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Haematological, Biochemical and Coagulation Changes in Calves with Endotoxemia

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Abstract: The purpose of the study was to determine alterations in the hematological, biochemical and coagulation parameters in calves with Lipopolysaccharide (LPS)-induced experimental endotoxemia. Endotoxemia was induced via intravenous administration of LPS (0.1 μg kg^{-1}) in 50 mL of physiological 0.9% NaCl >30 min. The levels of serum Alanine aminotransferase (ALT), Gamma-glutamyl Transferase (GGT), Aspartate aminotransferase (AST), Blood Urea Nitrogen (BUN), Total Protein (TP), Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP), total bilirubin, glucose, Creatinine (Cre), Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT) and fibrinogen were measured. Severe abnormality were observed in some of these parameters. Changes in hematological, biocehmical and coagulation findings were related to endotoxemia. The LPS infusion resulted in significant changes of haematological, biochemical and coagluation parametrers.

Key words: Eendotoxemia, calves, haematology, coagulation, biochemical parameters, Turkey

INTRODUCTION

The presence of endotoxins in the blood is called endotoxemia. It is seen in patients with sepsis and septic shock and can also be seen experimentally with Lipopolysaccharide (LPS) infusion (Mackay, 1996). LPS given to calves induces hemodynamic, respiratory, metabolic and pyrogenic responses which are similar to those seen in calves with naturally occuring sepsis (Templeton *et al*., 1988; Biniek *et al*., 1998).

LPS has been used to induce endotoxemia in animals such as rabbits (Yazar *et al*., 2004; Turgut *et al*., 2006), horses (Danek, 2006) and cattle (Templeton *et al*., 1988; Adams *et al*., 1990; Semrad, 1993; Gerros *et al*., 1995; Biniek *et al*., 1998; Jacobsen *et al*., 2005). Endotoxin which is a part of the cell wall of gram-negative bacteria, initiates acute inflamation when injected *in vivo* (Lohuis *et al*., 1988a). LPS causes physiological acute phase responses such as fever, systemic hypotension, bradycardia, Disseminated Intravascular Coagulation (DIC), tissue necrosis and the production of inflammatory mediators Including Tumor Necrosis Factor-α (TNF-α), interferon, eicosanoids and Interleukin-1β (IL-1β) and IL-6 (Adams *et al*., 1990).

Endotoxins contribute to the development of conditions commonly observed with gram-negative infections such as coliform mastitis, neonatal coliform septicemia, pasteurellosis and salmonellosis. In addition, endotoxins are associated with non-infectious diseases such as ruminal acidosis, laminitis and abomasal displacement (Jacobsen *et al*., 2005). Endotoxemia is most commonly associated with bacteremia or septicemia due to gram-negative organisms, especially *E. coli* (Constable, 2007). The clinical symptoms of severe endotoxemia (Lohuis *et al*., 1988a; Gerros *et al*., 1995; Constable, 2007) include depression, hyperthermia following hypothermia, tachycardia followed by decreased cardiac output, decreased systemic blood pressure, cool skin and extremities, diarrhea, congested mucosae with an increased capillary refill time and muscular weakness leading to recumbency. The purpose of this study was to determine the changes in the haematological paramaters biochemical parameters and coagulation profiles in calves with LPS-induced experimental endotoxemia.

MATERIALS AND METHODS

Calves: Eight Holstein breed calves were used in the study. These calves were between 25-42 days of age (Median 35, 0) and 35-60 kg (Median 45) body weight of the calves. They were kept unrestrained in stalls that were bedded with wood shavings for 1 week before experiment. The routine clinical and hematological findings of all calves were recorded at 1 week before the experiment. The calves were fed whole milk (60 mL kg^{-1}) twice a day. The

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calves had access to fresh water at all times. According to clinical and hematological findings, healthy calves were included in the study.

Experimental design: A jugular venous catheter was placed aseptically into each calf at 24 h before LPS infusion. Endotoxemia was induced via intravenous administration of LPS (0111:B4, Sigma) (0.1 μg kg^{-1})[a] in 50 mL of physiological 0.9% NaCl for >30 min. Time zero (baseline) is meaning start time of LPS infusion.

Blood sample collection: Venous blood samples were collected anaerobically in 8 mL non-heparinized plastic syringes. Blood samples were collected from the jugular vein of calves at 0 (before LPS infusion, baseline), 0.5, 1, 2, 3, 4, 6, 8, 12, 18, 24, 36 and 48 h after LPS infusion. An aliquot of blood was collected in a glass tube for serum biochemical analysis; the tubes were centrifuged after clotting and the serum was harvested and stored at -20°C until analysis. A second aliquot of blood was collected in a plastic tube containing EDTA for routine hematologic examination. A third aliquot of blood was incubated in a plastic tube containing sodium citrate for coagulation parameters. A fourth aliquot of blood was placed in a plastic tube containing sodium heparin for blood gas analysis.

Hematological and biochemical analysis: Hematologic analysis was performed using an automated hematology cell counter (Medonic CA 530). Blood gas was analyzed in heparinized blood samples using a blood gas analyzer (GEM Premier Plus) within 15 min. The levels of serum Alanine aminotransferase (ALT), Gamma-glutamyl Transferase (GGT), Aspartate aminotransferase (AST), Blood Urea Nitrogen (BUN), Total Protein (TP), Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP), total bilirubin, glucose and Creatinine (CRE) were measured using an automatic analyzer (Vettest Chemystery analyser 8008). Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT) and fibrinogen were measured within 24 h (BTR Dade Behring).

Preparation of blood smears: Four smears were prepared from the heparinized blood samples obtained from each animal and dried at room temperature. Differential leukocyte counts were performed on May-Grunwald-Gimsa-stained blood smears. The distinguish of B lymphcytes and T lymphocytes were made to determining of ANAE enzyme activities (Sen *et al*., 2002).

Statistical analysis: Data are expressed as means±SD or median (range). The level of statistical significance was set at $p<0.05$. Baseline value compared to every other value by one way analysis of variance and mean values were compared by used of a Tukey test. A Statistical Software Program (SPSS 10.0) was used for statistical analysis.

RESULTS AND DISCUSSION

Laboratory findings: Severe leukopenia developed after LPS infusion in all calves. The total leukocyte count before LPS infusion was 10,363 cells μL^{-1} which decreased to 1800 cell μL^{-1} within 1 h after LPS infusion. Severe leukopenia followed by significant leukocytosis developed 12 h after LPS infusion. These changes in total leukocyte counts mostly reflected changes in the neutrophil count. Similar changes were obseved in the lymphocyte count (Table 1). Blood pH, P_vO_2 and O_2% saturation decreased 30 min after LPS infusion and then returned to initial levels within 6 h. However, P_vCO_2 levels were only slightly increased 30 min after LPS infusion (Table 2). Fibrinogen concentration was decreased at 30 min after infusion of LPS and increased at 6 h compared to the baseline values. APTT and PT levels were prolonged for 30 min after LPS infusion compared with baseline values and continued for 24 h (Table 2).

The levels of serum total protein, albumin, globulin, glucose BUN and creatinine and their statistical significances are shown in Table 2. The serum glucose concentrations increased within 30 min after LPS infusion and then markedly decreased between 3 and 12 h. There were also small changes in the levels of serum LDH, GGT, AST, ALP, ALT and total biluribin in during the experiment. However, there were no significant changes in these parameters. Gram-negative sepsis and endotoxemia have a strong association with mortality in cattle, particularly in neonatal calves (Deldar *et al*., 1984; Gerros *et al*., 1993; Semrad and Dubielzig, 1993; Gerros *et al*., 1995). Metabolic, hematological and pathological changes that occur in cases of LPS-induced experimental endotoxemia show similarities with the results of naturally occurring cases of sepsis (Templeton *et al*., 1988; Gerros *et al*., 1995; Biniek *et al*., 1998). In the present study, experimental endotoxemia was induced in calves by intravenous administration of LPS at a dose of 0.1 μg kg^{-1}.

The development of endotoxemia in calves is related to respiration, hematology, blood gases and changes in serum biochemical values. The acid-base balance is also disturbed in the acute phase of endotoxemia. Constable *et al.* (1991) stated in their study on calves with experimentally induced endotoxemia (0.1 μg kg^{-1} i.v. LPS) that the initial pH of venous blood (7.42) decreased within 30 min after endotoxin administration and a significant decrease occurred (pH 7.25) from 120 min.

Table 1: Changes in haematological parameters in calves (n = 8) with Lipopolysaccharide (LPS)-induced experimental endotoxemia (mean±SD)

	Time after LPS administration (h)					
Parameters	0	0.5	1	2	4	6
Total leukocyt count (cells μL^{-1})	10363 ± 2224	4925±2110**	1800±407***	1750±463***	1300±593***	1975±778***
Lymphocyt count (cells μL^{-1})	6620±1514	3941±1731**	1546±319***	1528±431***	1124±589***	1603±701***
T lymp. count (cells μL^{-1})	4804±1204	2840±1143*	1193±254***	1210±332***	862±436***	1199±496***
B lymp. count (cells μL^{-1})	1815±394	1100±599	351±92***	317±108***	261±158***	403±213***
Seg. neut. count (cells μL^{-1})	3054±847	711±314***	166±124***	159±59***	96±48***	223±67***
Band neut. count (cells μL^{-1})	236±129	103±60	26±14	23±6	30±13	63±31
Toxic neut. count (cells μL^{-1})	60±42	63±21	39±43	23±31	28±13	55±34
RBC 10^3 (cells μL^{-1})	7542±1837	9294±2165	8751±2058	8325±1788	7851±1880	8302±2352
PLT 10^3 cells μL^{-1})	466±91	395±29**	352±45**	314±67**	305±65**	311±79**
PCV (%)	26.28±4.50	31.27±4.51	30.23±5.51	28.15±3.77	27.97±5.00	28.60±4.71
Hgb (g dL^{-1})	10.39±1.28	11.79±1.64	11.51±2.00	10.50±1.35	10.90±1.42	10.66±1.14
Parameters	8	12	18	24	36	48
Total leukocyt count (cells μL^{-1})	3338±842***	7888±2726	15413±4167*	15963±4643**	16588±4643***	14313±3624
Lymphocyt count (cells μL^{-1})	2422±648***	4313±1595	6637±1753	8334±2588	7940±2634	7987±2074
T lymp. count (cells μL^{-1})	1843±450***	3283±1096	5090±1397	6312±2003	5540±2214	5705±1578
B lymp. count (cells μL^{-1})	578±224***	1029±554*	1546±463	2021±638	2399±711	2281±553
Seg. neut. count (cells μL^{-1})	581±156***	2120±987	5060±1709***	4754±1717***	4925±1583***	3880±1211
Band neut. count (cells μL^{-1})	131±73	781±501	1619±1033**	1745±840**	1975±1205**	1175±620
Toxic neut. count (cells μL^{-1})	122±65	587±357	1397±638***	1100±712**	1481±1200***	1097±894**
RBC 10^3 (cells μL^{-1})	8138±2186	8395±2119	8426±2428	7921±1833	7028±3228	8131±2579
PLT 10^3 (cells μL^{-1})	305±82**	293±72**	283±92**	267±80**	344±67**	392±72
PCV (%)	28.92±3.94	30.40±3.09	30.44±4.25	28.12±2.45	29.46±4.01	28.45±5.38
Hgb (g dL^{-1})	10.45±1.12	11.24±1.29	11.01±1.20	10.09±1.55	10.41±1.41	9.91±1.17

Table 2: Changes in venous blood gas, biochemical values and coagulation parameters in calves (n = 8) with Lipopolysaccharide (LPS) induced experimental endotoxemia (mean±SD)

	Time after LPS administration (h`)					
Parameters	0	0.5	1	2	4	6
GLU (mg dL^{-1})	65.13±20.35	138.25±51.37***	102.50±22.93*	99.75±26.14	26.63±17.27*	47.25±12.23
BUN (mg dL^{-1})	20.35±6.50	20.70±6.52	22.45±7.65	23.86±7.94	25.26±6.86	25.26±6.00
CRE (mg dL^{-1})	1.09±0.13	1.05±0.20	1.00±0.16	1.00±0.16	1.03±0.17	1.04±0.17
TP (g L^{-1})	6.36±0.65	6.11±0.58	6.06±0.55	5.88±0.49	5.79±0.65	5.73±0.69
ALB (g L^{-1})	3.58±0.37	3.44±0.49	3.30±0.33	3.24±0.35	3.25±0.39	3.28±0.35
GLB (g L^{-1})	2.79±0.77	2.68±0.69	2.76±0.67	2.64±0.66	2.54±0.73	2.45±0.86
pH	7.41±0.03	7.36±0.05	7.37±0.05	7.38±0.04	7.39±0.04	7.40±0.04
P_vCO_2 (mmHg)	38.48±4.35	42.88±3.35	40.66±2.53	40.25±3.32	38.17±3.04	38.70±3.76
P_vO_2 (mmHg)	33.84±4.00	22.49±4.73**	28.31±5.56	28.38±7.12	31.01±7.15	31.48±5.47
O_2 sat (%)	65.11±8.72	36.81±12.91**	47.09±19.78	51.38±17.71	57.20±16.20	59.99±13.84
PT (sec)	28.18±3.30	32.14±5.63	34.41±9.90	32.7±3.62	38.99±8.93	41.24±6.54*
APTT (sec)	70.00±25.48	76.10±25.19	101.38±33.36	117.40±46.48*	98.56±16.60	96.55 ±19.70
FB (g L^{-1})	2.81±0.29	2.99±0.26	2.28±0.17	1.96±014	1.38±0.10	1.14±0.10*
PLT 10^3 (cells μL^{-1})	466±91	395±29**	352±45**	314±67**	305±65**	311±79**
Parameters	8	12	18	24	36	48
GLU (mg dL^{-1})	49.63±8.71	51.13±12.59	61.50±15.17	74.13±14.47	70.75±17.55	77.63±14.48
BUN (mg dL^{-1})	27.71±5.29	30.17±6.99	31.22±5.70	33.68±6.54*	26.31±6.86	23.50±6.35
CRE (mg dL^{-1})	1.06±0.16	1.04±0.12	1.04±0.12	1.08±0.15	1.13±0.19	1.06±0.19
TP (g L^{-1})	5.91±0.67	6.19±0.59	6.13±0.59	6.00±0.64	6.28±0.58	6.09±0.53
ALB (g L^{-1})	3.35±0.32	3.45±0.35	3.48±0.39	3.40±0.33	3.50±0.46	3.41±0.39
GLB (g L^{-1})	2.56±0.80	2.74±0.78	2.65±0.79	2.60±0.74	2.78±0.61	2.68±0.61
pH	7.41±0.03	7.45±0.03	7.45±0.03	7.45±0.03	7.44±0.03	7.43±0.05
P_vCO_2 (mmHg)	39.93±2.53	38.79±2.37	39.62±2.28	38.48±2.73	39.64±2.47	40.4±2.17
P_vO_2 (mmHg)	29.54±5.42	31.69±3.62	31.21±4.80	31.90±5.63	33.26±2.26	30.11±4.21
O_2 sat (%)	55.86±12.79	63.40±8.05	62.16±11.44	63.43±13.76	66.29±4.93	58.78±11.25
PT (sec)	41.78±9.63	41.63±6.49*	44.89±9.63**	45.05±10.91**	31.61±4.92	30.41±3.03
APTT (sec)	91.41±24.50	89.78±24.04	92.87±30.86	87.73±23.23	73.90±15.88	81.85±20.95
FB (g L^{-1})	1.27±0.17	1.81±0.29	1.96±0.30	2.53±0.35	3.66±0.54	3.31±0.82
PLT 10^3 (cells μL^{-1})	305±82**	293±72**	283±92**	267±80**	344±67**	392±72

Data are presented as mean±SD. Aterisked (*) mean values are significantly different (*p<0.05, **p<0.01, ***p<0.001)

P_vO_2 values significantly de creased within 30 min but reached normal levels after 30 min; P_vCO_2 values slightly increased after 30 min. In this study, venous blood pH decreased from 30-180 min compared to initial values (Table 2). A significant decrease was observed in P_vO_2 levels whereas a slight increase was observed in P_vCO_2 levels at 30 min. It is likely that these changes observed in blood gas parameters are related to the effect

of the endotoxin on the type and rate of respiration. In this study, O_2% saturation decreased 30 min after LPS infusion and then returned to initial levels within 6 h. Hypoxia occurs due to the loss in ventilation and perfusion and lung edema contributes to increased hypoxia (Lohuis *et al*., 1988b). The causes of hypoxia could be hypoventilation, diffusion impairment, ventilation-perfusion inequality or a shunt (Constable *et al*., 1991).

The characteristic pathophysiological changes observed in endotoxemia are hyperglycemia followed by hypoglycemia and leukopenia followed by leukocytosis and thrombocytopenia (Nagaraja *et al*., 1979). Templeton *et al*. (1988) and Deldar *et al*. (1984) observed significant decreases in the number of total leukocytes, neutrophils and lymphocytes in calves with experimentally induced endotoxemia. In this study, significant decreases in total leukocyte, neutrophil and lymphocyte and thrombocyte counts were observed within 30 min following LPS administration and leukocyte counts increased after 12 h (Table 1).

The most important cause of leukopenia observed in endotoxemia is related to the adhesion of granulocytes, thrombocytes and monocytes to the endothelial cell surface and their margination or aggregation. Significant increases were observed in some biochemical parameters in cattle with experimentally induced endotoxemia with low or moderately high LPS administrations (Constable *et al*., 1991; Kumar and Malik, 2001). In this study, small increases were observed in serum GGT, AST, ALP, total bilirubin and LDH levels in LPS-administered calves. However, these increases were found to be statistically insignificant (Table 2).

There were significant changes in serum glucose and BUN levels. The increase in serum enzyme levels might indicate that some systemic organs, the liver in particular were affected. The increase observed in glucose levels within the 1st 30 min after infusion might be related to the stress caused by endotoxemia in animals. The probable cause of the subsequent decrease in glucose levels could be related to an increase in glucose consumption due to metabolic events that occur in the body as a result of endotoxemia. Disseminated Intravascular Coagulation (DIC) is observed as a result of hemostatic abnormalities in various diseases such as neoplasia, sepsis, shock and pancreatitis. Septicemia in particular is the most common clinical disorder associated with DIC. DIC might develop in >50% of patients in cases of sepsis caused by gram-negative bacteria (Jain, 1993; Stokol *et al*., 2000). A significant increase in APTT and PLT values, a slight increase in PT values and a significant increase in fibrinogen in calves with suspected septic shock have been observed (Irmak *et al*., 2006).

Col and Durgun reported significant increases in APTT and PT levels and significant decreases in fibrinogen levels in endotoxemia induced in rabbits. In this study, a significant increase in APTT and PT values, a significant decrease in PLT values and decrease in fibrinogen concentration were observed (Table 2). In sepsis, coagulation factors are activated due to the existence of bacterial liposaccharides. The first response of the body to endotoxins is the activation of coagulation (Jain, 1993; Stokol *et al*., 2000).

CONCLUSION

Result from this study showed that although, there were individual differences among the calves, endotoxemia was induced by the intravenous administration of LPS at a dose of 0.1 μg kg^{-1}. Changes in an abnormal coagulation profile and biochemical enzymes were related to endotoxemia. The LPS infusion resulted in significant changes of haematological, biochemical and coagluation parametrers. The result of the study could be of practical use to investigators in the field of host response to infection as baseline data for developing future trials.

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