

# Effects of polymyxin B on clinical signs, serum TNF- $\alpha$ , haptoglobin and plasma lactate concentrations in experimental endotoxaemia in sheep

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## Abstract

**Introduction:** The experiment evaluated the effects of intravenous administration of polymyxin B on experimental endotoxaemia in sheep. **Material and Methods:** Twenty clinically healthy fat-tailed sheep were randomly divided into: a group treated with 6,000 U/kg of polymyxin B, a group at 12,000 U/kg, and positive and negative controls. Endotoxaemia was induced by intravenous administration of lipopolysaccharide (LPS) from *E. coli* serotype O55:B5 at 0.5  $\mu\text{g}/\text{kg}$ . Polymyxin was infused intravenously along with 2.5 L of isotonic intravenous fluids at 20 mL/kg/h. The positive control group received LPS and 2.5 L of isotonic fluids, the negatives receiving just 2.5 L of isotonic fluids. Clinical signs were evaluated before and at 1.5, 3, 4.5, 6, 24, and 48 h after LPS administration. Blood was also sampled at the denoted hours and serum haptoglobin, tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), and plasma lactate concentrations were assayed. **Results:** The serum concentration of TNF- $\alpha$  in the positive control group increased significantly up to 48 h after LPS administration. The concentration of TNF- $\alpha$  was significantly different from those of the polymyxin B and positive control groups from 3 to 48 h; also, the concentrations of haptoglobin at different times in the polymyxin groups were lower than those of the positive control group and were significant at hours 3 to 48 ( $P < 0.05$ ). Following the LPS administration, haptoglobin and TNF- $\alpha$  concentrations changed without significant difference between the two polymyxin B groups. **Conclusion:** Polymyxin B (6,000 U/kg) restrained blood lactate concentrations. Furthermore, it significantly improved the clinical signs in endotoxaemic animals, including rectal temperature and heart and respiratory rates. Polymyxin B may be an antiendotoxic in fat-tailed sheep.

**Keywords:** sheep, lipopolysaccharide, polymyxin B, clinical signs, inflammatory biomarkers.

## Introduction

Gram-negative bacteria, especially *Escherichia coli*, play a major role in the development of endotoxaemia among sheep and other livestock (8, 20, 34). Current data suggest that endotoxin is liberated and enters general circulation during bacteriolysis or periods of rapid growth. Endotoxaemia is the usual form of toxemia, which leads to multiple organ dysfunctions such as cardiovascular, respiratory, and haemostatic and biochemical changes, may eventually result in death, and is a significant cause of mortality in farm animals. Lipopolysaccharides (LPS), also known as endotoxins, are an integral part of the outer membrane of the cell wall in Gram-negative bacteria (28). LPS stimulate many adverse systemic reactions and trigger the release of pro-inflammatory mediators (27, 32). Over the past years,

several therapeutic strategies have been designed for treatment and prevention of endotoxaemia. Therapies suggested for Gram-negative sepsis and septic shock involve utilisation of antibiotics to control the infection and intensive care support to correct the underlying dysfunctions in circulatory, ventilatory, and other organ systems. However, only a few of these therapies have been proved to be effective.

One of the approaches used for the treatment of endotoxaemia is inactivation of LPS, which can be attained by neutralisation of endotoxin. The inactivation goal may be achieved through administration of polymyxin B, which is a bactericidal antibiotic belonging to the group of peptide antibiotics that has long been known to specifically target LPS and provide protection against LPS lethality (4). Lipid A is the biologically active portion of LPS. Polymyxin B has the

capacity to bind with high affinity to this active portion of LPS and neutralise its biological activity, which leads to restriction of the proinflammatory cytokine cascade (14, 19). Treatment with polymyxin B to attenuate clinical endotoxaemia is frequently used in adult horses for conditions such as strangulating intestinal lesions, pleuropneumonia, and postpartum metritis, with beneficial effects (34). The effects of intravenous applications of pentoxifylline and polymyxin B on the acute-phase-response to *Escherichia coli* endotoxin in dwarf goats have been evaluated (29). However, no study has been carried out on the clinical impact and anti-inflammatory effects of polymyxin B alone on endotoxaemia in sheep. The present study has evaluated the clinical manifestations and some circulating inflammatory biomarkers, including haptoglobin, TNF- $\alpha$ , and lactate, in an ovine experimental model in which endotoxaemia was induced by LPS of *Escherichia coli* serotype O55:B5.

## Material and Methods

**Animal care.** Twenty clinically healthy five-month-old Iranian fat-tailed sheep, with an average body weight of  $20 \pm 1.5$  kg, were randomly selected for the project. All sheep had normal physical examination results and fibrinogen concentrations and complete blood count (CBC) results. Four weeks before the start of the experiments, each sheep was treated orally with albendazole (15 mg/kg; Dieverm 600, Razak Pharmaceutical Co, Iran) and subcutaneously with ivermectin (0.2 mg/kg; 1% Erfamectin, Erfan Pharmaceutical Co, Iran) to control probable internal and external parasites. All sheep were maintained in open-shed barns with free access to water, food, and shade. The ration included mainly alfalfa hay, corn silage, corn, and barley. At the beginning of the study the animals were not fasted. Subsequently, sheep were randomly assigned into four equal experimental groups comprising animals treated with polymyxin B at a dose of 6,000 U/kg (BI) or 12,000 U/kg (BII), negative controls, and positive controls.

**Chemicals and drugs.** Phenol-extracted LPS from *Escherichia coli* serotype O55:B5 (product no. L2880, Sigma-Aldrich, Germany) were used at a dose of 0.5  $\mu$ g/kg as a source of endotoxin, inducing endotoxaemia in sheep (36). The endotoxin was diluted in sterile phosphate buffered saline (PBS, pH 7.4, Sigma-Aldrich, Germany) and then divided into 15 equal doses each containing 0.5  $\mu$ g/kg of the endotoxin, and stored at  $-80^{\circ}\text{C}$  until LPS administration (5, 6). Polymyxin B (6,000 and 12,000 U/kg) was intravenously administered to the sheep as a bolus according to an established experimental design (32). The intravenous fluid used was 5% dextrose plus 0.45% sodium chloride (Shahid Ghazi Pharmaceutical Co., Iran).

**LPS administration and treatment of endotoxaemia.** A 5.1 cm rinsing catheter was secured in

the left jugular vein and used for blood samplings and endotoxin and drug infusions. All sheep underwent a complete physical examination before and at 1.5, 3, 4.5, 6, 24, and 48 h after LPS injection. Clinical variables including rectal temperature and heart and respiratory rates were monitored during the experiments. Thawed LPS was diluted in 250 mL of normal saline and then infused intravenously at the rate of 10 mL/kg/h. Fluid therapy was performed in all experimental groups over a 30-min period after LPS injection by 5% dextrose plus 0.45% sodium chloride at the rate of 20 mL/kg/h. Immediately after the end of LPS infusion, polymyxin B was used along with the fluid therapy over 60 min. Polymyxin B was infused separately at 6,000 U/kg or 12,000 U/kg doses. Five sheep were randomly selected as the positive control group and received only LPS and an equal volume of saline solution (IV) instead of drugs. Similarly, another five sheep were randomly selected as the negative control group and received just intravenous fluid without any drugs or LPS.

**Blood sampling and biochemical assays.** Blood samples of all sheep were collected using fixed rinsing catheters before and at 1.5, 3, 4.5, 6, 24, and 48 h after LPS injection and kept in plain anticoagulant-containing (potassium oxalate/sodium fluoride) tubes. Immediately after collections, sera and plasma were separated by centrifugation at  $3,000 \times g$  for 10 min and stored at  $-22^{\circ}\text{C}$  until being assayed. Clinical variables including heart rate, respiratory rate, and rectal temperature were recorded at the same time as blood sample collection.

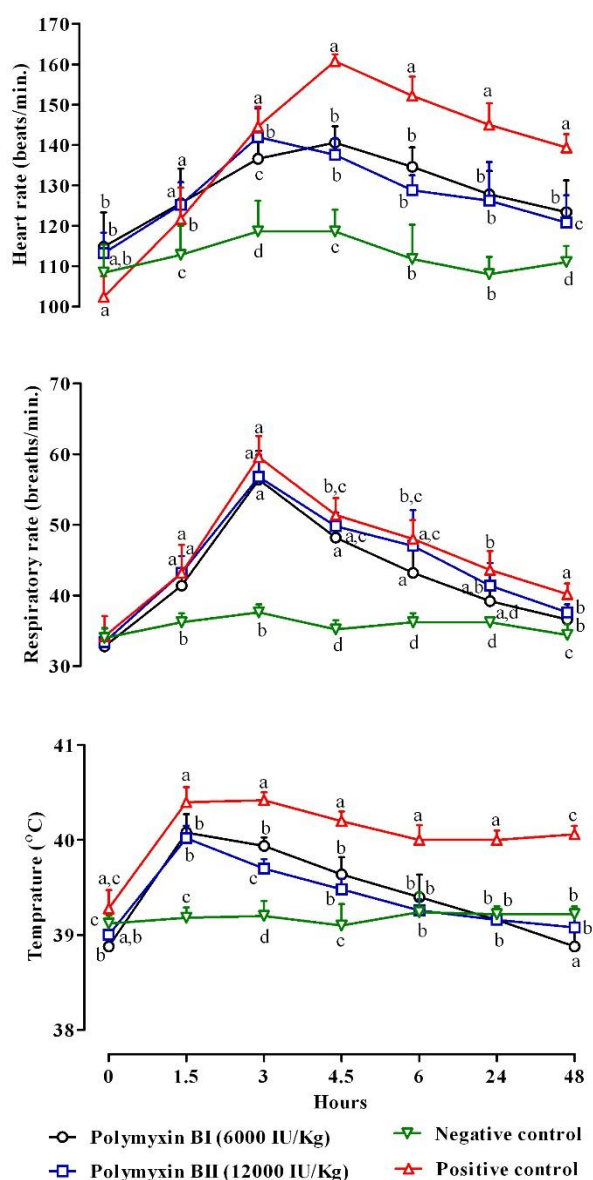
**Haptoglobin, TNF- $\alpha$ , and lactate determination.** Serum haptoglobin (Hp) was measured according to the preservation of peroxidase activity of haemoglobin procedure, which is directly proportional to the amount of Hp (Tridelta Development Ltd., Ireland). The lowest measuring interval of this test has been determined as 15.6 mg/L by the manufacturer. Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) was measured in serum by a solid phase sandwich ELISA (product no. AbC 606 Eurobio AbCys S.A., France). For TNF- $\alpha$ , a cut-off point of 129 ng/L represented 96% sensitivity and 93% specificity. Measurement of lactate was performed in plasma taken from potassium oxalate/sodium fluoride anticoagulant, using a Lactate Assay Kit considering the guidelines provided by the manufacturer (lactate monoliquid, product no. BXCO622, Biorexfars, Iran). The lactate had a sensitivity of 90% and specificity of 87%.

**Statistical analyses.** Data were expressed as mean  $\pm$  standard deviation (SD). To compare mean concentrations of different parameters among different experimental groups, over similar periods, one-way ANOVA with an LSD post-hoc test was used. As a multifactorial ANOVA test, a repeated measures ANOVA was used to estimate the trend change of each parameter in separate groups from hour 0 to hour 48. In this test, the time was considered as a covariable and the changing pattern of each parameter was evaluated during the study. A paired samples *t*-test was used to

determine the differences between two different time periods. The level of significance was set at  $P < 0.05$ . Statistical analysis was performed using SPSS software (SPSS for Windows, version 11.5, SPSS Inc, USA). Results by parameter are presented in Table 2.

## Results

**Clinical variables.** Clinical manifestations observed after LPS administrations were depression, incoordination, tremors, rapid laboured breathing, tachycardia, groaning, increased body temperature, anorexia, diarrhoea, and prostration. The results of monitored clinical variables are presented in Fig. 1.



**Fig. 1.** Clinical parameters (mean  $\pm$  SD) at different hours after intravenous LPS administration and in all experimental groups. Different letters indicate significant difference among groups ( $P < 0.05$ )

Heart rate, respiratory rate, and rectal temperature all increased significantly after LPS infusion in all LPS-treated groups. The increase in respiratory rate after LPS administration was obvious in all groups, with the exception of the negative control group. The values of vital signs returned to almost normal after 24 h in polymyxin B groups. In the polymyxin BI group, rectal temperature was significantly lower than that of the other endotoxin-treated groups at h 48 ( $P < 0.05$ ) (Fig. 1). Rectal temperature was significantly different between polymyxin B groups at hours 3 and 48 ( $P < 0.05$ ) (Fig. 1). The trend change of rectal temperature in both treatment groups altered significantly at different times (Fig. 1). After LPS injection at hour 1.5, respiratory rates in the polymyxin B groups significantly increased as compared with those of the negative control group ( $P < 0.05$ ). Significant differences in respiratory rates were not observed between the polymyxin B groups at the different denoted times. However, respiratory rates of positive controls and the polymyxin BI group were significantly different at 4.5, 6, 24, and 48 h ( $P < 0.05$ ). The heart rates in the polymyxin BII group were significantly lower at hour 48 than those of other LPS-treated groups ( $P < 0.05$ ) (Fig. 1). Although heart rates in the positive control group had an increasing trend up to 4.5 h and subsequently decreased, they were still higher at hour 48 in comparison with hour 0. There were no significant differences in heart rates between polymyxin B groups, with the exception of hours 3 and 48 ( $P < 0.05$ ). The trend change of heart rate in the positive control group significantly altered during the study at different times (Fig. 1).

**TNF- $\alpha$ .** Serum concentrations of TNF- $\alpha$  in the experimental groups at different times are presented in Table 1. No significant changing pattern was observed in the negative control group. The concentration of TNF- $\alpha$  increased rapidly after LPS administration in all experimental groups except the negative control group. Rapid elevations of TNF- $\alpha$  were observed and they were significantly different between hour 0 and the first time after endotoxin infusion ( $P < 0.05$ ). After LPS administration in the positive control group, serum concentrations of TNF- $\alpha$  elevated significantly up to 48 h and the trend of the changes was significant (Table 3), while the rate of increase in serum concentrations of TNF- $\alpha$  in the polymyxin B-treated groups presented a declining trend. Statistically significant differences in TNF- $\alpha$  concentration were observed separately between the polymyxin B and positive control groups from hour 3 to 48 after LPS administration. However, in this regard there were no significant differences between the polymyxin BI and polymyxin BII groups ( $P > 0.05$ ).

**Haptoglobin.** Serum concentrations of Hp at different times in each of the experimental groups are presented in Table 1. The concentrations elevated rapidly after LPS administration in all experimental groups ( $P < 0.05$ ), with the exception of the negative control group. There was no significant changing pattern in the negative control group (Table 1). The highest serum

**Table 1.** TNF- $\alpha$ , Hp, and lactate concentrations (mean  $\pm$ SD) at different hours after intravenous LPS administration in all experimental groups

Parameters	Groups	Hours						
		0	1.5	3	4.5	6	24	48
TNF- $\alpha$ (ng/L)	Negative control	169 $\pm$ 85	178 $\pm$ 79	183 $\pm$ 77 <sup>ac</sup>	179 $\pm$ 79 <sup>a</sup>	182 $\pm$ 83 <sup>a</sup>	184 $\pm$ 85 <sup>a</sup>	190 $\pm$ 85 <sup>a</sup>
	Positive control	178 $\pm$ 31	223 $\pm$ 57	270 $\pm$ 37 <sup>b</sup>	325 $\pm$ 16 <sup>b</sup>	337 $\pm$ 10 <sup>b</sup>	336 $\pm$ 13 <sup>b</sup>	325 $\pm$ 14 <sup>b</sup>
	Polymyxin BI	169 $\pm$ 50	186 $\pm$ 47	191 $\pm$ 51 <sup>a</sup>	202 $\pm$ 53 <sup>a</sup>	191 $\pm$ 55 <sup>a</sup>	183 $\pm$ 50 <sup>a</sup>	180 $\pm$ 48 <sup>a</sup>
	Polymyxin BII	168 $\pm$ 25	182 $\pm$ 21	199 $\pm$ 38 <sup>ab</sup>	233 $\pm$ 63 <sup>a</sup>	213 $\pm$ 36 <sup>a</sup>	200 $\pm$ 20 <sup>a</sup>	194 $\pm$ 15 <sup>a</sup>
Hp (mg/L)	Negative control	232 $\pm$ 11	242 $\pm$ 14	246 $\pm$ 23 <sup>a</sup>	253 $\pm$ 21 <sup>c</sup>	259 $\pm$ 29 <sup>c</sup>	253 $\pm$ 33 <sup>c</sup>	253 $\pm$ 34 <sup>c</sup>
	Positive control	254 $\pm$ 33	301 $\pm$ 32	395 $\pm$ 50 <sup>c</sup>	448 $\pm$ 39 <sup>b</sup>	524 $\pm$ 44 <sup>b</sup>	565 $\pm$ 19 <sup>b</sup>	589 $\pm$ 14 <sup>b</sup>
	Polymyxin BI	248 $\pm$ 51	300 $\pm$ 100	330 $\pm$ 113 <sup>ac</sup>	374 $\pm$ 130 <sup>a</sup>	397 $\pm$ 141 <sup>a</sup>	439 $\pm$ 142 <sup>a</sup>	442 $\pm$ 150 <sup>a</sup>
	Polymyxin BII	261 $\pm$ 38	282 $\pm$ 50	299 $\pm$ 49 <sup>a</sup>	312 $\pm$ 56 <sup>ac</sup>	327 $\pm$ 65 <sup>ac</sup>	339 $\pm$ 68 <sup>ac</sup>	362 $\pm$ 83 <sup>ac</sup>
Lactate (g/L)	Negative control	0.67 $\pm$ 0.01	0.90 $\pm$ 0.01 <sup>b</sup>	1.23 $\pm$ 0.02 <sup>a</sup>	1.33 $\pm$ 0.03 <sup>c</sup>	1.35 $\pm$ 0.01 <sup>a</sup>	1.03 $\pm$ 0.01 <sup>a</sup>	0.65 $\pm$ 0.01 <sup>b</sup>
	Positive control	0.63 $\pm$ 0.01	0.68 $\pm$ 0.01 <sup>b</sup>	2.51 $\pm$ 0.03 <sup>b</sup>	3.50 $\pm$ 0.04 <sup>b</sup>	4.30 $\pm$ 0.01 <sup>c</sup>	1.80 $\pm$ 0.04 <sup>b</sup>	0.80 $\pm$ 0.01 <sup>bc</sup>
	Polymyxin BI	0.74 $\pm$ 0.01	1.21 $\pm$ 0.03 <sup>a</sup>	1.12 $\pm$ 0.06 <sup>a</sup>	2.06 $\pm$ 0.02 <sup>a</sup>	2.10 $\pm$ 0.07 <sup>a</sup>	1.15 $\pm$ 0.01 <sup>a</sup>	1.10 $\pm$ 0.03 <sup>ac</sup>
	Polymyxin BII	0.59 $\pm$ 0.01	0.65 $\pm$ 0.01 <sup>b</sup>	1.51 $\pm$ 0.04 <sup>a</sup>	2.48 $\pm$ 0.05 <sup>a</sup>	3.15 $\pm$ 0.08 <sup>b</sup>	1.70 $\pm$ 0.02 <sup>b</sup>	1.30 $\pm$ 0.02 <sup>a</sup>

Different letters in each column indicate significant difference ( $P < 0.05$ )

**Table 2.** P value of trend change of each parameter in separate groups from hour 0 to hour 48

Groups	Parameters					
	Hp	TNF- $\alpha$	Lactate	Heart rate	Respiratory rate	Temperature
Negative control	0.213	0.294	0.093	0.4	0.942	0.165
Positive control	0.001*	0.002*	0.001*	0.001*	0.287	0.049
Polymyxin BI	0.01*	0.071	0.021	0.03	0.249	0.012*
Polymyxin BII	0.009*	0.146	0.001*	0.566	0.888	0.001*

\* Stars indicate significant trend change for each parameter in different groups ( $P < 0.05$ ).

In this table the time is considered as a covariable

concentrations of Hp (589  $\pm$  14 mg/L) occurred at hour 48 after injection of LPS in the positive control group. Serum concentrations of Hp at different times in polymyxin B groups were significantly lower than those of the positive control group. Significant differences were generally found between hour 3 and hour 48 ( $P < 0.05$ ). There were no significant differences between Hp concentrations in the two polymyxin B groups (Table 1). Hp increased significantly in the positive control and both treatment groups in different times (Table 2).

**Lactate.** In all experimental groups, with the exception of negative control group, serum concentrations of lactate at the first time after LPS administration were significantly higher than the basis line concentrations at hour 0 ( $P < 0.05$ ) (Table 1). Blood lactate concentrations increased after LPS administration in the positive control group up to 6 h and subsequently declined after 48 h. The trend change of lactate increased significantly in the positive control group at different times (Table 2). In comparison with the positive control group, the polymyxin BI group revealed a significant reduction of plasma lactate concentration at different times. It is obvious that the polymyxin BI group's dose was able to restrain elevated concentrations of blood lactate. Although a statistically significant difference was noticed between the polymyxin groups at hour 6 and 24, it seems that the polymyxin BI dose had a better impact on reducing blood lactate concentrations than that of the polymyxin BII group (Table 1).

## Discussion

LPS is the major part of the outer membrane of Gram-negative bacteria and is released when bacteria

die or multiply. This process results in extreme production and liberation of inflammatory cytokines and subsequent adverse effects (3, 22). Current therapies suggested for Gram-negative sepsis and septic shock involve utilisation of antibiotics to control the infection, and intensive care support to correct the underlying dysfunctions of the circulatory and ventilatory systems, and other organ systems. The present experimental study was designed to assess the anti-endotoxic impacts of polymyxin B in an ovine model. The clinical effects of various anti-inflammatory drugs on clinical manifestations of experimentally-induced endotoxaemia in sheep have been investigated in several studies (5, 23, 24, 33, 34). In the present study, heart and respiratory rates and rectal temperature significantly increased following LPS administration in sheep. These clinical variables improved following polymyxin B administrations (6,000 and 12,000 U/kg) at different sampling periods. In two studies respectively addressing anti-inflammatory effects of dimethyl sulfoxide and pentoxifylline, it was found that these drugs had significant improving effects on rectal temperature and respiration and heart rates (23, 24). Wong *et al.* (33) reported the clinical efficacy of polymyxin B on experimentally-induced endotoxaemia in a group of foals and confirmed that polymyxin B improved clinical symptoms following injection of endotoxin. Van Miert *et al.* (29) studied the effects of polymyxin B on the acute phase response to *Escherichia coli* endotoxin in dwarf goats and found that pretreatment with polymyxin B significantly reduced body temperature, heart rate, and rumen motility. Infusion of polymyxin B alone caused tachycardia and inhibition of ruminal contraction in goats.

TNF- $\alpha$  is considered to be the most important pro-inflammatory cytokine (2, 3). Research addressing the

pathogenesis of endotoxaemia points to TNF- $\alpha$  as a dominant mediator of endotoxicity (31). In the present study, significant and rapid elevation of serum TNF- $\alpha$  concentration was observed before commencing intravenous fluid therapy in all endotoxin-treated groups. Infusion of polymyxin B was followed by marked and significant depression of serum concentration of TNF- $\alpha$  in polymyxin B groups compared with the positive control group. There were no significant differences between polymyxin B groups in terms of decreasing the concentrations of TNF- $\alpha$ . Increased serum concentration of TNF- $\alpha$  in response to endotoxaemia in sheep has been studied previously (6, 24). Parviainen *et al.* (17) used polymyxin B in treatment of experimentally-induced endotoxaemia in horses. They reported the dose-dependent effects of polymyxin B in doses of 1,100 and 5,200 U/kg in inhibiting effects of TNF- $\alpha$ . It was found that 3 h after polymyxin B injection, almost 75% of TNF- $\alpha$  activity decreased. MacKay *et al.* (13) stated that utilisation of polymyxin B (polymyxin B-dextran 70) in horses at a dose of 5,000 U/kg after injection of LPS caused a significant reduction in serum concentrations of TNF- $\alpha$  and IL-6 and reduced heart and respiration rates, as well as rectal temperature. The promising effects of using polymyxin B in intestinal strangulation injuries, pleuropneumonia, and postpartum metritis have been studied on adult horses (28). After an experimental induction of endotoxaemia in foals (single dose of 0.5  $\mu$ g LPS /kg), slow intravenous injection of polymyxin B (6,000 U/kg) resulted in a significant reduction of serum concentration of TNF- $\alpha$  and improvement of the clinical symptoms of endotoxaemia (33). The release of endotoxin in the blood stream is considered to be a stimulus for synthesis of acute-phase proteins (APPs) and inflammatory mediators. Due to the relatively short half-life of acute-phase proteins in the blood and the rapid response of APPs in sick animals, they can be considered to be a measure of animal health (7). In comparison with haematology and clinical tests, determination of acute-phase proteins for the diagnosis of various infectious diseases provides more sensitive results. APPs increase during the development of systemic inflammatory response syndrome and decrease in the recovery stages of inflammatory conditions (16). Acute-phase proteins, notably haptoglobin, and their alterations have been studied widely due to inflammatory and non-inflammatory conditions in various animal species including sheep (15, 25). Hp along with other APPs has been suggested as a marker of stress in animals (18). In ruminants, the concentration of circulating Hp is insignificant in healthy animals; however, it rises over 100-fold with immune stimulation (32). Additionally, Hp is a clinically useful variable for assessing the occurrence and severity of inflammatory diseases in sheep (25). In this research, the observed rapid meaningful rise in serum concentrations of Hp occurred in all sheep after 48 h following endotoxin infusion. This result agrees with experimentally induced

endotoxaemia in Iranian fat-tailed sheep where haptoglobin concentration significantly increased 48 h after infusion of endotoxin (23).

Although administration of polymyxin B decreased serum haptoglobin concentrations, there were no significant differences between the 6,000 and 12,000 U/kg groups in this regard. An increase in blood lactate concentrations occurred after LPS administration. It has been reported that blood lactate concentrations increased in the cases of sepsis and were frequently observed as the evidence of tissue hypoxia and/or lack of oxygen secondary to hypoperfusion (26). Acute circulatory failure and poor oxygenation of blood (*e.g.* hypotension) which occur after septic shock can cause development of lactic acidosis or underutilisation of lactate (11). As the shock progresses, metabolic acidosis aggravates and blood pH decreases. Hyperlactatemia is a marker of disease severity and a strong predictor of mortality (12). In a group of rabbits with experimentally induced endotoxaemia, serum lactate concentration increased following the injection of LPS of *Escherichia coli* (10). In the present study, the effect of polymyxin B on serum lactate concentration in fat-tailed sheep after LPS administration was found to be dose-dependent. In comparison with the positive control group, the polymyxin BI group (6,000 U/kg) experienced a significantly reduced blood lactate concentration. Wong *et al.* (33) reported similar findings in neonatal foals treated with intravenous administration of polymyxin B after a challenge with experimental endotoxaemia. Yamamoto *et al.* (35) evaluated the effect of direct haemoperfusion (DHP) (using polymyxin B-immobilised fibres as an extracorporeal blood filter) on endotoxaemic anaesthetised sheep and reported that it improved shock and deteriorated oxygenation during endotoxaemia, probably through the suppression of nitric oxide production. Van Miert *et al.* (29) established that most of the haematological and blood biochemical effects of *Escherichia coli* LPS can be reduced by polymyxin B pretreatment in dwarf goats.

Polymyxin B is a cationic, polypeptide antibiotic which possesses bactericidal activity against gram-negative bacteria. It has an extra advantage of binding to lipid A LPS and interacts with the lipid A-keto-3-deoxyoctulosonate region of the LPS molecule by forming inactive polymyxin B-LPS complexes (29). Although a number of anti-endotoxin proteins and peptides have been reported (1, 9, 21, 29), there is still no anti-endotoxin agent certified for clinical use in endotoxaemia in sheep.

In conclusion, polymyxin B acts as an anti-endotoxic drug by attenuating the acute-phase response following LPS administration *via* binding the circulating endotoxins. Based on the results of the present research, pro-inflammatory cytokine (TNF- $\alpha$ ) and pro-inflammatory hepatic APP (Hp) decreased following polymyxin B infusion at different doses after *Escherichia coli* serotype O55:B5 LPS administration in Iranian fat-tailed sheep. According to our results,

polymyxin B did not induce its effects in a dose-dependent manner and its anti- and pro-inflammatory effects at 6,000 and 12,000 U/kg doses were statistically similar except in lactate concentration, which reduced more in polymyxin B at 6,000 U/kg. Finally, it may be suggested that polymyxin B could be used as an anti-endotoxic drug against endotoxaemia in Iranian fat-tailed sheep. However, further investigations including prospective clinical trials may be required to confirm the role of polymyxin B in treatment of farm animal endotoxaemia. The results of the present study may suggest a new potential therapeutic regimen for endotoxaemia in small ruminant medicine.

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