Indian Journal of Animal Sciences **88** (1): 69–00, January 2018/Review Article
https://doi.org/10.56093/ijans.v88i1.79502

Lipopolysaccharide-induced changes in physiological and haematological variables of Jakhrana goats

S P SINGH[1], R NATESAN, N SHARMA[3] and A KUMAR[4]

ICAR- Central Institute for Research on Goats, Mathura, Uttar Pradesh 281 122 India

Received: 15 May 2017; Accepted: 12 July 2017

Key words: Lipopolysaccharide, Haematology, Physiological variables, Jakhrana goat

Bacterial infection adversely affects farm animal productivity by increasing morbidity and is a major cause of economic losses in livestock. Lipopolysaccharide (LPS), also known as endotoxin, is the major virulence factor of gram-negative bacteria (Heumann and Roger 2002). LPS is capable of stimulating immunocompetent cells and activating cytokines pathway that leads to the secretion of pro-inflammatory mediators, which initiate responses that involve in fever and metabolic alterations. Some animal species like mice and Rhesus monkey have a capability to develop a certain level of tolerance against endotoxin even when a load of the pathogen is high (Warren *et al.* 2010). However, some other animals likerabbit and small ruminants are relatively more sensitive to the endotoxin (Schiffer *et al.* 2002,Warren *et al.* 2010). Circulating monocytes and neutrophils are the main target cells among the leukocytes that are involved in protection against LPS and these cells regularly express CD-14 and toll-like receptor 4, which are essential for recognition of LPS and activation of innate immune system (Park *et al.* 2013).

However, information abouttime dependent effectsof LPS challenge on physiological and haematological response in tropical goat breed is not available in literature. Therefore, the aim of present study was to identify LPS-induced changes in certain physiological and haematological responses in Jakhranagoats so that most efficient indicators of acute bacterial infection in tropical goats can be identified for longitudinal studies.

Eight clinically healthy Jakhrana goats (3 to 4 years of age; male, 41.9±1.9 kg BW) were included in this study. Goats were housed and given free access to hay and green fodder in shade. The animals were allowed to acclimate for 10 days prior to the study. Thereafter, equal no. of goats were divided randomly into two groups i.e. control (CON; n = 4) and treatment (LPS; n = 4). Sterile physiological saline (20 µL/kg BW) or bacterial LPS (1 µg/kg body weight; *Escherichia coli*0111: B4, Sigma chemicals, St. Louis MO) was intravenouslyadministered into the animals of CONorLPS group, respectively. The dose of LPS was selected based on our previous pilot trial; in which similar dose of LPS generate febrile condition with certain behavioural changes such as reduction in feeding and rumination, but without any very serious manifestation or shock to the animals. The stock solution of LPS was prepared by dissolving 200 µg LPS in 4 mL of pyrogen-free sterile physiological saline. Thus, injected volume of LPS solution ranged from 680µL to 970 µL, depending on the BW ofindividual animal. Blood sampling and recording of cardinal physiological responses [rectal temperature (RT), respiratory rate (RR) and heart rate (HR)] were done at about 10 min before (0 h) and at different time intervals after LPS or saline infusion.

Recordings of RT, RR and HR were performed at 10 min before(0 h),and at 2, 4, 6, 8, 26, 32 and 50 h after LPS infusion. All measurements of physiological variables were done under relatively calm and quietcondition with minimun undue stress to the animals. Recording of RT (°C)was done with the help of clinical digital thermometer, which was inserted about 3–4 cm into the rectum for about 60 sec. The HR (beats per minute) and RR (breath per minute) were recorded using a medical stethoscope (Amex Macro Tone Stethoscope) at the fourth left inter-costal space behind the animal's left elbow and above the animal's left elbow over a period of 1 min., respectively. The recording of RR was done before the recording of HR and RT.

Whole blood samples were collected by jugular venipuncture into 15 mL centrifuge tubes containing K_2EDTA about 10 min before(0 h) and at 2, 4, 6, 8, 26 and 32h afterLPS infusion. Samples were packaged on ice immediately after collection andwere taken to the laboratory in ice box for complete haematological analysis that included quantification of white blood cells (WBC; $\times 10^3$/mm^3), red blood cells (RBC;$\times 10^6$/mm^3), haematocrit (Hct;%), mean corpuscular volume (MCV; fL), haemoglobin (Hb; g/dL), mean corpuscular haemoglobin (MCH; fmol/cell), mean corpuscular haemoglobin concentration (MCHC; g/dL), red blood cells distribution

Present address: [1]Scientist (spsinghmail1@gmail.com), [2]Senior Scientist (ramacirg@gmail.com), [3]Research Fellow (sharmanandini.2188@gmail.com), Animal Physiology and Reproduction Division; [4]Principal Scientist (akumar63 @gmail.com), Goat Health Division.

width (RDW;%), lymphocytes (%), monocytes (%), granulocytes (%) and platelet indices [platelet count (×1000/µL), mean platelet volume (MPV; fL), plateletcrit (Pct;%), platelet distribution width (PDW;%)]. Blood cells analysis was made with the automatic haematologicalanalyzer (MS4SE, Melet Schloesing Laboratories, Osny, France).

To account for multiple mean comparisons of physiological and haematological variables, an independent sample t-test or corresponding non-parametric test (Mann–Whitney U test) was performed for individual time point. Variations were assessed by probability value (*P*) with the following levels of significance: $P < 0.05$, $P < 0.01$, $P < 0.001$ and a trend $P \leq 0.1$. Statistical analyses were performed with SPSS (version 16.0, SPSS Inc., Chicago, IL) software. Data are presented as a mean±standard error of the mean.

Figure 1a, b and c represent the time course of changes in RT, RR and HRafter saline or LPSinfusionin CON and LPS goats, respectively. Increased RR and HR in LPS goatswere observed after 2 h of LPS administration compared to the CON animals($P< 0.05$). However, RT was not significantly ($P = 0.180$)different among the groups at this time point (2 h). Effect of LPS on RT and RR was not observed later than 6 h after LPS infusion whereas, HR remains significantly ($P< 0.05$) higherup to 26 hin LPS goats compared to the CON goats.

Among different haematological variables, WBC countand its subpopulation (lymphocytes, monocytes and granulocytes) were greatly affected by LPS infusion. Thesignificant difference in these variables was observed as early as 2 h post-LPS infusion and this difference remains

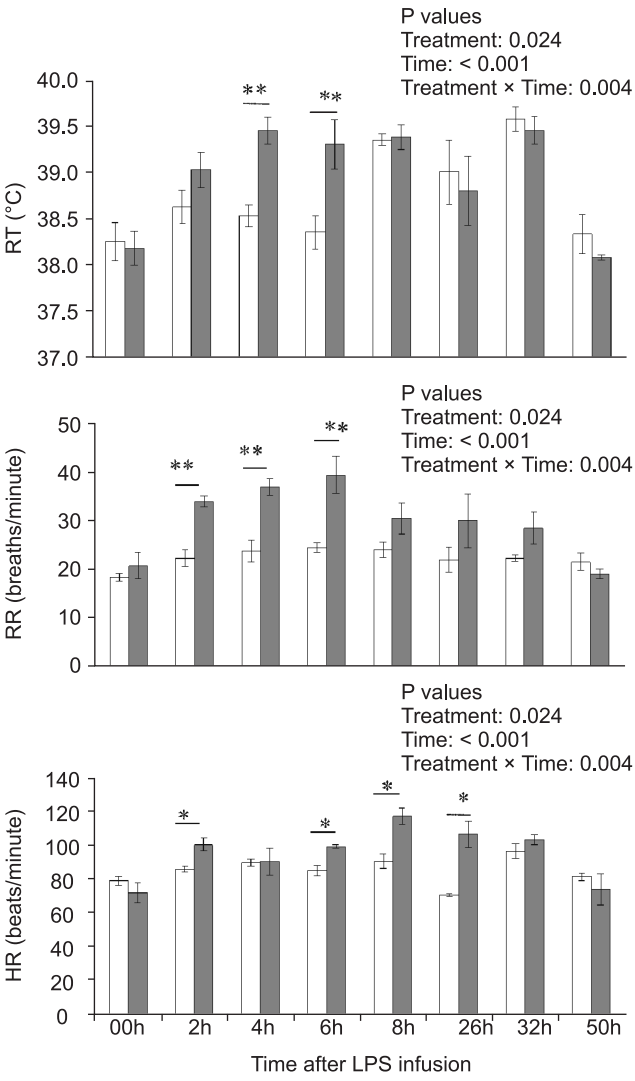

Fig. 1. Physiologicalvariables [Fig. 1a: rectal temperature (RT); Fig 1b;respiration rate (RR); Fig. 1c:heart rate (HR)] at different time intervals before and aftereithernormal saline (CON; n=4,)or LPS infusion (LPS, n=4,).

*:P<0.05; **:P ≤0.01, ***: P ≤0.001

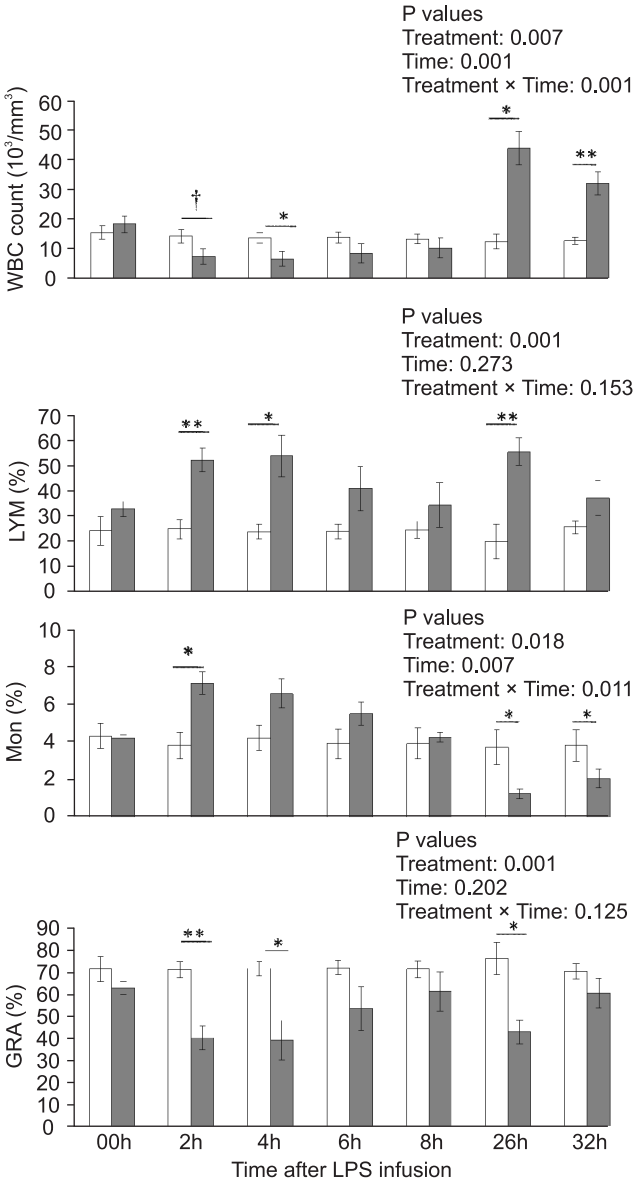

Fig. 2. Haematological variables [Fig. 2a: White blood cell (WBC) count; Fig 2b; lymphocyte (LYM); Fig. 2c: monocyte (MON) and Fig. 2d: granulocyte (GRA)] at different time intervals before and aftereithernormal saline (CON, n=4,)or LPS infusion (LPS, n= 4,).

*:P<0.05; **:P ≤0.01, ***: P≤0.001, †:≤0.1

Table 1. Time dependent changes in haematological variables (mean ± SEM) after infusion ofeither physiological saline (CON; n = 4)or LPS (LPS; n = 4)

Time(h)	Group	RBC($\times10^6$/mm^3)	Hct(%)	MCV(fL)	Hb(g/dL)	RDW(%)	MCH(fmol/cell)	MCHC(g/dL)
0	1	11.61 ±0.39	15.98 ±0.87	13.78 ±0.32	5.75 ±0.30	18.00 ±0.33	4.90 ±0.11	35.95 ±0.18
	2	11.91 ±0.04	16.98 ±0.57	14.28 ±0.48	6.08 ±0.14	25.13 ±1.01	5.08 ±0.13	35.78 ±0.55
2	1	11.94 ±0.41	16.90 ±0.85	14.18 ±0.21	5.70 ±0.30	25.08 ±0.35	4.70 ±0.09*	33.65 ±0.18
	2	12.42 ±0.31	18.65 ±0.91	15.05 ±0.51	6.40 ±0.27	23.33 ±1.91	5.10 ±0.14	34.33 ±0.43
4	1	11.36 ±0.39	16.13 ±1.03	14.18 ±0.42	5.43 ±0.35	21.63 ±1.73	4.70 ±0.15	33.60 ±0.30
	2	12.20 ±0.52	18.38 ±0.82	15.13 ±0.44	6.03 ±0.22	23.65 ±0.82	4.90 ±0.21	32.83 ±0.69
6	1	11.71 ±0.36	16.80 ±0.72	14.38 ±0.26	5.50 ±0.41	20.15 ±1.93†	4.65 ±0.26	32.70 ±1.56
	2	12.23 ±0.40	18.50 ±0.81	15.18 ±0.49	5.83 ±0.26	24.93 ±0.41	4.70 ±0.20	31.45 ±0.41
8	1	11.62 ±0.47	16.78 ±0.87	14.48 ±0.23	5.48 ±0.43	22.15 ±1.65	4.65 ±0.22	32.53 ±1.25
	2	12.27 ±0.61	18.63 ±1.10	15.23 ±0.52	6.15 ±0.33	26.33 ±3.02	4.98 ±0.21	33.03 ±0.40
26	1	10.45 ±1.36	14.38 ±2.22	13.68 ±0.39	4.83 ±0.84	20.85 ±2.01†	4.48 ±0.30	33.10 ±1.76
	2	12.09 ±0.96	17.90 ±1.15	14.98 ±0.82	6.50 ±0.33	25.50 ±1.01	5.40 ±0.49	36.40 ±1.25
32	1	11.71 ±0.38	17.25 ±0.75	14.75 ±0.23	5.25 ±0.32	23.08 ±2.24	4.43 ±0.17	30.33 ±0.69
	2	12.80 ±0.75	19.55 ±1.50	15.28 ±0.50	6.33 ±0.54	24.55 ±0.68	4.88 ±0.24	32.25 ±0.71

RBC= red blood cells, Hct = haematocrit, MCV = mean corpuscular volume, Hb = haemoglobin, RDW = red blood cell distribution width,MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular hemoglobin concentration

* and† indicate significant difference or a trend, respectively, between CON and LPS goats at specific time point in the column

* :P< 0.05; ** : P< 0.01, †: 0.05<Pd" 0.1

at other time points during the study i.e. 4 h, 6 h, 26 h and 32 h (Fig 2a, b, c and d). 1.9 fold (14.38±2.28 vs 7.51±2.58; P = 0.093) and 2.1 fold (13.92±1.76 vs 6.77±2.49; P = 0.024) decline in WBC count of LPS goats was observed compared to CON goats, at 2 h and 4 h post-LPS administration, respectively. Nonetheless, WBC count of LPS goatsincreased by 3.5 fold (12.56±2.43 vs 44.25±5.56; P = 0.002) and 2.5 fold (12.93±1.11 vs 32.4±3.91; P = 0.003) at 26 h and 32 h after LPS administration compared to the CON goats (Fig 2a). Significant increase in lymphocyte (2.08 fold, P = 0.004) and monocyte (1.86 fold, P = 0.011) fraction, and decrease in granulocyte fraction (1.76 fold, P= 0.003) was observed in LPS goats compared to CON goats at 2 h post-LPS infusion (Fig 2b,c,d).

Effect of LPS infusion on platelet indices (platelet count, MPVandPct) was observed only at 2 h after LPS infusion and their values were remain similar among the groups at subsequent time points (Table 2). Platelet count and Pct in LPS goats decreased by about 30% (P= 0.013) and 26.1% (P= 0.008) at 2 h compared to the CON goats. No significant difference in RBC count, Hct, MCV, Hb, MCH, MCHC and PDW was observed among groups during the entire study period (Table 1 and 2).

Although there are few studies that have examined metabolic effects of LPS in farm animals, the responses of physiological and haematological variables to systemic exposure to endotoxin in tropical goats remained unknown. Accordingly, the present study was designed for evaluation of time dependent changes in cardinal physiological and haematological responses to intravenousLPSinfusion in adult Jakhrana goats. The dose of LPS that is used in this study (1 µg/kg BW) has producedchanges in certain variables that lasted for at least 32 h post injection.

Higher RR and HR in LPS goats were observed at 2 h that remain lasted up to 6 h and 26 h post-LPS

Table 2. Time dependent changes in platelet indices (mean ± SEM) after infusion of either physiological saline (CON; n = 4) or LPS (LPS; n = 4)

TIME (h)	Group	Platelet count (×1000/µL)	MPV (fL)	Pct (%)	PDW (%)
0	1	323.25± 39.54	5.53± 0.11	0.18± 0.02	3.75± 0.37
	2	388.25± 46.75	5.10± 0.14	0.20± 0.02	4.43± 0.69
2	1	471.25± 39.74*	4.93± 0.09†	0.23± 0.01**	7.05± 0.29
	2	331.25± 5.41	5.18± 0.08	0.17± 0.00	6.50± 0.98
4	1	394.00± 62.63	5.18± 0.15	0.20± 0.03	5.55± 1.11
	2	359.75± 65.41	5.10± 0.12	0.18± 0.03	7.35± 0.32
6	1	443.75± 80.97	4.88± 0.32	0.22± 0.03	6.08± 0.99
	2	388.50± 40.73	4.95± 0.09	0.19± 0.02	7.68± 0.46
8	1	471.50± 75.08	4.93± 0.14	0.23± 0.03	7.30± 0.35
	2	372.25± 57.43	5.03± 0.13	0.19± 0.03	7.73± 0.47
26	1	397.25± 38.37	5.20± 0.15	0.20± 0.01	5.80± 0.76
	2	376.50± 77.11	5.10± 0.16	0.19± 0.03	6.65± 1.09
32	1	492.00± 85.18	4.85± 0.23	0.23± 0.03	6.70± 0.70
	2	396.00± 35.68	4.43± 0.51	0.20± 0.02	8.23± 0.09

MPV=mean platelet volume, Pct = plateletcrit, PDW = platelet distribution width

* and† indicate significant difference or a trend, respectively, between CON and LPS goats at specific time point in the column

* :P< 0.05; ** : P< 0.01, †: 0.05 < P d" 0.1

administration, respectively. Whereas, significantly higher RT was observed only at 4 h and at6 h after LPS treatment, thereafter no difference in RT among the groups was observed. This is in accordance with earlier reports, in which higher HR after LPS injection was observed in calves (Konigsson*et al.* 2002, Husler and Blum 2001) and ewe (Yates *et al.* 2011). Due to the fact observed in the present study that the effect of LPS on HR is started early (at 2 h) and remain lasted longer(up to 26 h post-LPS infusion) compared to RT and RR,change in HR may be considered asthe most appropriatephysiological variable, among those studied, for monitoring time dependent effects of LPS in goats.

Effect of endotoxins on RBC count, Hband Hct are reported by earlier researchers in ewe (Yates *et al.* 2011),pigs (Thorgersen*et al.* 2010).In contrast to this, mean RBC count, Hct, Hb, MCV in LPS goats was similar to those CONgoats at all time points, suggesting them as relatively more stable haematological variables and the LPS dosage (1 mg/kg BW) used in the study may not sufficient enough to affect erythropoiesis and/or destruction of RBCs in this tropical goat breed.

The effect of LPS was more pronounced on WBCs and its subpopulation (granulocytes, lymphocytes and monocytes) even after26 h of LPS administration.

Significant rise in monocyte fraction in LPS goatsat 2 h of LPS treatment compared to CON goats and subsequent release of monokinesmay result into increased number of WBCs and lymphocyte at 26 and 32 h post-LPS administration. Increase number of lymphocytes and monocytes in circulation may provide defences system in the body to counteract harmful effects of LPS.

In this study, significant reduction of platelet count and Pct, and increase in MPV was observed within 2 hof LPS administration in LPS goats compared to the CON goats. However, the effect of LPS on platelet indices was not observed during subsequent samplings. This is in agreement with previous reports that administration of LPS decreases the number of platelets in circulation within a short period in mice (Jayachandran*et al.* 2007) and pigs (Kvidera*et al.* 2016). This is perhaps due to increased accumulation of platelets in the liver and lungs (Ohtaki*et al.* 2003) or as a result of amplifying secretion-dependent platelet aggregation in blood (Zhang *et al.* 2009). These findings indicate that the platelet indices, more specifically,platelet count, MPV and Pctare appropriate to follow up only short term early effects of low dose of bacterial infection in goats.

Bacterial endotoxins from gram-negative bacteria are potent mediators for in vivo induction and release of cytokines such as interleukin-1, tumor necrosis factor, interleukin-6 and colony stimulating factor. Though the mechanism by which this occurs is not fully understood in farm animals including goats, it becomes apparent that many of the LPS-related physiological and haematological responses are mediated directly or indirectly by these endogenous cytokines individually or in combination with other mediators of inflammation secreted by different activated cells including leukocytes.

SUMMARY

The present study showstime dependent variations in different physiological and haematological variables in response to systemic LPS exposurein goat. The results ofthis study revealed thatRT, RR and HR were significantly increased at different time points until 26 h post-LPS exposure. All leukocyte variables including WBC count and leucocyte fractions i.e. lymphocyte, monocyte and granulocytes, responded to LPS at some point within 32 h of LPS administration. However, effect of LPS on platelet indices (platelet count, MPV and Pct) was observed only during early period of LPS infusion. No difference in RBC count, Hct, MCV, Hb, MCH and MCHC was observed among the groups, in present experimental conditions. This study emphasizes the significance of considering HR and certain haematological variables such as WBC count and its fractions i.e. lymphocytes and monocyteswhen working with longitudinal effects of acute bacterial diseases and pathophysiology of LPS associated illness in tropical goat breeds.

ACKNOWLEDGMENTS

The authors are thankful to the support extended by Director of the Institutefor providing necessary facilities to carry out this study.The technical assistance by Mr. Dinesh Bhatt and help from Dr. Deepak Dwivedi,Mr. Ranjeet Singh and Ms. Monica Soni are also duly acknowledged. The study was supported by a grant from SERB-DST, Govt. of India, New Delhi (Grant No.YSS/2015/001518).

REFERENCES

Heumann D and Roger T. 2002. Initial responses to endotoxins and Gramnegative bacteria. *Clinica Chimica Acta* **323**: 59–72.

Hüsler B R and Blum J W. 2001. Nitrite/Nitrate responses to endotoxin in calves. *Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine* **48**: 487–500.

Jayachandran M, Brunn G J, Karnicki K, Miller R S, Owen W G and Miller V M. 2007. *In vivo* effects of lipopolysaccharide and TLR4 on platelet production and activity: implications for thrombotic risk. *Journal of Applied Physiology* **1**: 429–33.

Königsson K, Odensvik K and Kindahl H. 2002. Endocrine, metabolic and clinical effects of intravenous endotoxin injection after pre-treatment with Meloxicam in heifers. *Journal of Veterinary Medicine* **49**: 408–14.

Kvidera S K, Horst E A, Laughlin E J, Mayorga J, Seibert J T, Abuajamieh M, Al-Qaisi M, Lei S, Keating A F, Ross J W and Baumgard L H. 2016. Effects of repeated LPS infusion on hematologic, metabolic, and intestinal histology parameters. *FASEB Journal* **30**: 925–13.

Ohtaki Y, Shimauchi H, Yokochi T, Takada H and Endo Y. 2003. *In vivo* platelet response to lipopolysaccharide in mice: proposed method for evaluating new antiplatelet drugs. *Thrombocyte Research* 108: 303–09.

Park B S and Lee J O. 2013. Recognition of lipopolysaccharide

pattern by TLR4 complexes. *Experimental and Molecular Medicine* **45**: e66.

Schiffer E R, Reber G D, Moerloose P and Morel D R. 2002. Evaluation of unfractionated heparin and recombinant hirudin on survival in a sustained ovine endotoxin shock model. *Critical Care Medicine* **30**: 2689–99.

Thorgersen E B, Hellerud B C, Nielsen E W, Barratt-Due A, Fure H, Lindstad J K, Pharo A, Fosse E, Tonnessen T I, Johansen H T, Castellheim A and Mollnes T E. 2010. CD14 inhibition efficiently attenuates early inflammatory and hemostatic responses in *Escherichia coli* sepsis in pigs. *FASEB Journal* **24**: 712–22.

Warren H S, Fitting C, Hoff E, Adib-Conquy M, Beasley-Topliffe L and Tesini B. 2010. Resilience to bacterial infection: difference between species could be due to proteins in serum. *Journal of Infectious Diseases* **201**: 223–32.

Yates D T, Löest C A, Ross T T, Hallford D M, Carter B H and Limes S W. 2011. Effects of bacterial lipopolysaccharide injection on white blood cell counts, hematological parameters, and serum glucose, insulin, and cortisol concentrations in ewes fed low- or high protein diets. *Journal of Animal Science* **89**: 4286–93.

Zhang G, Han J, Welch E J, Ye R D, Voyno-Yasenetskaya T A, Malik A B, Du X and Li Z. 2009. LPS stimulates platelet secretion and potentiates platelet aggregation via TLR4/MyD88 and the cGMP-dependent protein kinase pathway. *Journal of Immunology* **182**: 7997–8004.