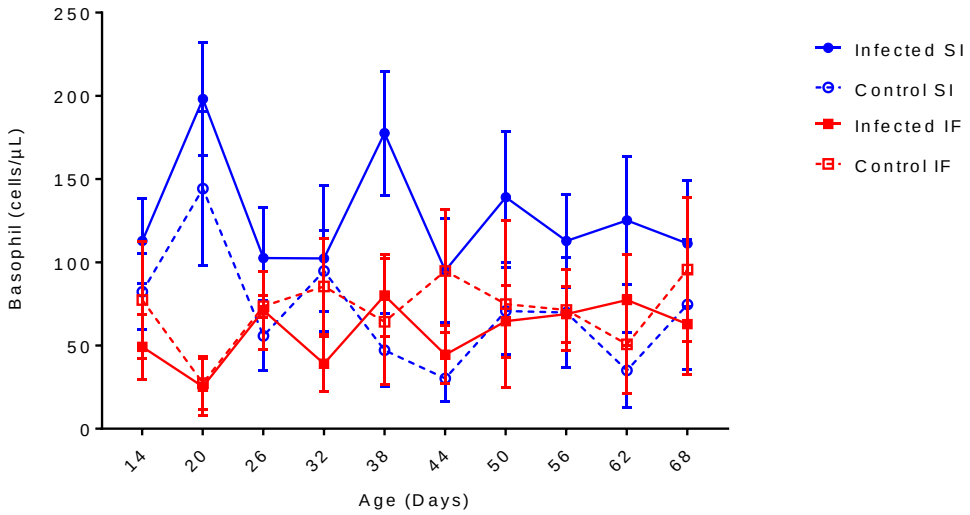

Figure 12. Basophil averages (± standard error) of Ile de France (IF) and Santa Inês (SI) suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

There was time effect (*P* < 0.0001) on the lymphocytes values (Figure 13). IIF lambs presented higher lymphocytes numbers than ISI at 26 and 44 days old (*P* = 0.0117 and *P* = 0.0043, respectively). There was breed effect on lymphocyte counting at 26, 44, 50, 56 and 62 days old, and infection status effect at 20 and 62 days old. Significant Breed x Infection status effect was found only at 68 days old. Infected SI presented a higher lymphocyte count than non-infected SI lambs (*P* = 0.0001) at 62 days old (Figure 13).

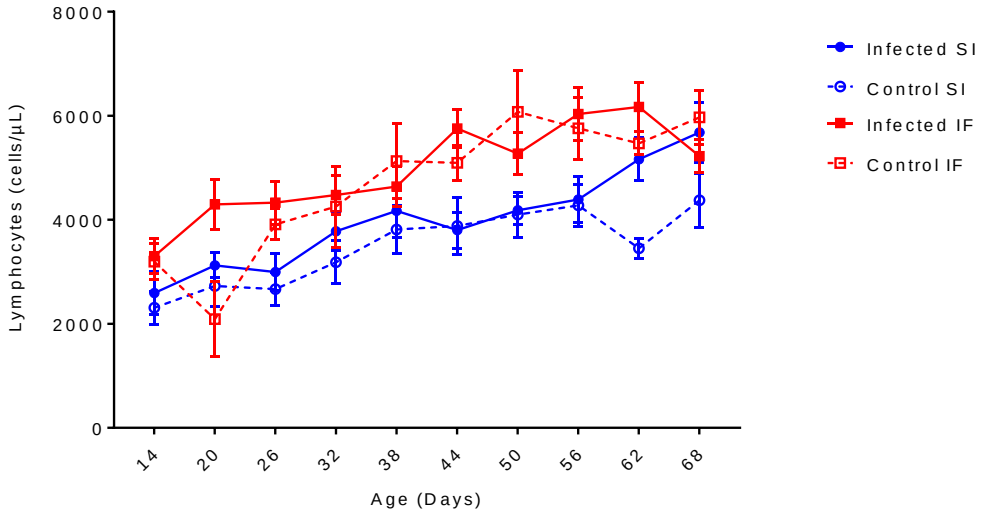

Figure 13. Lymphocyte averages (± standard error) of Ile de France (IF) and Santa Inês (SI) suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

With regards to monocytes means (Figure 14), there was an infection status effect at 32 days old (P = 0.0321). A significant Breed x Infection interaction, and a breed effect (P = 0.002) were observed at 50 days old. IIF presented higher number of monocytes than non-infected IF lambs at 50 days old (P = 0.0051).

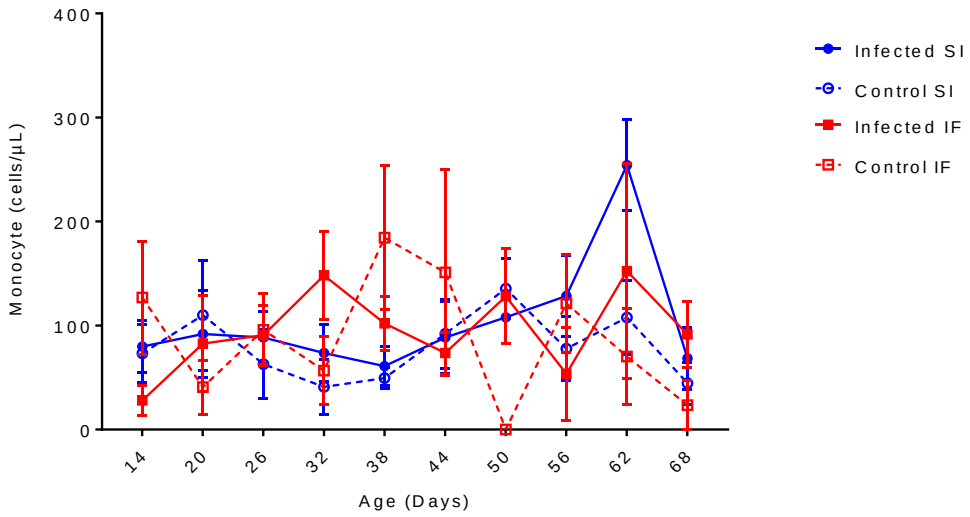

Figure 14. Monocyte averages (± standard error) of Ile de France (IF) and Santa Inês (SI) suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Regarding IgG against antigens of *H. contortus* L3 (Figure 15), there was time effect ($P < 0.0001$) during the serial infection period. Although ISI lambs had higher IgG levels than non-infected SI lambs IgG against *H. contortus* L3, there was no statistical difference between the groups.

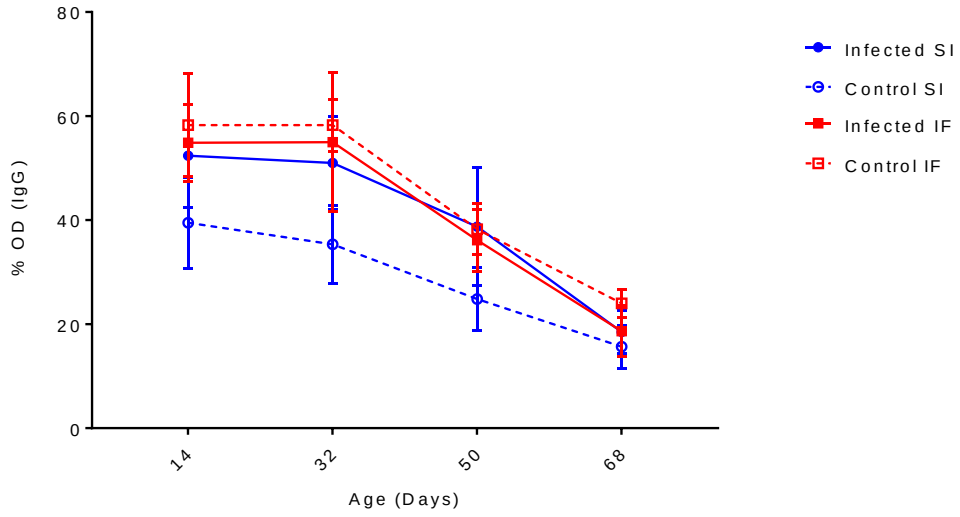

Figure 15. Percentages of Optical Density (± standard error) of IgG against *Haemonchus contortus* L3 level of Ile de France (IF) and Santa Inês (SI) suckling lambs experimentally infected and non-infected control.

Regarding to IgA against *H. contortus* L3 antigens in the abomasal mucus all groups presented averages similar to blank values.

Abomasal lymph nodes weights are presented in Figure 16. It was observed a significant Breed x Infection status interaction (P = 0.0371) on weight measurement, with the infected SI lambs presenting the highest average in comparison with the other groups (P < 0.05).

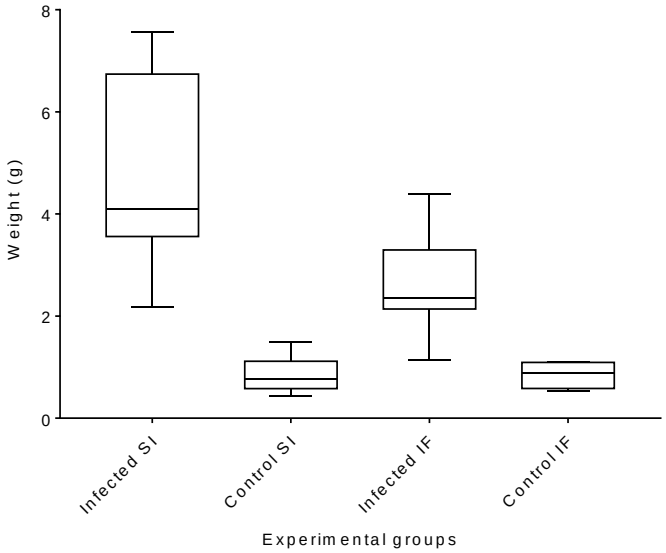

Figure 16. Abomasal lymph nodes weight of the Ile de France (IF) and Santa Inês (SI) suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control. The ends of the box are the upper and lower quartiles; the median is marked by a vertical line inside the box; and the two lines outside the box extend to the highest and lowest observations.

Cellular infiltration of immune cells was significantly more pronounced in Santa Inês suckling lambs than in Ile de France lambs (Figure 17, Figure 18).

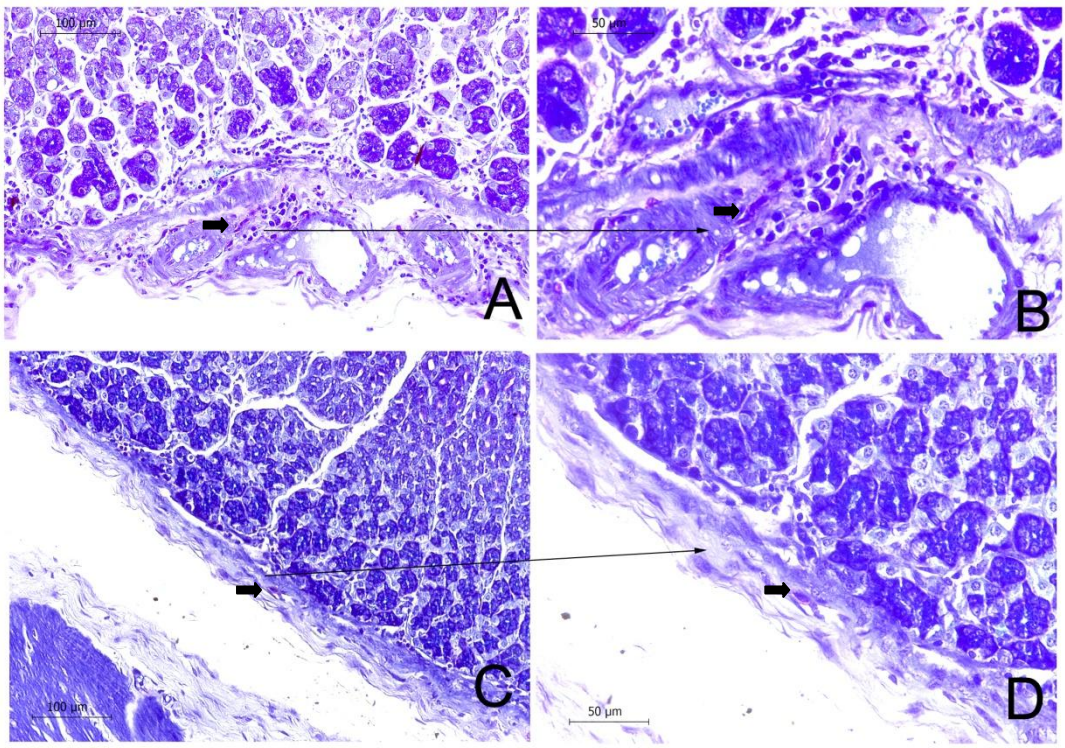

Figure 17. Histopathology (1% toluidine blue stained) of abomasum tissue sections showing mast cells in lamina propria of the mucosa. The micrographics A (×200 magnification) and B (×400 magnification) show tissue from the Santa Ines suckling lamb with a *Haemonchus contortus* burden of 20; while C (×200 magnification) and D (×400 magnification) show tissue from Ile de France suckling lamb with a *H. contortus* burden of 1809 worms. Arrow keys indicate mast cells.

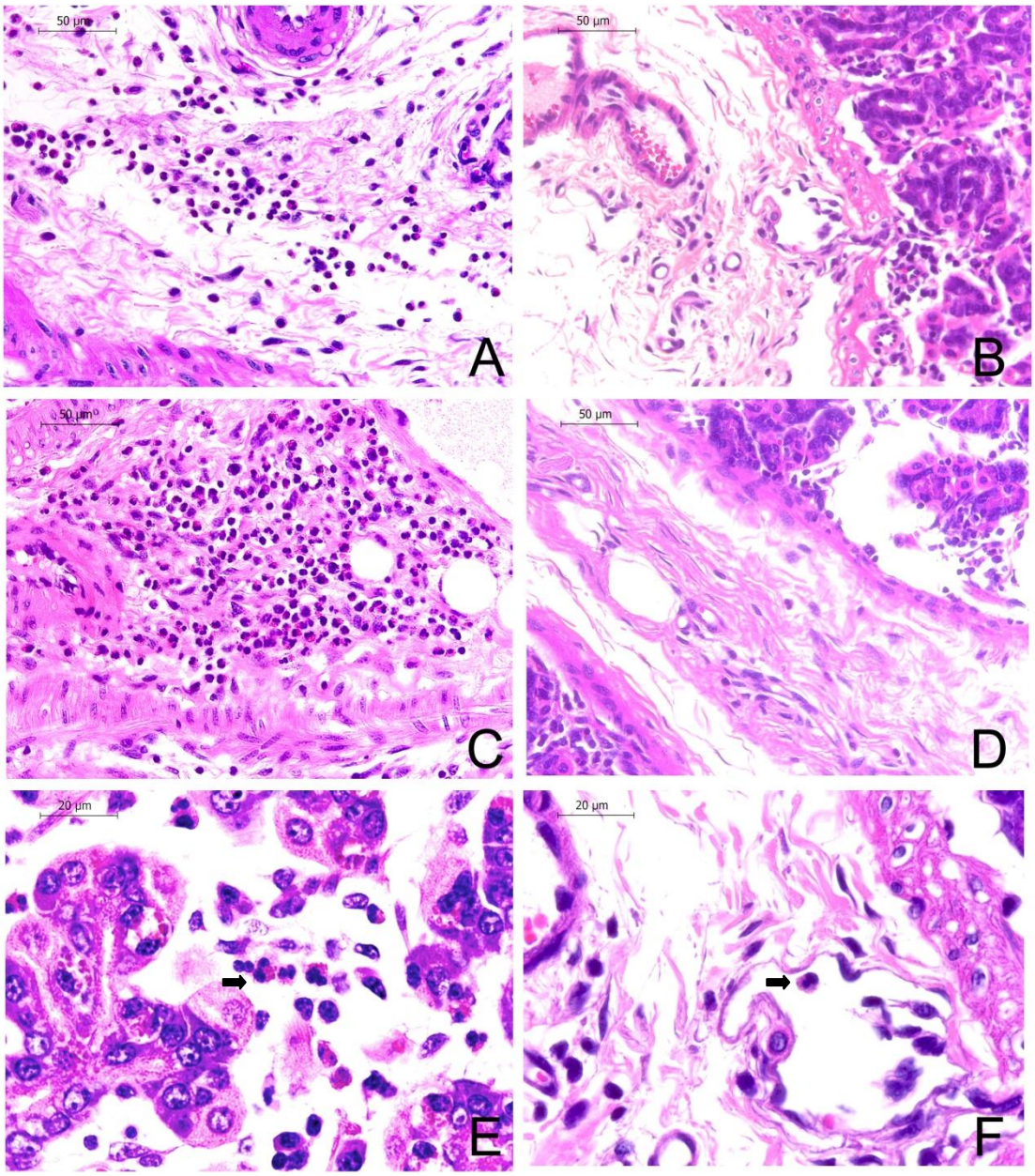

Figure 18. Histopathology (H&E stained) of abomasum tissue sections showing eosinophils in lamina propria of the mucosa. The micrographics A, C (×400 magnification) and E (×1000 magnification) show tissue from the Santa Ines suckling lamb with a *Haemonchus contortus* burden of 1, 42 and 20 worms, respectively; while B, D (×400 magnification) and F (×1000 magnification) show tissue from Ile de France suckling lamb with a *H. contortus* burden of 1404, 1809 and 1404 worms, respectively. Arrow keys indicate eosinophils.

The presence of eosinophils and mast cells were more pronounced in the lamina propria and abomasums mucosa, while globule leukocytes were in abomasums mucosa.

There was a breed and infection status effect, and a significant breed x infection status interaction on eosinophils, mast cells and globule leukocytes counting in the abomasums tissue.

Infected SI suckling lambs presented the greatest means of eosinophils, mast cells and globule leukocytes in the abomasums tissue (Figure 19).

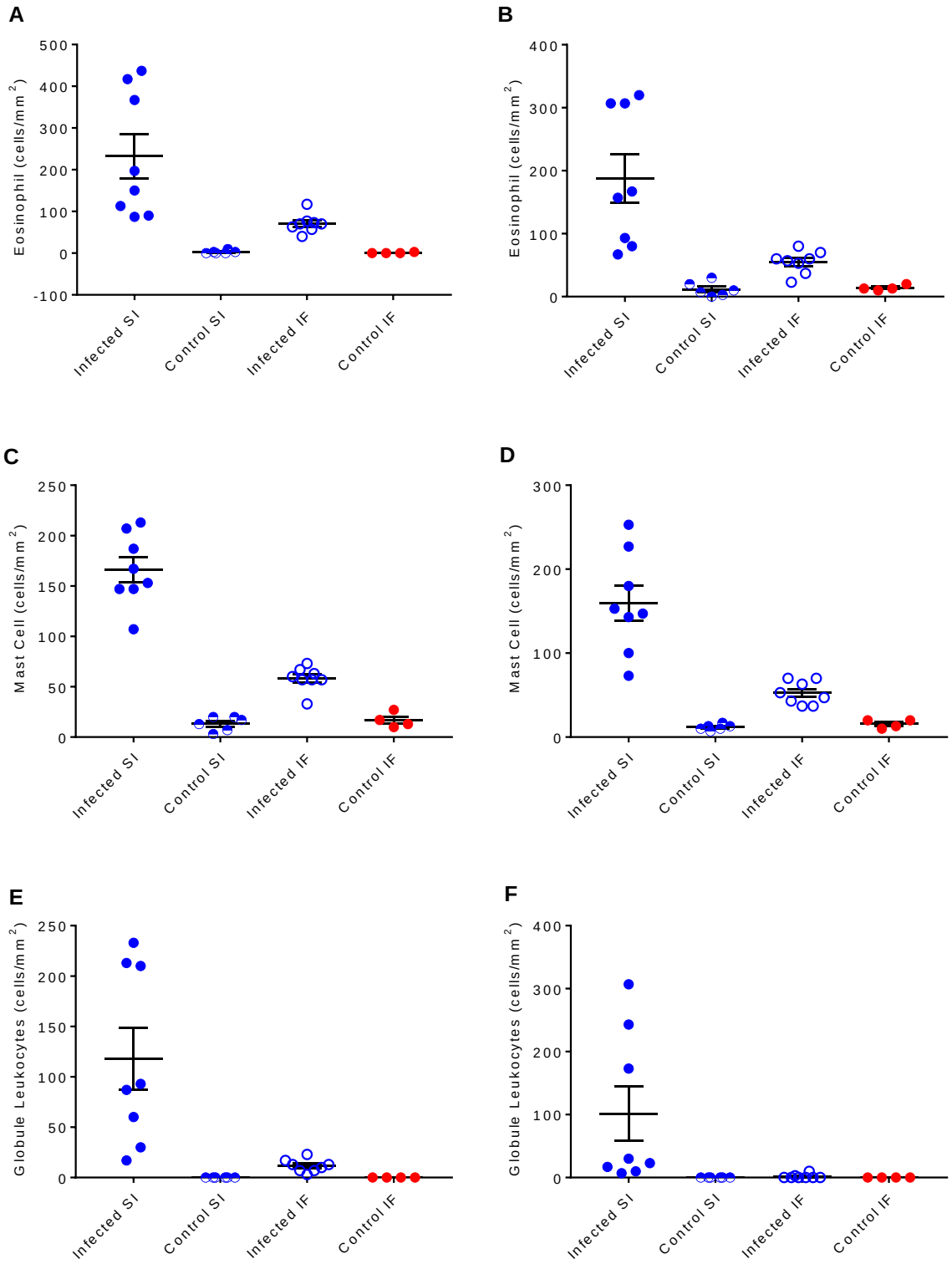

Figure 19. Averages (± standard error) of eosinophils (cells/mm^2), mast cells (cells/mm^2) and globule leukocytes (cells/mm^2) in the fundic (A, C, E) and pyloric (B, D, F) regions of the abomasal tissue of Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Non-infected groups of both breeds did not present globule leukocytes in the randomly selected fields of view. Numbers of globule leukocytes were similar between IIF and non-infected IF suckling lambs.

As we observed three infected Santa Inês suckling lambs more resistant than the other ISI lambs, we decided deepening the statisticall analysis in some of their results. There was a significant difference on blood eosinophils only at 20 days old (Figure 20).

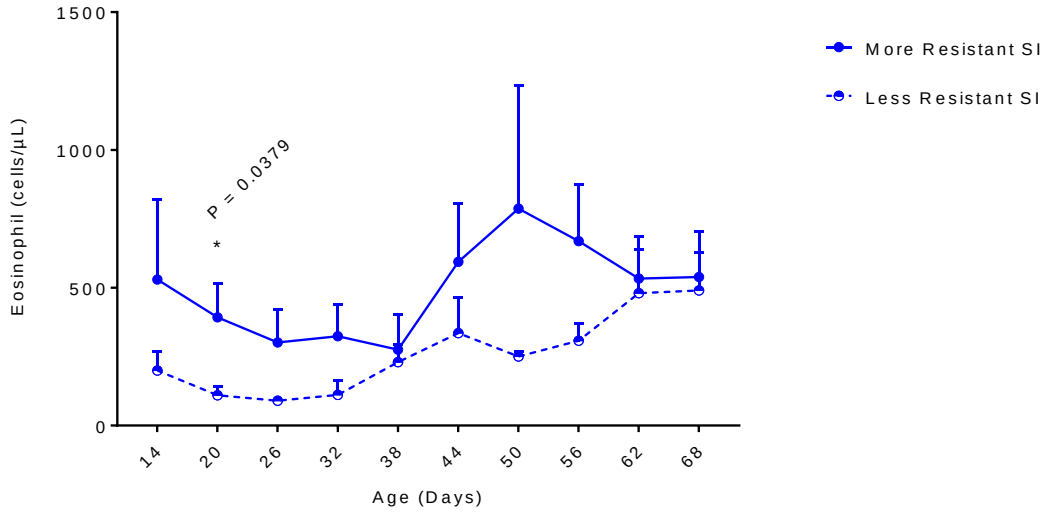

Figure 20. Eosinophil averages (± standard error) of infected Santa Inês suckling lambs experimentally infected with *Haemonchus contortus*.

Percentages of optical density of IgG against antigens of *H. contortus* L3 on plasma samples were significantly greater in the more resistant ISI lambs at 32 and 68 days old (Figure 21).

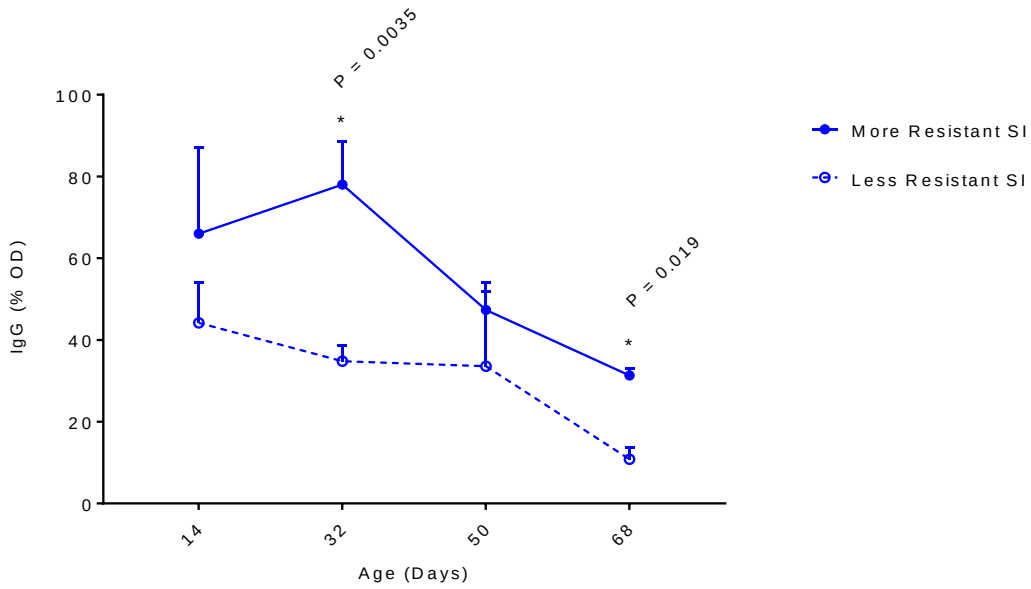

Figure 21. Percentages of Optical Density (± standard error) of IgG against *Haemonchus contortus* L3 level of infected Santa Inês suckling lambs artificially infected with *Haemonchus contortus*.

Regarding the abomasal lymph nodes weight averages, the more resistant ISI lambs presented similar average weight of the less resistant ISI lambs (Table 1).

Table 1. Abomasal lymph nodes weight averages (± standard error) of infected Santa Inês suckling lambs experimentally infected with *Haemonchus contortus*.

Infected Santa Ines	
More Resistant (n=3)	**Less Resistant (n=5)**
5.603 g (±0.89)	4.246 g (±0.9)

Arithmetic means in row did not differ by T test ($P < 0.05$).

These three infected Santa Inês suckling lambs had the highest numbers of eosinophils, mast cells and globule leukocytes (Figure 22) in the tissue ($P < 0.05$).

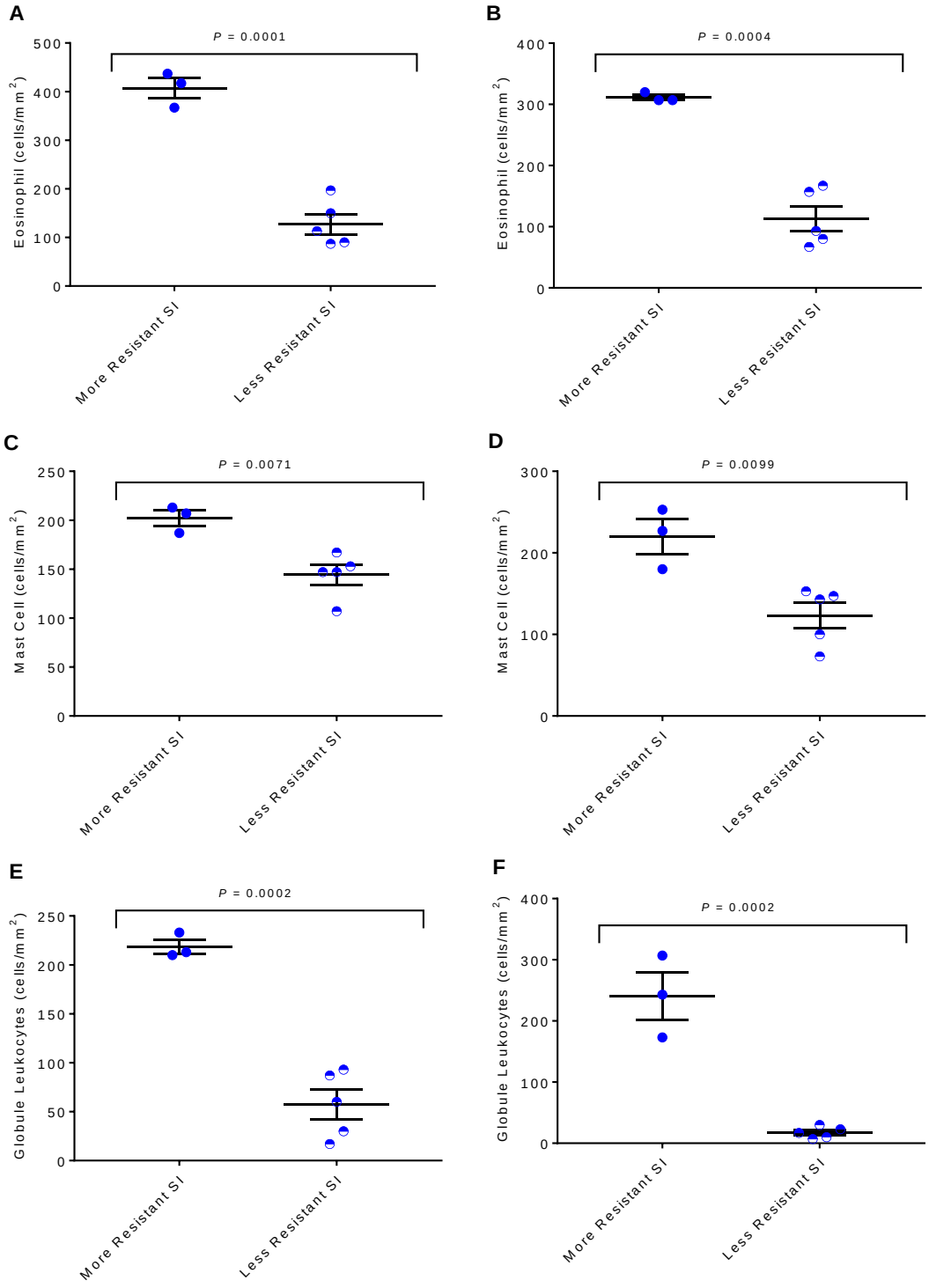

Figure 22. Averages (± standard error) of eosinophils (cells/mm^2), mast cells (cells/mm^2) and globule leukocytes (cells/mm^2) in the fundic (A, C, E) and pyloric (B, D, F) regions of the abomasal tissue of infected Santa Inês suckling lambs experimentally infected with *Haemonchus contortus*.

4. Discussion

SI lambs showed to have the ability of controlling *H. contortus* infection at an age earlier than what has been reported for development of immune response competency against GIN in most commercial sheep breeds (Greer and Hamie, 2016). Similar results were reported in suckling Native lambs in USA (Bahirathan et al., 1996) and in sucking SI, that despite acquiring natural infections with gastrointestinal nematodes displayed greater capacity to tolerate adverse effects of the infection than IF lambs (Rocha et al., 2005).

Due to progressive increase of worms reaching maturity and in the number of L3 used in the artificial serial infections, the EPG averages increased over the samplings, with Ile de France lambs always presenting the highest means. The *H. contortus* L3 establishment rate was higher in Ile de France (22.9%) than in Santa Inês lambs (11.1%). However, the infection dynamics and the worm burden at the end of the trial showed expressive intrabreed differences. In the Santa Ines group, we found three highly resistant lambs. They stopped shedding eggs on faeces from 50 days of age and showed a very low worm establishment rate (0.37%, 0.02% and 0.78% in lambs 1, 2 and 4, respectively). The immune response did not cause the complete elimination of the helminths. Even resistant animals harbor worms but they have a much lower parasitic load than susceptible animals (Alba-Hurtado and Muñoz-Guzmán, 2013).

Within Ile de France breed, one lamb (number 23) also was more resistant to *H. contortus* infection in comparison with the other animals of the same group. This lamb 23 started shedding eggs latter at 62 days of age. Additionally, it presented the lowest establishment rate (12.1%) among IF lambs. This data indicate that even in certain susceptible breeds there are individuals with higher capacity to develop immunity against parasites. Gastrointestinal parasite resistance is a genetically controlled characteristic that could be affected by age and breed, and may vary between individuals of the same population (McManus et al., 2014; Zvinorova et al., 2016).

The immune response development against *H. contortus* infection requires a continuous challenge that may prevent the establishment of incoming larvae. Other manifestations of the immune response include arrested

development, reduction in fecundity and elimination of the adult worms (Balic et al., 2000a; Shakya et al., 2011; Santos et al., 2014).

In accordance with our results, in another study, IF suckling lambs naturally infected with GIN showed higher EPG counts than SI lambs after 43 days of age (Rocha et al., 2005). Amarante et al. (2004) working with lambs naturally infected with GIN, observed that SI animals showed *H. contortus* EPG means below 1000 eggs, while IF and Suffolk sheep presented means higher than 1000 EPG on several occasions. Additionally, in that trial, IF and Suffolk sheep required frequent salvage anthelmintic treatment to prevent deaths due to haemonchosis and, at the end of the trial, IF and Suffolk sheep presented higher *H. contortus* total burden than SI sheep (Amarante et al., 2004). In another study, with lambs artificially infected, SI lambs, mainly those fed with high-protein diet, showed lower *H. contortus* than IF lambs fed with high-protein diet and than SI and IF lambs fed with moderate-protein diet (Bricarello et al., 2005).

Genetics may play an important role in regulating host resistance (Zhang et al., 2019). Different genomic regions show to be associated to several genes that are involved in the immune system development and inflammatory response against gastrointestinal nematodes (Benadives et al., 2016; Berton et al., 2017). *H. contortus* infection in sheep was associated with large changes in gene expression in abomasal tissue and lymph nodes (MacKinnon et al., 2015).

Our results indicate that Santa Inês breed has ability of rapid development of resistance. At 68 days old, ISI lambs had the greatest abomasal lymph node weight. Continuous stimulation of the host immune response by nematode parasitism induces increase in the abomasal lymph node size due CD4+ T cells hyperplasia (Balic et al., 2000b). Balic et al. (2000b) working with Merino lambs at 5 months old, found that lymph nodes of lambs artificially infected with 10000 L3 *H. contortus* and euthanized 27 – 36 days post infection, presented higher weight than those of non-infected lambs. Differences in abomasal lymph node of resistant and/or susceptible may reflect physiological differences between breeds and individuals in how they develop resistance (McRae et al., 2016).

In contrast, Shakya et al. (2011) observed that there was no significant difference in means of abomasal lymph node weights, between Gulf Coast

Native (resistant) and Suffolk (susceptible) lambs artificially infected with *H. contortus*. It is difficult to compare our results with those of the Shakya et al. (2011) due to differences in the protocol of infections and age of the animals, because, Shakya et al. (2011) evaluated older lambs (6 months old) and euthanatized earlier, 14 and 21 days post-infection, than in our trial.

According to McRae et al. (2015), in helminth infections, nematode antigens are recognized by antigen-presenting cells (APC), followed by a migration of these cells to the regional lymph nodes where they present antigens to naive T cells. T-cell differentiation results in the release of Th2-associated cytokines and the recruitment of effector cells such as eosinophils and mast cells to the site of infection. Abomasal lymph nodes are also closely related to immune cell chemotaxis and cell proliferation in abomasal infection (MacKinnon et al., 2010).

In the present experiment, highest ISI abomasal lymph node weight mean may indicate an increase in the percentage of T cells in the local lymph nodes. CD4+ T cells and other Th2 cells are important regulators of all aspects of eosinophil development and function (Weiss and Wardrop, 2010), and lymph node weight should be related to the increase in the number of such cells, and consequently in a higher recruitment of eosinophils into the abomasal tissue. The immune response of sheep to infection with *H. contortus* may be characterized by quantitative and qualitative changes in the percentage of activated CD4+ T cells in the local lymph node (Balic et al., 2002).

In the present trial, infected suckling SI lambs presented higher counts of eosinophils, mast cells and globule leukocyte to the abomasal tissue than Ile de France lambs. Similarly, in a *H. contortus* chronic infection, naturally infected Santa Inês lambs (8 months old) presented higher number of mast cells, eosinophils and globule leukocytes in abomasum mucosa than Ile de France lambs, however only globule leukocytes counts were statistically different between breeds (Albuquerque et al., 2019).

The more intense local immune responsiveness presented by SI lambs against *H. contortus* infection may explain our findings of low means of EPG and total worm burden, and heavier abomasal lymph nodes. Genetic resistance against GIN is regulated by the presence of mast cells, eosinophils, and globules leukocytes in the abomasum mucosa (Meeusen et al., 2005). Mast

cells and eosinophils can limit parasite growth, decrease fecundity of the parasite, and also damaging it morphologically (Vogel et al., 2018). Globule leukocytes and mast cells are involved in the expulsion of *H. contortus*, through a strong inflammatory response that can be initiated by the nonspecific degranulation of mast cells and release of vasoactive amines, activation of the alternative complement pathway and also presence of complement-derived peptides (Huntley et al., 1992; Balic et al., 2002; Kemp et al., 2009; Robinson et al., 2010; Alba-Hurtado and Muñoz-Guzmán, 2013).

In our study, non-infected lambs of both breeds presented low and similar number of eosinophils and also mast cells. Similar concentrations of eosinophils in abomasal tissues of non-infected control Caribbean wool and hair lambs were reported by MacKinnon et al. (2010).

Blood parameters to assess immune response against *H. cortortus* infection in this experiment had shown very little differences between IF and SI suckling lambs. Comparing to their respective control groups (non- infected), ISI lambs presented higher circulating WBC than IFI lambs, but such differences were not significant. In studies focusing on differences between susceptible and resistant breeds, similar results were observed by Bowdridge et al. (2015) on previous infected lambs and then artificially infected with *H. contortus*, where St. Croix (resistant) showed greater WBC than wool lambs (susceptible).

Regarding to blood eosinophil count, even identifying differences between SI groups in two moments, there were no consistent differences between ISI and IIF lambs. Rocha et al. (2005) in study with SI and IF suckling lambs naturally infected by gastrointestinal nematodes, found SI presented higher eosinophil counts than IF lambs, at 29, 43 and 57 days old, however differences between breeds were not significant.

Gastrointestinal nematodes (GIN) infection may induce transcription factors and cytokines to stimulate stem cell of bone morrow into eosinophil differentiation (Park and Bochner, 2010), and its number increases significantly in the blood (Tizard, 2014). Furthermore, after getting into circulation, eosinophils rapidly migrate into tissues to the site of infection (Anthony et al., 2007). Due to the absence of differences in blood eosinophil counts in contrast to pronounced difference in numbers of this cell in the mucosa, our histopathology findings support the hypothesis that SI suckling lambs present a

greater and faster eosinophil migration to the abomasal mucosa than in IF lambs, despite the absence of differences in blood eosinophil counts. However such hypothesis need to be better investigated in further studies.

Regarding to lymphocyte counts, infected groups presented higher means than their respective non-infected control groups, however, ISI lambs presented greater number of cells than non-infected SI only at 62 days old. Increases in lymphocyte number and WBC count may be related to the recruitment of defence Th2 cells to the tissue. Interleukins secreted by Th2 cells, such as IL5 and IL13, are respectively related to increased production of eosinophils by bone morrow, and to the recruitment of them to tissues by cytokines.

Lambs are generally considered to be born with no innate immunity to gastrointestinal nematodes (Greer and Hamie, 2016). Once innate immune system has been activated by the presence of gastrointestinal nematodes, adaptive immunity is induced in local lymph nodes by the cells of the innate immunity, such as conventional dendritic and T cells (Lamb, 2012; Sorobetea et al., 2018).

Our results indicated that IgG and IgA were not involved in protection against *H*. *contortus* in the young SI and IF suckling lambs. In older weaned lambs, although IgA antibody concentrations in the mucus against third-stage larvae antigen (L3) of *H. contortus* were detected in the lambs, no significant differences were observed by Bricarello et al. (2005) between SI and IF groups. Bowdridge et al. (2015) found that analysis of circulating IgG and crude worm antigen IgG and IgA did not reveal differences between St. Croix hair (resistant) and wool (susceptible) lambs.

Detected immunoglobulin G against *H. contortus* in this experiment is related to the transference of maternal immunoglobulin by colostrums. For this reason, IgG levels decreased over the time because this passive humoral response is not a long-lasting immunity. The same may have occurred with anti-parasitic immunoglobulin A that was not detected in the abomasal mucus.

In this trial, there was no time for production of anti-parasite immunglobulins by suckling lambs. If the present experiment had lasted longer, ISI lambs would initiate an effective adaptive immune response through the production of nematode-specific antibodies. Nevertheless, suckling lambs

immunized with 5 µg or 50 µg vaccine antigen of *H. contortus* gut presented a marked detectable anti-vaccine antibody response after 58 days old (Bassetto et al., 2014). Similar results were found in suckling lambs immunized with 1 ml of a commercial Barbervax® vaccine (Bassetto et al., 2018). These findings may be explained by the high exposure of the lambs to antigen of *H. contortus* of the vaccine.

Effectiveness of humoral immune response, throughout IgG and IgA production against *H. contortus* antigen may be prominent in weaned lambs and older sheep, after primary infections (Shakya et al., 2011; Bowdridge et al., 2013; Hernández et al., 2016).

In *H. contortus* infections, the complement system is activated as one of the first innate immune responses. Interestingly, in the presence of vasoactive and chemotactic peptides, eosinophils are recruited to the site of infection and in the absence of specific antibodies, they have their cytotoxicty against larvae in early infection stages mediated by the complement system activation (Alba-Hurtado and Muñoz-Guzmán, 2013).

Infections by *H. contortus* might cause reductions in haematological parameters such as anaemia, apathy and submandibular oedema (Amarante et al., 2004). Such clinical signal or physiopathological effects were absent in the SI and IF suckling lambs of the present study. The number of infective larvae used did not cause significant reduction in PCV, STP and RBC averages over the experiment. Considering only control groups of both breeds as reference, low PCV and RBC values might be a racial characteristic of SI lambs during the first few days of life, as long as, both parameters for IF lambs remained stable. For the age category and breeds studied in this experiment, our results indicated that healthy SI and IF suckling lambs up to 68 days old, present STP mean between 5.0 and 6.2 g/dL.

Suckling Santa Ines lambs showed a greater genetic resistance than Ile de France lambs, reflected on the development of a very robust innate immune response against *H. contortus* infection. In addition, resistant animals may be identified in an early age, before weaning.

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APÊNDICES

APÊNDICE I

Averages (minimum and maximum values) of *Haemonchus contortus* eggs per gram of faeces (EPG) of Ile de France and Santa Inês suckling lambs serially infected.

Days post the first infection	Age	Ile de France (n=8)	Santa Inês (n=8)	P-value
24	38 days	588 (0-1400)	225 (0-600)	
		2.13 (±0.47)	1.78 (±0.4)	0.58
30	44 days	863 (0-1600)	800 (100-1700)	
		2.63 (±0.38)	2.77 (±0.15)	0.73
36	50 days	2250 (0-4300)	813 (0-2900)	
		2.99 (±0.44)	2.39 (±0.38)	0.31
42	56 days	3113 (0-9700)	1650 (0-4900)	
		3.11 (±0.46)	2.39 (±0.54)	0.32
48	62 days	5888 (200-11700)	1950 (0-5400)	
		3.66 (±0.21)	2.4 (±0.55)	0.04
54	68 days	8175 (2000-15500)	2963 (0-7900)	
		3.87 (±0.1)	2.25 (±0.66)	0.03

Means (± standard error) of data transformed using log (x + 1).
Not significant ($P > 0.05$)

APÊNDICE II

Haemonchus contortus averages (minimum and maximum values) of Ile de France and Santa Inês suckling lambs experimentally infected.

***H. contortus* stages**	**Ile de France**	**Santa Inês**	
	Infected (n=8)	**Infected (n=8)**	**Effects (P-value)**
L4 early	75 (3-173)	64 (0-140)	
	#1.73 (±0.17)	1.56 (±0.25)	0.56
Female late L4	92 (33-181)	47 (0-135)	
	1.91 (±0.09)	1.12 (±0.34)	0.0421
Male late L4	61 (10-10)	42 (0-120)	
	1.71 (±0.12)	0.95 (±0.36)	0.06
Female early L5	199 (95-311)	60 (0-164)	
	2.26 (±0.07)	1.09 (±0.36)	0.0067
Male early L5	240 (156-327)	115 (0-312)	
	2.37 (±0.04)	1.47 (±0.36)	0.0286
Adult female	327 (80-630)	158 (0-297)	
	2.45 (±0.1)	1.54 (±0.42)	0.05
Adult male	244 (62-403)	110 (0-254)	
	2.32 (±0.1)	1.39 (±0.41)	0.0441
Total worm burden	1237 (653-1809)	597 (1-1284)	
	3.07 (±0.05)	2.25 (±0.37)	0.0443

#Means (± standard error) of data transformed using log (x + 1).
Not significant ($P > 0.05$)

APÊNDICE III

Total Plasma Protein (g/dL) averages (± standard error) of Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Age	**Ile de France**		**Santa Inês**	
	Infected (n=8)	**Control (n=4)**	**Infected (n=8)**	**Control (n=6)**
14 days	5.6 (±0.12)	5.9 (±0.24)	5.8 (±0.19)	6.1 (±0.34)
20 days	5.6 (±0.15)	5.9 (±0.18)	5.8 (±0.23)	5.9 (±0.35)
26 days	5.6 (±0.17)	5.8 (±0.13)	5.7 (±0.14)	5.7 (±0.37)
32 days	5.8 (±0.17)	5.7 (±0.22)	5.5 (±0.15)	5.5 (±0.36)
38 days	5.6 (±0.20)	5.9 (±0.13)	5.5 (±0.19)	5.6 (±0.29)
44 days	5.5 (±0.24)	5.5 (±0.15)	5.7 (±0.17)	5.6 (±0.35)
50 days	5.7 (±0.29)	5.9 (±0.05)	5.5 (±0.18)	5.7 (±0.32)
56 days	5.7 (±0.28)	6.0 (±0.15)	5.8 (±0.17)	5.8 (±0.26)
62 days	5.8 (±0.24)	6.0 (±0.14)	5.9 (±0.22)	5.9 (±0.26)
68 days	5.8 (±0.23)	6.2 (±0.14)	6.0 (±0.21)	6.0 (±0.23)

There were not breed and infection status effects, and breed x infection status interaction on TPP values ($P > 0.05$).

APÊNDICE IV

Packed Cell Volume in percentage (%) averages (± standard error) of Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Age	Ile de France		Santa Ines		Effects (P-value)	
	Infected (n=8)	Control (n=4)	Infected (n=8)	Control (n=6)	Breed	Infection status
14 days	36 (±1.11) a	35 (±1.03) ab	30 (±1.87) ab	29 (±1.94) b	0.0071	ns
20 days	34 (±1.12) a	34 (±0.33) a	28 (±1.87) bc	25 (±1.61) c	0.0002	ns
26 days	32 (±1.05) a	34 (±1.49) ab	27 (±1.82) bc	25 (±1.15) c	0.0007	ns
32 days	32 (±1.32) ab	35 (±0.96) b	27 (±1.1) ac	26 (±1.08) c	0.0002	ns
38 days	32 (±1.03)	37 (±0.25)	30 (±1.85)	30 (±1.33)	0.0153	ns
44 days	33 (±0.74) ab	36 (±0.91) a	31 (±1.16) b	32 (±1.33) ab	0.01	ns
50 days	33 (±0.53)	36 (±2.29)	33 (±1.0)	35 (±1.77)	ns	ns
56 days	34 (±0.81)	38 (±1.35)	34 (±0.85)	36 (±2.19)	ns	0.0293
62 days	34 (±1.32)	37 (±2.04)	34 (±1.13)	35 (±2.29)	ns	ns
68 days	36 (±1.13)	39 (±1.03)	35 (±1.43)	38 (±2.25)	ns	ns

In each row, arithmetic means with different lower case letters are significantly different by Tukey test ($P < 0.05$).
ns = not significant ($P > 0.05$).
There was no significant interaction between breed x infection status ($P > 0.05$).

APÊNDICE V

Red Blood Cell (x 10^6/ µL) averages (minimum and maximum values) of Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Age	Ile de France		Santa Ines		Effect (P-value)
	Infected (n=8)	Control (n=4)	Infected (n=8)	Control (n=6)	Breed
14 days	8.7 (7.6-9.9)	7.9 (7.4-8.6)	8.0 (5.8-9.82)	8.2 (6.8-9.7)	
	#0.98 (±0.01)	0.95 (±0.01)	0.95 (±0.02)	0.96 (±0.02)	ns
20 days	9.5 (8.2-11)	9.16 (8.3-10.0)	8.3 (7.11-9.97)	7.8 (6.8-9.0)	
	1.02 (±0.01) a	0.98 (±0.03) ab	0.97 (±0.02) ab	0.94 (±0.01) b	0.0173
26 days	9.4 (8.4-11.3)	9.1 (8.1-9.9)	8.8 (7.63-10.5)	8.3 (7.7-9.5)	
	1.01 (±0.01)	1.0 (±0.02)	0.99 (±0.01)	0.97 (±0.01)	ns
32 days	9.4 (7.3-10.4)	9.6 (8.7-10.5)	9.4 (8.25-10.2)	8.4 (7.4-9.7)	
	1.02 (±0.01)	1.03 (±0.02)	1.02 (±0.01)	0.97 (±0.02)	ns
38 days	9.1 (6.9-10.6)	10.3 (9.5-10.8)	9.8 (9.1-12.6)	10.0 (9.6-10.6)	
	1.0 (±0.02)	1.05 (±0.01)	1.03 (±0.01)	1.04 (±0.006)	ns
44 days	9.3 (7.5-10.6)	10.0 (9.7-10.4)	10.9 (9.1-12.6)	11.1 (9.4-12.6)	
	1.01 (±0.01) a	1.04 (±0.007) ab	1.07 (±0.02) b	1.08 (±0.02) b	0.0074
50 days	9.6 (7.3-10.6)	9.8 (8.6-10.4)	11 (8.7-14.1)	11.9 (10.1-13.7)	
	1.02 (±0.02) a	1.03 (±0.02) ab	1.08 (±0.02) ab	1.11 (±0.02) b	0.0052
56 days	9.4 (5-12.5)	10.1 (8.3-12.0)	12.3 (9.7-15.1)	12.1 (8.9-14.0)	
	1.01 (±0.04) a	1.04 (±0.03) ab	1.12 (±0.02) b	1.11 (±0.03) ab	0.0094
62 days	10.2 (8.7-13.0)	10.5 (9.0-12.2)	12.0 (10-14.5)	12.0 (9.7-17.2)	
	1.05 (±0.02)	1.06 (±0.02)	1.11 (±0.02)	1.11 (±0.03)	0.0329
68 days	10.3 (8.4-14.4)	11.7 (10.4-12.4)	12.9 (9.9-15.7)	13.8 (10.3-16.2)	
	1.05 (±0.02) a	1.1 (±0.02) ab	1.14 (±0.03) ab	1.16 (±0.03) b	0.0129

#Means (± standard error) of data transformed using log (x + 1).
In each row, arithmetic means with different lower case letters are significantly different by Tukey test ($P < 0.05$).
ns = not significant ($P > 0.05$).

APÊNDICE VI

White Blood Cell (cells/ µL) averages (minimum and maximum values) of Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Age	Ile de France		Santa Ines		Effects (P-value)
	Infected (n=8)	Control (n=4)	Infected (n=8)	Control (n=6)	Breed
14 days	6544 (4200-8700)	7025 (5550-8450)	6175 (3850-10700)	6025 (4500-8000)	
	#3.8 (±0.04)	3.84 (±0.05)	3.76 (±0.06)	3.77 (±0.05)	ns
20 days	7481 (4300-10200)	6500 (5400-8550)	6238 (4050-8100)	9458 (5300-13500)	
	3.9 (±0.04)	3.8 (±0.06)	3.78 (±0.04)	3.95 (±0.07)	ns
26 days	7109 (4875-10200)	7363 (5800-8950)	6225 (3300-11500)	6592 (3950-9950)	
	3.84 (±0.04)	3.86 (±0.04)	3.76 (±0.06)	3.8 (±0.06)	ns
32 days	7188 (5250-8600)	9738 (6100-11550)	6613 (3950-11650)	5792 (4600-6350)	
	3.85 (±0.03)	3.98 (±0.06)	3.8 (±0.05)	3.76 (±0.02)	0.0182
38 days	7863 (6300-11250)	9663 (7300-11200)	7625 (3500-10850)	6525 (4950-9500)	
	3.89 (±0.03)	3.98 (±0.04)	3.85 (±0.06)	3.81 (±0.04)	ns
44 days	8344 (6700-11750)	9213 (8000-10400)	7338 (4750-10450)	7333 (5250-9050)	
	3.92 (±0.03)	3.96 (±0.02)	3.85 (±0.05)	3.86 (±0.04)	ns
50 days	9581 (5050-16800)	9375 (7000-11200)	7756 (5650-10750)	8175 (6200-9800)	
	3.96 (±0.05)	3.97 (±0.05)	3.88 (±0.03)	3.91 (±0.03)	ns
56 days	9525 (6600-12150)	9650 (8300-10950)	8631 (4800-12850)	8700 (6700-10150)	
	3.97 (±0.04)	3.98 (±0.03)	3.92 (±0.05)	3.93 (±0.03)	ns
62 days	10000 (7600-17300)	9363 (8500-10600)	9969 (5500-15600)	8483 (6200-11950)	
	3.98 (±0.04)	3.97 (±0.02)	3.98 (±0.05)	3.92 (±0.04)	ns
68 days	8244 (6300-12350)	9275 (8400-10500)	10131 (6450-16000)	9358 (6000-12050)	
	3.9 (±0.04)	3.97 (±0.02)	3.99 (±0.05)	3.96 (±0.05)	ns

#Means (± standard error) of data transformed using log (x + 1).
ns = not significant (p>0.05).

APÊNDICE VII

Eosinophil (cells/ µL) averages (minimum and maximum values) of Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Age	Ile de France		Santa Inês		Effects (P-value)		
	Infected (n=8)	Control (n=4)	Infected (n=8)	Control (n=6)	Breed	Infection status	Breed x Infection status
14 days	398 (49-959)	355 (111-761)	323 (52-1071)	254 (0-576)			
	#2.42 (±0.16)	2.42 (±0.18)	2.31 (±0.16)	1.71 (±0.54)	ns	ns	ns
20 days	492 (61-918)	311 (171-540)	216 (41-568)	80 (0-212)			
	2.54 (±0.17) a	2.55 (±0.15) a	2.18 (±0.14) a	1.1 (±0.49) b	0.0037	ns	ns
26 days	286 (0-918)	484 (116-1074)	169 (48-504)	199 (0-624)			
	1.85 (±0.42)	2.54 (±0.21)	2.11 (±0.12)	1.52 (±0.51)	ns	ns	ns
32 days	139 (0-453)	423 (217-941)	191 (0-557)	139 (46-315)			
	1.82 (±0.28)	2.54 (±0.15)	1.95 (±0.3)	2.06 (±0.12)	ns	ns	ns
38 days	105 (0-258)	469 (91-1232)	247 (70-508)	95 (0-285)			
	1.16 (±0.44)	2.48 (±0.24)	2.3 (±0.11)	1.39 (±0.45)	ns	ns	0.0064
44 days	220 (0-668)	474 (80-1128)	433 (95-1012)	223 (67-496)			
	1.79 (±0.4)	2.5 (±0.24)	2.52 (0.12)	2.25 (±0.14)	ns	ns	ns
50 days	133 (0-344)	405 (87-959)	452 (170-1656)	198 (0-496)			
	1.62 (±0.37)	2.46 (±0.22)	2.52 (±0.11)	1.63 (±0.52)	ns	ns	0.0240
56 days	210 (0-495)	350 (83-657)	443 (120-994)	108 (0-335)			
	2.0 (±0.30) ab	2.37 (±0.24) ab	2.57 (±0.10) a	1.14 (±0.52) b	ns	ns	0.0126
62 days	244 (79-519)	225 (106-425)	500 (96-1212)	228 (91-372)			
	2.31 (±0.1)	2.30 (±0.12)	2.59 (±0.13)	2.31 (±0.1)	ns	ns	ns
68 days	138 (0-247)	458 (281-672)	508 (160-867)	148 (97-279)			
	1.69 (±0.37) a	2.63 (±0.08) ab	2.63 (±0.10) b	2.14 (±0.07) ab	ns	ns	0.0095

#Means (± standard error) of data transformed using log (x + 1).
In each row, arithmetic means with different lower case letters are significantly different by Tukey test ($P < 0.05$).
ns = not significant ($P > 0.05$).

APÊNDICE VIII

Neutrophil (cells/ µL) averages (minimum and maximum values) of Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Age	Ile de France		Santa Inês		Effects (P-value)
	Infected (n=8)	Control (n=4)	Infected (n=8)	Control (n=6)	Infection status
14 days	2769 (1308-4300)	3337 (2498-4452)	3055 (1519-5243)	3306 (1786-5076)	
	#3.41 (±0.06)	3.51 (±0.06)	3.44 (±0.07)	3.49 (±0.07)	ns
20 days	2584 (1333-3672)	3311 (1404-5643)	2617 (1134-4350)	6396 (2096-10004)	
	3.39 (±0.06) a	3.45 (±0.3) ab	3.39 (±0.06) a	3.73 (±0.12) b	0.0481
26 days	2334 (975-3978)	2797 (2280-3491)	2869 (825-6670)	3606 (2054-6048)	ns
	3.32 (±0.08)	3.44 (±0.17)	3.39 (±0.09)	3.51 (±0.09)	
32 days	2385 (1304-4043)	4919 (2074-8138)	2480 (672-6058)	2332 (1260-3422)	ns
	3.35 (±0.06)	3.63 (±0.04)	3.31 (±0.1)	3.33 (±0.08)	
38 days	2936 (1935-5063)	3815 (2628-5159)	2981 (945-4557)	2519 (1040-4275)	ns
	3.45 (±0.05)	3.57 (±0.13)	3.42 (±0.09)	3.35 (±0.09)	
44 days	2249 (1192-3525)	3375 (3008-3620)	2921 (1140-5225)	3108 (1628-4446)	ns
	3.32 (±0.06)	3.53 (±0.06)	3.42 (±0.07)	3.47 (±0.07)	
50 days	3985 (1414-12432)	2819 (2450-3136)	2858 (1541-4623)	3671 (2146-6174)	ns
	3.5 (±0.1)	3.45 (±0.02)	3.44 (±0.05)	3.54 (±0.06)	
56 days	3159 (1188-5429)	3602 (2883-5037)	3570 (1440-6570)	4167 (3082-5887)	ns
	3.45 (±0.08)	3.55 (±0.05)	3.5 (±0.07)	3.61 (±0.04)	
62 days	3434 (1238-9688)	3548 (2635-4346)	3927 (1540-9204)	4661 (2604-7768)	ns
	3.44 (±0.1)	3.54 (±0.06)	3.53 (±0.08)	3.64 (±0.07)	
68 days	2667 (704-4940)	3172 (2415-4602)	3762 (1812-9760)	4718 (2280-8556)	ns
	3.35 (±0.1)	3.49 (±0.06)	3.51 (±0.08)	3.64 (±0.08)	

#Means (± standard error) of data transformed using log (x + 1).
In each row, arithmetic means with different lower case letters are significantly different by Tukey test ($P < 0.05$).
ns = not significant ($P > 0.05$).
There were not breed effect and breed x infection status interaction on Neutrophil count ($P > 0.05$).

APÊNDICE IX

Basophil (cells/ µL) averages (minimum and maximum values) of Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Age	Ile de France		Santa Inês		Effects (P-value)
	Infected (n=8)	Control (n=4)	Infected (n=8)	Control (n=6)	Breed
14 days	49 (0-137)	77 (0-168)	113 (0-230)	82 (0-144)	
	#0.99 (±0.38)	1.48 (±0.5)	1.81 (±0.27)	1.64 (±0.33)	ns
20 days	25 (0-126)	37 (0-56)	198 (52-355)	144 (64-368)	
	0.5 (±0.33) a	1.16 (±0.58) ab	2.24 (±0.09) b	2.08 (±0.11) b	0.0001
26 days	71 (0-146)	74 (58-90)	103 (0-220)	56 (0-126)	
	1.27 (±0.38)	1.87 (±0.04)	1.56 (±0.35)	1.27 (±0.4)	ns
32 days	39 (0-125)	86 (0-122)	102 (0-375)	95 (0-173)	
	0.93 (±0.36)	1.54 (±0.52)	1.34 (±0.4)	1.69 (±0.34)	ns
38 days	80 (0-210)	64 (0-146)	178 (0-305	47 (0-118)	
	1.49 (±0.33)	1.05 (±0.61)	1.99 (±0.29)	0.98 (±0.44)	ns
44 days	45 (0-118)	95 (0-181)	95 (0-244)	30 (0-67)	
	0.97 (±0.37)	1.56 (±0.52)	1.53 (±0.34)	0.89 (±0.39)	ns
50 days	65 (0-157)	75 (0-213)	139 (0-335)	71 (0-166)	
	1.24 (±0.37)	1.07 (±0.62)	1.68 (±0.37)	1.34 (±0.43)	ns
56 days	69 (0-122)	72 (0-110)	113 (0-257)	70 (0-185)	
	1.47 (±0.32)	1.48 (±0.49)	1.8 (±0.27)	1.07 (±0.48)	ns
62 days	77 (0-210)	51 (0-106)	125 (0-312)	35 (0-120)	
	1.29 (±0.38)	1.0 (±0.58)	1.62 (±0.36)	0.67 (±0.43)	ns
68 days	63 (0-247)	96 (0-210)	111 (0-269)	75 (0-241)	
	1.02 (±0.39)	1.55 (±0.52)	1.39 (±0.41)	1.07 (±0.48)	ns

#Means (± standard error) of data transformed using log (x + 1).
In each row, arithmetic means with different lower case letters are significantly different by Tukey test ($P < 0.05$).
ns = not significant (p>0.05).
There were no infection status effect and breed x infection status interaction on Basophil count ($P > 0.05$).

APÊNDICE X

Lymphocyte (cells/ µL) averages (minimum and maximum values) of Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Age	Ile de France		Santa Inês		Effects (P-value)		
	Infected (n=8)	Control (n=4)	Infected (n=8)	Control (n=6)	Breed	Infection status	Breed x Infection status
14 days	3299 (2040-4611)	3194 (2280-3972)	2593 (1360-5136)	2310 (1350-3360)			
	#3.5 (±0.05)	3.5 (±0.05)	3.38 (±0.06)	3.34 (±0.06)	ns	ns	ns
20 days	4297 (2623-5765)	2787 (2331-3294)	3123 (2273-4131)	2728 (1960-4001)			
	3.61 (±0.05) a	3.44 (±0.04) ab	3.49 (±0.03) ab	3.42 (±0.06) b	ns	0.0377	ns
26 days	4328 (3045-6624)	3912 (3190-4560)	2996 (2138-4715)	2668 (1857-3980)			
	3.62 (±0.04) a	3.59 (±0.03) ab	3.46 (±0.05) b	3.41 (±0.05) b	0.004	ns	ns
32 days	4476 (3379-6439)	4253 (2496-5957)	3780 (2491-5476)	3185 (2204-4725)			
	3.64 (±0.03)	3.6 (±0.08)	3.56 (±0.04)	3.49 (±0.05)	ns	ns	ns
38 days	4640 (3577-6586)	5130 (3620-6882)	4172 (2485-6192)	3814 (2596-5320)			
	3.66 (±0.03)	3.7 (±0.06)	3.6 (±0.05)	3.56 (±0.05)	ns	ns	ns
44 days	5757 (4416-7638)	5095 (4480-6032)	3801 (2744-5638)	3880 (2747-6210)			
	3.75 (±0.03) a	3.7 (±0.03) ab	3.57 (±0.04) b	3.57 (±0.06) b	0.0016	ns	ns
50 days	5272 (3535-6870)	6076 (4200-7840)	4183 (3221-5698)	4101 (2542-5307)			
	3.71 (±0.04)	3.77 (±0.06)	3.62 (±0.03)	3.59 (±0.05)	0.0110	ns	ns
56 days	6034 (4123-8505)	5757 (4897-7437)	4391 (2976-6682)	4277 (3948-5700)			
	3.77 (±0.04)	3.75 (±0.04)	3.63 (±0.04)	3.62 (±0.04)	0.0048	ns	ns
62 days	6169 (4582-8362)	5470 (4947-6042)	5162 (3300-7028)	3452 (2730-4160)			
	3.78 (±0.03) a	3.74 (±0.02) a	3.7 (±0.04) a	3.53 (±0.02) b	0.0012	0.0105	ns
68 days	5224 (3564-6669)	5969 (4872-7350)	5681 (3999-9104)	4373 (3133-6612)			
	3.71 (±0.03)	3.77 (±0.04)	3.7 (±0.04)	3.63 (±0.05)	ns	ns	0.0319

#Means (± standard error) of data transformed using log (x + 1).
In each row, arithmetic means with different lower case letters are significantly different by Tukey test ($P < 0.05$).
ns = not significant ($P > 0.05$).

APÊNDICE XI

Monocyte (cells/ µL) averages (minimum and maximum values) of Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Age	Ile de France		Santa Inês		Effects (P-value)		
	Infected (n=8)	Control (n=4)	Infected (n=8)	Control (n=6)	Breed	Infection status	Breed x Infection status
14 days	28 (0-87)	127 (0-252)	80 (0-180)	73 (0-160)			
	#0.7 (±0.34)	1.64 (±0.55)	1.47 (±0.33)	1.33 (±0.43)	ns	ns	ns
20 days	83 (0-378)	55 (0-108)	92 (0-303)	110 (0-350)			
	1.1 (±0.41)	1.26 (±0.64)	1.1 (±0.42)	1.42 (±0.46)	ns	ns	ns
26 days	91 (0-210)	96 (0-152)	89 (0-208)	63 (0-199)			
	1.52 (±0.34)	1.57 (±0.53)	1.53 (±0.34)	1.03 (±0.46)	ns	ns	ns
32 days	148 (0-344)	57 (0-122)	74 (0-239)	41 (0-127)			
	1.87 (±0.29)	1.03 (±0.59)	1.43 (±0.32)	0.7 (±0.44)	ns	0.0321	ns
38 days	102 (0-225)	185 (0-333)	61 (0-124)	49 (0-70)			
	1.76 (±0.26)	1.79 (±0.6)	1.24 (±0.36)	1.48 (±0.3)	ns	ns	ns
44 days	74 (0-173)	151 (0-416)	89 (0-276)	92 (0-201)			
	1.48 (±0.33)	1.22 (±0.71)	1.47 (±0.34)	1.41 (±0.45)	ns	ns	ns
50 days	128 (0-336)	0 (0-0)	108 (0-201)	136 (74-261)			
	1.61 (±0.36) a	0 (±0.0) b	1.79 (±0.27) a	2.09 (±0.08) a	0.002	ns	0.0071
56 days	54 (0-363)	121 (0-219)	129 (0-359)	78 (0-201)			
	0.55 (±36)	1.64 (±0.55)	1.82 (±0.28)	1.36 (±0.43)	ns	ns	ns
62 days	152 (0-865)	70 (0-194)	254 (126-468)	108 (0-239)			
	1.34 (±0.41)	1.06 (±0.55)	2.36 (±0.07)	1.71 (±0.35)	ns	ns	ns
68 days	91 (0-247)	23 (0-94)	68 (0-227)	45 (0-97)			
	1.51 (±0.34)	1.06 (±0.61)	1.05 (±0.4)	0.98 (±0.44)	ns	ns	ns

#Means (± standard error) of data transformed using log (x + 1).
In each row, arithmetic means with different lower case letters are significantly different by Tukey test ($P < 0.05$).
ns = not significant ($P > 0.05$).

APÊNDICE XII

Averages (± standard error) of IgG against *Haemonchus contortus* L3 level of Ile de France and Santa Inês suckling lambs experimentally infected with *H. contortus* and non-infected control.

Age	Ile de France		Santa Ines	
	Infected (n=8)	**Control (n=4)**	**Infected (n=8)**	**Control (n=6)**
14 days	1.72 (±0.07)	1.75 (±0.08)	1.67 (±0.08)	1.49 (±0.18)
32 days	1.66 (±0.1)	1.77 (±0.04)	1.67 (±0.07)	1.48 (±0.14)
50 days	1.51 (±0.1)	1.58 (±0.05)	1.47 (±0.13)	1.3 (±0.16)
68 days	1.16 (±0.15)	1.39 (±0.05)	1.2 (±0.11)	1.12 (±0.15)

Means (± standard error) of data transformed using log (x + 1).
There were no breed and infection status effects, and breed x infection status interaction on IgG level ($P > 0.05$).

APÊNDICE XIII

Abomasal lymph nodes weight averages (± standard error) of Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Ile de France		**Santa Ines**	
Infected (n=8)	**Control (n=4)**	**Infected (n=8)**	**Control (n=6)**
2.58 g (±0.35) a	0.86 g (±0.13) a	4.76 g (±0.67) b	0.85 g (±0.15) a

In each row, arithmetic means with different lower case letters are significantly different by Tukey test ($p<0.05$).

APÊNDICE XIV

Averages (minimum–maximum values) of eosinophils (cells/mm^2), mast cells (cells/mm^2) and globule leukocytes (cells/mm^2) in the fundic region of the abomasal tissue of Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Cell	**Ile de France**		**Santa Inês**		**Effects (P-value)**		
	Infected (n=8)	**Control (n=4)**	**Infected (n=8)**	**Control (n=6)**	**Breed**	**Infection status**	**Breed x Infection status**
Eosinophils	70.83 (40-117)b	0.83 (0-3)b	232.08 (87-437)a	2.78 (0-10)b	0.0289	0.0003	0.0326
Mast cells	58.33 (33-73)b	16.67 (10-27)c	165.83 (107-213)a	17.78 (3-20)c	<0.0001	<0.0001	<0.0001
Globule leukocytes	11.63 (3-23)b	0b	117.91 (17-233)a	0b	0.0015	0.0041	0.0155

In each row, arithmetic means with different lower case letters are significantly different by Tukey test ($P < 0.05$).

APÊNDICE XV

Averages (minimum–maximum values) of eosinophils (cells/mm^2), mast cells (cells/mm^2) and globule leukocytes (cells/mm^2) in the piloric region of the abomasal tissue of Ile de France and Santa Inês suckling lambs experimentally infected with *Haemonchus contortus* and non-infected control.

Cell	**Ile de France**		**Santa Inês**		**Effects (P-value)**		
	Infected (n=8)	**Control (n=4)**	**Infected (n=8)**	**Control (n=6)**	**Breed**	**Infection status**	**Breed x Infection status**
Eosinophils	55 (23-80)b	14.17 (10-20)b	187.1 (67-320)a	11.67 (0-30)b	0.0183	0.0003	0.0147
Mast cells	52.5 (37-70)b	15.83 (10-20)b	159.58 (73-253)a	11.67 (7-17)b	0.0015	<0.0001	0.0007
Globule leukocytes	1.67 (0-10)b	0b	101.21 (7-307)a	0b	0.0004	<0.0001	0.0004

In each row, arithmetic means with different lower case letters are significantly different by Tukey test ($P < 0.05$).

ANEXO I

Aprovação do Projeto pela Comissão de Ética no Uso de Animal

unesp UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"

fmvz - unesp
Faculdade de Medicina Veterinária e Zootecnia
Campus de Botucatu

ATESTADO

Atesto que o Projeto "RESPOSTA IMUNOLÓGICA E DESEMPENHO DE CORDEIROS LACTENTES SANTA INÊS E ILE DE FRANCE INFECTADOS ARTIFICIALMENTE COM Haemonchus contortus" **Protocolo CEUA 0118/2018** , a ser conduzido por JOSÉ GABRIEL GONÇALVES LINS, responsável/orientador ALESSANDO FRANCISCO TALAMINI DO AMARANTE, para fins de pesquisa científica/ensino - encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal - CONCEA.

Finalidade	PESQUISA CIENTÍFICA
Vigência do projeto	15/10/2018 a 23/12/2019
Nome Comum / Espécie / Linhagem	OVINA / OVIS ARIES / Ambas as raças com linhagem para produção de carne
Raça	Santa Inês e Ile de France
Nº de animais machos	18
Nº de animais fêmeas	18
Nº de animais sexo indefinido	0
Peso médio de animais machos	5kg
Peso médio de animais fêmeas	4,5kg
Peso médio de animais sexo indefinido	0
Idade	0 ano(s) e 0 mes(es) e 14 dia(s).
Procedência	Os animais serão comprados de um ovinocultor.

Projeto de Pesquisa aprovado em reunião da CEUA em 21/06/2018

JOSÉ NICOLAU PRÓSPERO PUOLI FILHO
Presidente da CEUA da FMVZ, UNESP - Campus de Botucatu

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ANEXO II

Composição da ração comercial utilizada como suplemento alimentar