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ORIGINAL ARTICLE

Plasma lipopolysaccharide elevations in cattle associated with early-stage infection by *Fasciola hepatica*

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Fasciolosis is an endemic zoonotic parasitic disease with significant impacts on human health and both animal health and production. Early post-infection impacts on the host remain unclear. The objective of this study was to determine the changes, if any, to levels of endotoxin in cattle plasma in response to early-stage infection with *Fasciola hepatica*. Thirty-six (36) commercial bred cattle were experimentally infected with approximately 400 viable metacercariae. Plasma lipopolysaccharide (endotoxin) levels were examined on 24 occasions from 0 h before infection to 336 h after infection using the Limulus Amoebocyte Lysate chromogenic end point assay and compared with that of six (6) uninfected control animals. Peak lipopolysaccharide levels in infected animals were reached at 52 h after infection and returned to pre-infection levels at time 144 h after infection. Infected animals had significantly elevated lipopolysaccharide levels between 24 and 120 h after infection when compared to uninfected animals. The mean change in endotoxin units (EU)/mL over time after infection was statistically significant in infected animals. Elevations of lipopolysaccharide occurred in all infected animals suggesting a possible repeatable and titratable endotoxemia conducive to therapeutic agent model development.

Keywords cattle; endotoxin; *F. hepatica*; fasciolosis; lipopolysaccharide; migration

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Introduction

Fasciolosis is a disease affecting at least 50 countries worldwide and resulting in an estimated economic cost of US$3.2B due to loss of production.[1] Data relating to the prevalence and intensity of infection rates are extremely limited across district, regional, and national scales.[2] While the introduction and implementation of flukicides has seen a reduced incidence of wide-scale mortality due to liver fluke infection, it is also hypothesised that infection rates have risen with an increase in grazing of irrigated land.[3]

Studies have highlighted the significant production impacts of fasciolosis within the meat and livestock industry.[3–5] Production losses are clearly associated with migration of near-adult to adult flukes in the hepatic parenchyma; however, little published evidence has specifically implicated the early phase of infection with production loss. Orally ingested metacercariae excyst in the duodenum following digestion of the cyst wall. The presence of subadult fluke in the hepatic parenchyma has been demonstrated by 4–6 days[6] where they develop to sexual maturity[7]; however, juvenile flukes appear in the abdominal cavity within 2 h of experimental ingestion, via direct penetration of the duodenal mucosa.[6] The migration through the intestinal mucosa has been postulated to impact the integrity of the intestinal epithelium, resulting in concomitant loss of integrity of the intestinal barrier. The concept of low-level damage to the intestinal mucosa, with resultant increase in permeability to luminal contents, has been postulated to reflect a condition called 'leaky gut syndrome'.

Lipopolysaccharide (endotoxin) is a major component of the outer cell membrane of Gram-negative bacteria. The significance of elevated levels of endotoxin, that is, endotoxemia, is profound to both human and animal health. Endotoxin levels in circulating plasma are typically extremely low.[8] For example, in a study of healthy human blood donors, whilst always measurable, the range of levels was between 0.01 and 1.0 EU/mL.[9] Similarly, in healthy cattle, endotoxin levels have been shown to average at 0.7 EU/mL (range: 0–0.82 EU/mL).[10] Endotoxemia is a condition associated with high mortality in humans[11,12] and significant economic impact on production species.[13–16]

The presence of high endotoxin levels within the entire lumina of the gastrointestinal tract reflects the ubiquitous presence of Gram-negative bacteria. It also reflects the effectiveness of both the structural innate immune system (the gastrointestinal mucosal barrier) and systemic inflammatory responses.[17] The host immune response to fluke infection in domestic sheep and cattle has been examined in previous studies; however, impact of infection on host plasma endotoxin levels have not been reported.[18–22] The objective of this study was to determine the changes, if any, to levels of endotoxin in cattle plasma in response to early stage infection with *Fasciola hepatica*.

Materials and Methods

Animals and parasite

Uninfected animals. Six (6) castrated beef cross-bred cattle 4 months of age with an average body weight of 167 kg (±6 SD) were enrolled in the uninfected control treatment group. On Day 0, each animal received a sham infection of water. Uninfected animals were handled and maintained the same as infected animals to

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ensure that any changes in biomarkers were a true result of the infection and not a result from stress from handling.

Infected animals. Thirty-six (36) castrated male beef cross-bred cattle between the ages of 8 and 20 months and with an average body weight of 265 kg (±45 SD) were enrolled into the infected treatment group. Metacercariae were examined microscopically for viability on storage petri dishes, prior to pooling and suspension in 0.4% carboxymethylcellulose. On Day 0, each animal was orally infected a final dose of 10 mL containing approximately 400 metacercariae, generated from a laboratory strain of triclabendazole-susceptible *F. hepatica* referred to as 'Palmers Oaky'. This strain was isolated in 2014 from sheep grazing near Oberon, NSW, Australia. On Days 105–107 after infection, six animals from the cohort were euthanised, and the total count of adult flukes was calculated in accordance with WAAVP Guidelines to determine adequate infection.[23] In short, intact livers and gall bladders were recovered from cattle at necropsy. The gall bladder was removed and examined for presence of adult *F. hepatica*. Bile ducts were massaged to encourage adult flukes to emerge. The organ was than sliced into 1–2 cm transverse segments, which were individually squeezed to encourage remaining parasites to emerge. These slices were than incubated in warm saline at 37°C for a minimum of 2 h. Slices were then squeezed once to recover any remaining parasites in the parenchyma. The saline was sieved, and the remaining debris caught in the sieve was examined for parasites. Intact, heads and tails of the recovered parasites were counted. The final total comprised of the number of intact flukes and whichever count was higher between heads and tails.

Maintenance of animals. Animals were confirmed as clinically healthy by a veterinarian and enrolled in this study. Animals were maintained in a feedlot facility and fed approximately 2% of body weight/head/day with roughage mix and approximately 1% of body weight/head/day with Lucerne pellets. Faecal samples from all animals collected 4 days prior to infection were examined by sedimentation and confirmed free of *F. hepatica* eggs. It was anticipated that minimal stress or discomfort would arise from the proposed procedures. Animals were acclimatised to the facilities for a minimum of 1 week prior to the commencement of the study. All infections were designed to be subclinical and were thus not anticipated to produce any signs of parasitism. Had any cattle develop clinical signs of liver fluke disease, such as impaired health, then that animal would have been treated with an appropriate flukicide (e.g. triclabendazole) and withdrawn from the study. All personnel were trained or accredited to carry out the procedures they were allocated in order to reduce stress on the animals. The cattle in each pen were inspected per at least once daily throughout the study. At any stage, an animal could be withdrawn from the study on animal welfare grounds at the discretion of the examining veterinarian or Animal Welfare Officer (AWO). Animal Ethics was approved by the Elanco Animal Ethics Committee under AEC number ELAVV200287 and ELAVV200260.

Venepuncture

Blood samples were collected at before infection on Day −4 ($T = 0$) and after infection at 4, 8, 12, 24, 28, 32, 36, 48, 52, 56, 60, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, and 336 h. Collection time points were within ±10 min for all time points on Day 0 and ± 2 h for time points thereafter. Blood samples were collected aseptically in lithium heparin tubes. Specimens were centrifuged at approximately 4000×g for 15 min at 4°C. Plasma samples were then aliquoted into three 1.5-mL cryovials and stored at −80°C within 4 h of collection.

Endotoxin (lipopolysaccharide) analysis

Plasma endotoxin (lipopolysaccharide) was determined using the Limulus Amoebocyte Lysate (LAL) chromogenic end-point assay according to manufacturer's instructions (Hycult Biotech®, Wayne PA, USA. HIT302). In brief, two replicates were analysed for each sample, and the mean was compared with a standard curve produced on the plate from a serial dilution of the provided endotoxin standard. Samples that read above the standard curve on the plate in the initial reading were diluted in endotoxin-free water and the process was repeated (n = 242), and the results were then corrected for the appropriate dilution factor. Plate readings were accepted if the R^2 value of the calibration curve was ≥0.95. The sensitivity of the described method was 0.04 EU/mL.

Statistical analysis

Results were analysed by repeated measures ANCOVA (JMP® Version 16). Statistical models were analysed for and selected by the lowest Bayesian Information Criterion (BIC). To ensure the accuracy of the results, plasma endotoxin levels were analysed for normality using quantile plots and goodness-of-fit tests within collection time; statistical outliers (values outside 1.5 interquartile range from greater and lesser quartiles) were removed from the data set. This resulted in a reduction of 8% in BIC, which was an indication that the removal of outliers was appropriate. The linear relationship between age and body weight (BW) was evaluated and determined to be positive ($P < 0.001$). Therefore, BW was used as a random variable for the determination of mean endotoxin level for each time point. Multiple mean comparison was performed using Tukey's adjustment; the results are presented as least squared means ± SE.

Results

Parasite infection

The mean total fluke count (±SD) of the 6 euthanised animals was 78 (±11). This represented a 19.5% take for the parasite infections, above the recommended VICH guidelines[24] for an adequate infection (μ = 20 fluke). No animals presented with clinical signs of infection. All animals competed the study successfully.

Endotoxin (lipopolysaccharide) analysis

Low levels of endotoxin were detected before infection (least square mean: 0.007; range: 0–0.28; 0.01 SEM). A rise in endotoxin in infected animals was first detected at 12 h after infection (least square mean: 3.03; range = 1.41–5.48; 0.09 SEM; $P = 0.03$). Their peak endotoxin levels were reached at 52 h after infection (Figure 1, least square mean: 23.64; range = 4.16–51.66; 2.25 SEM; $P < 0.0001$) and returned to pre-infection levels at 168 h (least square mean: 1.24; range = 0.764–3.18; 0.10 SEM; $P = 0.21$). A second but smaller endotoxin peak was detected at 216 h after infection (least square mean: 3.42; range = 0.97–6.84; 0.25 SEM; $P = 0.01$). The mean change in EU/mL over time (at all-time points after infection) was statistically significant ($P < 0.0001$).

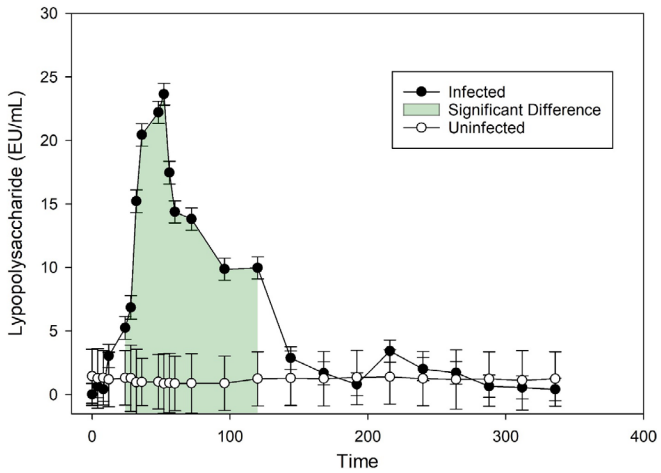

Figure 1. Average concentration of lipopolysaccharide presented in endotoxin units (EU) per mL over time. Coloured areas under the curve indicate that the average value was significantly different (P < 0.05) between infected and uninfected groups.

There was no change in levels of endotoxin within uninfected animals over time (range: 0.86–1.43, 0.18 SEM). Infected animals had significantly elevated lipopolysaccharide levels between 24 and 120 h after infection when compared to uninfected animals ($P < 0.05$). When comparing infected versus uninfected animals, time, treatment and treatment * time where all statistically significant ($P < 0.0001$).

Descriptions of hepatic lesions from recovered livers

Hepatic migration of *F. hepatica* resulted in clusters of varying sized abscesses. The lesions were necrotic and cavitated and were consistent with lesions frequently reported associated with fluke migration. Numerous lesions frequently contained mummified adult worms.

Discussion

The ruminant gastrointestinal tract, by the nature of its fermentative capabilities, contains high levels of luminal endotoxin.[13,25,26] Endotoxin levels have been shown to increase markedly during feeding of high grain diets.[25,27] In more controlled diets associated with sub clinical ruminal acidosis, concomitant elevations in plasma endotoxin are relatively modest (peak plasma endotoxin, 0.81 EU/mL). In health, a functioning intestinal mucosa prevents absorption of significant endotoxin across the mucosal barrier[17,28] and the low concentrations of endotoxin detectable in the portal circulation[28] are rapidly cleared by the normally functioning liver and acute phase proteins,[14] such as Lipopolysaccharide Binding Protein (LPS-BP). The degree to which a functioning gastrointestinal barrier prevents endotoxin leakage is impressive—peak ruminal endotoxin levels of over 100,000 EU/mL following induced subclinical acidosis were associated with plasma endotoxin levels of less than 1 EU/mL.[26]

The presence of endotoxin within the plasma is a significant immunological challenge to the host.[29,30] Egress of luminal endotoxin beyond the intestinal lumen can occur via paracellular or transcellular routes of transport.[8] Irrespective of the route, systemic translocation triggers an acute immune response with production of pro-inflammatory cytokines following activation of TLR4[28,31] and an entire cascade of acute phase responses, with clinical and production consequences.[13,14,32–37] This response elicits the clinical signs commonly associated with endotoxemia (including pyrexia, tachypnoea, and vasodilation)[14] and has been ascribed to pro-inflammatory mediators such as TNF-α and IL-6. In the current study, no such clinical signs were noted. This initial response to endotoxin is termed the acute phase response. The levels of detectable endotoxin that are associated with the acute phase response are low. In clinically normal cattle, including those on high grain diets, circulating plasma endotoxin is usually not detectable or, cited as less than 0.05 EU/mL.[26,38,39]

In the current study, elevation of plasma endotoxin was noted within 12 h after infection. Peak endotoxin levels were reached at 52 h after infection at an average of 23.64 EU/mL, with the highest individual value reaching 51.66 EU/mL. Between time points 12 h and 120 h, ALL individual infected animals had plasma endotoxin levels greater than 5 EU/mL. As a comparison, when cattle where injected intravenously with sufficient purified endotoxin to induce experimental endotoxic shock (and in one case death), the maximum plasma endotoxin detectable was 30.7 EU/mL (range, 1.6–30.7 EU/mL) and control animals had undetectable plasma endotoxin by 15 min after injection.[38] Similarly, calves injected repeatedly with 5 ug/kg body weight of endotoxin had undetectable plasma endotoxin within 3 min of injection,[40] which suggests a rapid and effective clearance mechanism. In stark contrast, the current study clearly demonstrated plasma endotoxin levels an order of magnitude higher than uninfected controls over prolonged periods of time. In the absence of any infectious process other than the controlled oral infestation with metacercariae, this is strong evidence that a localised compromise of the gastrointestinal barrier has led to a demonstrable increase in gastrointestinal barrier permeability. Furthermore, numerous studies have shown that various forms of liver injury including hepatitis and hepatic lipidosis can decrease LPS clearance.[38,41–45] In particular, hepatocyte lesions have been shown to reduce the percentage of hepatic LPS that the liver is able to clear.[42] As juvenile fluke have been shown to reach the liver parenchyma within 4–6 days, the resulting lesions from parasite migration could explain why LPS concentrations did not return to baseline within the study period.

Helminth parasites are known to damage intestinal barrier function, and specific mechanisms have been demonstrated in the case of fascioliasis.[6] The current study strongly supports existing evidence that direct damage to the gastrointestinal barrier is associated with the initial trans-intestinal migration phase of *F. hepatica* infection and that a consequence of this (presumably) localised compromise is also associated with endotoxin (LPS) levels more typically seen in clinically aggressive endotoxemia models. Recent research efforts, across multiple species, have focussed on numerous proteins produced by the excysted metacercaria or juvenile flukes, which have been shown to significantly dampen local immune responses, both innate and acquired.[21,22,46–57] The current study raises the possibility that localised immunomodulation by the parasite, presumably to maintain a cryptic status, has a low-level immune modulation effect which is systemic in its impact. This could be affirmed with the analysis of biomarkers from the acute phase response such as LBP,

haptoglobin, and Serum Amyloid. In the current model, this is manifested both by the longevity of plasma endotoxin after infection and also the lack of systemic clinical impact of endotoxemia. The reduction in circulating LPS levels likely reflects the temporally finite period in which juvenile fluke are viable within the gastrointestinal lumen, leaving a short period of time for penetration of the intestinal mucosa. The results suggest that any damage (or increased permeability) of the tract is reversible.

With regard to production losses, the study suggests a significant metabolic insult (endotoxemia). Acute endotoxemia in cattle has recently been demonstrated to cause a significant and quantifiable negative energy balance.[58] It is possible that an extended period of intestinal permeability following juvenile fluke damage, and the resultant endotoxemia, accounts for a significant and persistent negative energy balance which would be difficult to address.

To the authors' knowledge, this is the first demonstration of endotoxemia associated with *Fasciola* infection. The longevity of the response and the lack of systemic clinical impact on animals are in contrast to the high levels of endotoxin detected. Additionally, there is extensive research examining models attempting to induce an endotoxemic state (leaky gut in production animals and sub-acute ruminal acidosis in ruminants), with mixed results.[25,59,60] The current study suggests a simpler, less clinically adverse model for systemic endotoxemia in cattle. Its repeatability in other domestic ruminants should be assessed and other biomarkers associated with elevated endotoxin examined.

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Conflicts of interest or sources of funding

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the content of the manuscript, and there is no financial interest to report.

References

1. Mehmood K, Zhang H, Sabir AJ et al. A review on epidemiology, global prevalence and economical losses of fasciolosis in ruminants. *Microb Pathog* 2017;109:253–262.
2. Dargie J. The impact on production and mechanisms of pathogenesis of trematode infections in cattle and sheep. *Int J Parasitol* 1987;17:453–463.
3. Mazeri S, Rydevik G, Handel I et al. Estimation of the impact of *Fasciola hepatica* infection on time taken for UK beef cattle to reach slaughter weight. *Sci Rep* 2017;7:7319.
4. Malone JB, Loyacano AF, Hugh-Jones ME et al. A three-year study on seasonal transmission and control of *Fasciola hepatica* of cattle in Louisiana. *Prev Vet Med* 1984;3:131–141.
5. Hicks R, Gill DR, Owens F et al. *Impact of liver flukes on the performance of feedlot steers*. Oklahoma State University, USA, Miscellaneous publication-Agricultural Experiment Station, 1989.
6. Sangster NC, Martínez-Moreno A, Pérez J. Pathology, pathophysiology and clinical aspects. In: *Fasciolosis*. Nosworth Way, Wallingford, Oxfordshire UK, CABI Wallingford UK, 2021;145–179.
7. Hurtrez-Boussès S, Meunier C, Durand P et al. Dynamics of host–parasite interactions: the example of population biology of the liver fluke (*Fasciola hepatica*). *Microbes Infect* 2001;3:841–849.
8. Gnauck A, Lentle RG, Kruger MC. Chasing a ghost? – issues with the determination of circulating levels of endotoxin in human blood. *Crit Rev Clin Lab Sci* 2016;53:197–215.
9. Nádházi Z, Takáts A, Offenmüller K et al. Plasma endotoxin level of healthy donors. *Acta Microbiol Immunol Hung* 2002;49:151–157.
10. Wittek T, Fürll M, Constable PD. Prevalence of endotoxemia in healthy postparturient dairy cows and cows with abomasal volvulus or left displaced abomasum. *J Vet Intern Med* 2004;18:574–580.
11. Mayr FB, Yende S, Angus DC. Epidemiology of severe sepsis. *Virulence* 2014;5:4–11.
12. Bauer M, Gerlach H, Vogelmann T et al. Mortality in sepsis and septic shock in Europe, North America and Australia between 2009 and 2019- results from a systematic review and meta-analysis. *Crit Care* 2020;24:239.
13. Ametaj BN, Zebeli Q, Iqbal S. Nutrition, microbiota, and endotoxin-related diseases in dairy cows. *Revista Brasileira de Zootecnia* 2010;39:433–444.
14. Andersen PH. Bovine Endotoxicosis – some aspects of relevance to production diseases. A review. *Acta Vet Scand* 2003;44:S141.
15. Aschenbach JR, Seidler T, Ahrens F et al. Luminal salmonella endotoxin affects epithelial and mast cell function in the proximal colon of pigs. *Scand J Gastroenterol* 2003;38:719–726.
16. Chen J, Tellez G, Richards JD et al. Identification of potential biomarkers for gut barrier failure in broiler chickens. *Front Vet Sci* 2015;2:14.
17. Sekirov I, Russell SL, Antunes LC et al. Gut microbiota in health and disease. *Physiol Rev* 2010;90:859–904.
18. Van Milligen FJ, Cornelissen JBWJ, Bokhout BA. Protection against *Fasciola hepatica* in the intestine is highly correlated with eosinophil and immunoglobulin G1 responses against newly excysted juveniles. *Parasite Immunol* 1999;21:243–251.
19. Raadsma HW, Kingsford NM, Suharyanta STW et al. Host responses during experimental infection with Fasciola gigantica or *Fasciola hepatica* in Merino sheep I. Comparative immunological and plasma biochemical changes during early infection. *Vet Parasitol* 2007;143:275–286.
20. Rioux MC, Carmona C, Acosta D et al. Discovery and validation of serum biomarkers expressed over the first twelve weeks of *Fasciola hepatica* infection in sheep. *Int J Parasitol* 2008;38:123–136.
21. Moxon JV, Flynn RJ, Golden O et al. Immune responses directed at egg proteins during experimental infection with the liver fluke *Fasciola hepatica*. *Parasite Immunol* 2010;32:111–124.
22. Escamilla A, Bautista MJ, Zafra R et al. *Fasciola hepatica* induces eosinophil apoptosis in the migratory and biliary stages of infection in sheep. *Vet Parasitol* 2016;216:84–88.
23. Wood IB, Amaral NK, Bairden K et al. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Vet Parasitol* 1995;58:181–213.
24. Vercruysse J, Holdsworth P, Letonja T et al. International harmonisation of anthelmintic efficacy guidelines. *Vet Parasitol* 2001;96:171–193.
25. Andersen PH, Bergelin B, Christensen KA. Effect of feeding regimen on concentration of free endotoxin in ruminal fluid of cattle. *J Anim Sci* 1994;72:487–491.
26. Khafipour E, Krause DO, Plaizier JC. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. *J Dairy Sci* 2009;92:1060–1070.
27. Li S, Khafipour E, Krause DO et al. Effects of subacute ruminal acidosis challenges on fermentation and endotoxins in the rumen and hindgut of dairy cows. *J Dairy Sci* 2012;95:294–303.
28. Jacob AI, Goldberg PK, Bloom N et al. Endotoxin and bacteria in portal blood. *Gastroenterology* 1977;72:1268–1270.
29. Carroll JA, Reuter RR, Chase CC Jr et al. Profile of the bovine acute-phase response following an intravenous bolus-dose lipopolysaccharide challenge. *Innate Immun* 2009;15:81–89.

30. Carroll J, Burdick N, Reuter R et al. Differential acute phase immune responses by Angus and Romosinuano steers following an endotoxin challenge. *Domest Anim Endocrinol* 2011;41:163–173.
31. Raetz CR, Whitfield C. Lipopolysaccharide endotoxins. *Annu Rev Biochem* 2002;71:635–700.
32. Emmanuel DGV, Dunn SM, Ametaj BN. Feeding high proportions of barley grain stimulates an inflammatory response in dairy cows. *J Dairy Sci* 2008;91:606–614.
33. Gozho GN, Plaizier JC, Krause DO et al. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. *J Dairy Sci* 2005;88:1399–1403.
34. Plaizier JC, Krause DO, Gozho GN et al. Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. *Vet J* 2009;176:21–31.
35. Zebeli Q, Metzler-Zebeli BU, Ametaj BN. Meta-analysis reveals threshold level of rapidly fermentable dietary concentrate that triggers systemic inflammation in cattle. *J Dairy Sci* 2012;95:2662–2672.
36. Ceciliani F, Ceron JJ, Eckersall PD et al. Acute phase proteins in ruminants. *J Proteomics* 2012;75:4207–4231.
37. Xu T, Cardoso FC, Pineda A et al. Grain challenge affects systemic and hepatic molecular biomarkers of inflammation, stress, and metabolic responses to a greater extent in Holstein than Jersey cows. *J Dairy Sci* 2017;100:9153–9162.
38. Andersen PH, Jarløv N, Hesselholt M et al. Studies on in vivo endotoxin plasma disappearance times in cattle. *J Vet Med Series A* 1996;43:93–101.
39. Rodríguez-Lecompte J, Kroeker A, Ceballos-Márquez A et al. Evaluation of the systemic innate immune response and metabolic alterations of nonlactating cows with diet-induced subacute ruminal acidosis. *J Dairy Sci* 2014;97:7777–7787.
40. Maxie MG, Valli VE, Robinson GA et al. Studies with radioactive endotoxin. I. Clearance of 51Cr-labelled endotoxin from the blood of calves. *Can J Comp Med* 1974;38:347–366.
41. Andersen PH. Bovine endotoxicosis–some aspects of relevance to production diseases. A review. *Acta Veterinaria Scandinavica* 2003;44:1–15.
42. Chang G, Zhang K, Xu T et al. Feeding a high-grain diet reduces the percentage of LPS clearance and enhances immune gene expression in goat liver. *BMC Vet Res* 2015;11:1–11.
43. Garcia M, Bradford B, Nagaraja T. Invited review: ruminal microbes, microbial products, and systemic inflammation. *Prof Anim Sci* 2017;33:635–650.
44. Jirillo E, Caccavo D, Magrone T et al. The role of the liver in the response to LPS: experimental and clinical findings. *J Endotoxin Res* 2002;8:319–327.
45. Nolan JP. Endotoxin, reticuloendothelial function, and liver injury. *Hepatology* 1981;1:458–465.
46. Tran N, Ricafrente A, To J et al. *Fasciola hepatica* hijacks host macrophage miRNA machinery to modulate early innate immune responses. *Sci Rep* 2021;11:6712.
47. Celias DP, Corvo I, Silvane L et al. Cathepsin L3 from *Fasciola hepatica* induces NLRP3 inflammasome alternative activation in murine dendritic cells. *Front Immunol* 2019;10:552.
48. Aguayo V, Valdés Fernandez BN, Rodríguez-Valentín M et al. *Fasciola hepatica* GST downregulates NF-κB pathway effectors and inflammatory cytokines while promoting survival in a mouse septic shock model. *Sci Rep* 2019;9:2275.
49. Ramos-Benítez MJ, Ruiz-Jiménez C, Aguayo V et al. Recombinant *Fasciola hepatica* fatty acid binding protein suppresses toll-like receptor stimulation in response to multiple bacterial ligands. *Sci Rep* 2017;7:5455.
50. Noya V, Brossard N, Rodríguez E et al. A mucin-like peptide from *Fasciola hepatica* instructs dendritic cells with parasite specific Th1-polarizing activity. *Sci Rep* 2017;7:–40615.
51. Figueroa-Santiago O, Espino AM. *Fasciola hepatica* ESPs could indistinctly activate or block multiple toll-like receptors in a human monocyte cell line. *Ann Clin Pathol* 2017;5:1112.
52. Rodríguez E, Noya V, Cervi L et al. Glycans from *Fasciola hepatica* modulate the host immune response and TLR-induced maturation of dendritic cells. *PLoS Negl Trop Dis* 2015;9:e0004234.
53. Japa O, Hodgkinson JE, Emes RD et al. TGF-β superfamily members from the helminth *Fasciola hepatica* show intrinsic effects on viability and development. *Vet Res* 2015;46:29.
54. Guasconi L, Serradell MC, Garro AP et al. C-type lectins on macrophages participate in the immunomodulatory response to *Fasciola hepatica* products. *Immunology* 2011;133:386–396.
55. Dowling DJ, Hamilton CM, Donnelly S et al. Major secretory antigens of the helminth *Fasciola hepatica* activate a suppressive dendritic cell phenotype that attenuates Th17 cells but fails to activate Th2 immune responses. *Infect Immun* 2010;78:793–801.
56. Hamilton CM, Dowling DJ, Loscher CE et al. The *Fasciola hepatica* tegumental antigen suppresses dendritic cell maturation and function. *Infect Immun* 2009;77:2488–2498.
57. McGonigle L, Mousley A, Marks NJ et al. The silencing of cysteine proteases in *Fasciola hepatica* newly excysted juveniles using RNA interference reduces gut penetration. *Int J Parasitol* 2008;38:149–155.
58. Kvidera S, Horst E, Abuajamieh M et al. A procedure to estimate glucose requirements of an activated immune system in steers. *J Anim Sci* 2016;94:4591–4599.
59. Fink MP. Animal models of sepsis. *Virulence* 2014;5:143–153.
60. Poli-de-Figueiredo LF, Garrido AG, Nakagawa N et al. Experimental models of sepsis and their clinical relevance. *Shock* 2008;30(Suppl 1):53–59.

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