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RESEARCH ARTICLE

Investigation of the Concentrations of Some Essential Elements in LPS-Induced Septicemic Sheep

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ABSTRACT

Endotoxemia, which is defined as an organ disorder due to irregular immunological host response to infection, is a disease that causes serious economic losses as a result of high mortality and morbidity in sheep. In the study, macro (Ca, Mg, Na, K, P) and micro (Fe, Cu, Se, Zn) essential element concentrations of plasma and serum samples; taken from healthy (control) (n=6) and septicemic sheep (n=6) were determined. According to the results, the differences between the groups in terms of Ca, K, and Se concentrations were statistically significant ($p \leq 0.05$). Comprehensive studies on the concentrations and changes of elements using sensitive analysis methods such as ICP-MS are needed to successful diagnose and treat septic patients, identify new biomarkers, and explain their mechanisms.

Keywords: ICP-MS, Macro element, Micro element, Plasma, Septicemia, Serum.

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LPS ile Indüklenen Septisemik Koyunlarda Bazı Eser Element Konsantrasyonlarının İncelenmesi

ÖZ

Enfeksiyona karşı düzensiz immünolojik konak yanıtına bağlı meydana gelen organ bozukluğu olarak tanımlanan endotoksemi, koyunlarda yüksek mortalite ve morbidite sonucu ciddi ekonomik kayıplara neden olan bir hastalıktır. Çalışmada septisemi oluşturulmayan sağlıklı (kontrol) koyunlar (n=6) ile septisemi oluşturulan koyunlardan (n=6) alınan plazma ve serum örneklerinde makro (Ca, Mg, Na, K, P) ve mikro (Fe, Cu, Se, Zn) esansiyel element konsantrasyonları belirlendi. Sonuçlara göre Ca, K ve Se konsantrasyonları açısından gruplar arasındaki farklar istatistiksel olarak anlamlıydı ($p \leq 0.05$). Septik hastalarda başarılı teşhis ve tedavi, yeni biyobelirteç belirlenebilmesi ve mekanizmalarının açıklanabilmesi açısından ICP-MS gibi hassas analiz yöntemlerinin kullanıldığı elementler konsantrasyonları ve değişimleri üzerine kapsamlı çalışmalara ihtiyaç duyulmaktadır.

Anahtar kelimeler: ICP-MS, Makro element, Mikro element, Plazma, Septisemi, Serum.

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INTRODUCTION

Bacterial infections are one of the major factors reducing farm animal productivity and raising morbidity and mortality rates. When the immune response, which is a defense mechanism against these infections, becomes unbalanced and cannot be successfully controlled, it can lead to the development of sepsis, which is defined as organ dysfunction resulting from the host's irregular immunological response to the infection (Riquelme et al. 2018, Türkmen 2017).

Lipopolysaccharide (LPS), a bacterial endotoxin, is a glycolipid molecule found in the outer membrane of gram-negative bacteria, such as Escherichia coli (E. coli), and plays a significant role in the pathophysiology of septic shock (Riquelme et al. 2018).

Animal models induced by LPS endotoxin injection have been widely used in sepsis research for many years. Endotoxin's ease of use, practical storage, applicability for controlled experiments, and suitability for standardization are some of the advantages of the endotoxic shock model. When administered to animals, LPS simulates many of the pathological symptoms of septicemia, such as fever, leukopenia, hemodynamic changes, coagulopathies, and complement activation (Redl et al. 1993).

Elements necessary for the maintenance of vital physiological functions for living organisms are structural components of enzymes and cofactors that prevent nutritional deficiencies and diseases, support antioxidant defense, and regulate immune functions and gene expression. They are classified as macro or micro depending on the amount required (Strachan 2010). Calcium (Ca), phosphorus (P), potassium (K), chlorine (Cl), sodium (Na), magnesium (Mg), and sulfur (S) are macro elements that are required in large quantities for normal physiological processes while micro elements such as zinc (Zn), selenium (Se), iron (Fe), manganese (Mn), copper (Cu), chromium (Cr), iodine (I), molybdenum (Mo), lithium (Li) and vanadium (V) are found in trace amounts in organisms. While many of these elements (Cu, Se, Zn, etc.) may have a toxic effect above tolerable concentrations, others can become toxic by interacting with one another. Some elements, such as aluminum (Al), mercury (Hg), and cadmium (Cd), are not required by the organism and can have toxic effects when they exceed tolerable concentrations (Patrashkov 2003, Nordberg et al. 2015, Ali and Khan 2018, Tatara et al. 2018, Zoroddu et al. 2019).

The prevention of the excess of all these elements that may cause toxic effects or the deficiencies that may cause different physiological or pathological effects is provided by the homeostatic mechanism. The regulation of element storage, absorption, and excretion is constantly maintained by homeostasis processes (Goyer, 2004). While this mechanism can be influenced by a variety of factors (age, nutrition, genetics, etc.), some homeostasis issues may arise (Strachan, 2010). Deficiencies or excess states of elements can result in many functional disorders. Blood (whole blood, serum, plasma) concentrations can also determine this deficiency or excess before clinical symptoms arise. Measurement of element concentrations can be helpful in identifying some diseases. Routinely measured biochemical and hematological blood parameters, as well as element profiles, can provide benefits such as obtaining additional information about disease pathogenesis, diagnosis, and prognosis, and assessing the suitability of element supplementation in treatment (Cedeño et al. 2004, Mert et al. 2008, Nordberg et al. 2015, Tuncer et al. 2020). As a result, the goal of this study was to establish how plasma and serum element concentrations change in the event of septicemia/endotoxemia, which causes considerable economic losses and has high death rates, as well as their association with infection.

MATERIAL AND METHODS

Animal Material

The study included 12 sheep (Awassi breed), both experimentally healthy control and septicemia-induced. The sheep had free access to feed and water throughout the study. Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee approved the study permit with the date 30/03/2022 and number: 2022/03-03

Blood samples were taken before septicemia was induced in sheep, and plasma (control plasma) and serum (control serum) were obtained (n=12). The sheep were then given 30 minutes of intravenous LPS toxin (E. coli O55:B5) in 30 ml 10 μg kg-1 0.9% NaCl to induce septicemia. Blood samples from sheep with septicemia (n=12) were used to obtain plasma (septicemia plasma) and serum (septicemia serum).

6 ml blood samples were taken into tubes with heparin and without anticoagulant to obtain plasma and serum from healthy controls and sheep with septicemia. After centrifuging the blood samples at 4000 x g for 10 minutes, the plasma and serum samples were stored at -80 C until analysis.

Element analysis

The concentrations of macro (Ca, Mg, Na, K, P) and micro (Fe, Cu, Se, Zn) elements were determined in serum and plasma samples collected from sheep with and without septicemia. Organic parts of serum and plasma samples were prepared for analysis by burning in a microwave system. P concentrations in prepared solutions were determined using ultraviolet visible light absorption spectroscopy (UV-VIS), Ca, Mg, Na, and K concentrations were determined using atomic absorption spectrometry (AAS), and Fe, Cu, Se, and Zn concentrations were determined using inductively coupled plasma-mass spectrometry (ICP-MS).

Statistical analyzes

The study's findings were evaluated using a statistical program at the end of the study (SPSS, IBM, USA). ANOVA and the Tukey HSD test were used to statistically analyze the data. Significance concentration was accepted as $p \leq 0.05$.

RESULTS

Macro (Ca, Mg, Na, K, P) and micro (Fe, Cu, Se, Zn) element concentrations in plasma and serum samples from healthy and septicemia-induced sheep were measured in the study. Tables 1 and 2 compare plasma and serum macro and micro essential element concentrations (mg L-1) (mean±SE) between groups.

Table 1. Plasma and serum macro-essential element concentrations of the groups (mg L-1) (mean±SE)

	Ca	Mg	Na	K	P
Control plasma (n=6)	100.42±21.85^{a}	44.26±2.96	2771.67±148.64	184.58±10.58^{a}	92.08±11.77
Control serum (n=6)	67.08±5.38^{ab}	42.14±2.61	3620.83±462.76	182.50±5.16^{a}	114.58±16.06
Septicemia plasma (n=6)	58.33±5.83^{b}	42.24±2.90	3155.83±300.49	177.50±5.77^{a}	144.41±21.13
Septicemia serum (n=6)	55.42±2.92^{b}	47.16±2.05	2874.17±125.87	154.58±4.93^{b}	118.12±9.62
P	0.05	0.51	0.20	0.03	0.15
Reference ranges (Puls 1994, Radostits et al. 2006, Kaneko et al. 2008)	92-102	28-40	4647-4871	125-173	52-76

Differences in letters within the same column are statistically significant ($p \leq 0.05$).

Table 2. Plasma and serum micro (trace) essential element concentrations of the groups (mg L-1) (mean±SE)

	Fe	Cu	Se	Zn
Control plasma (n=6)	3.23±0.36	0.80±0.07	0.16±0.02^{a}	1.22±0.30
Control serum (n=6)	4.69±0.59	0.78±0.06	0.10±0.02^{a}	0.72±0.09
Septicemia plasma (n=6)	4.26±0.56	0.67±0.07	0.11±0.02^{ab}	0.64±0.05
Septicemia serum (n=6)	5.72±2.35	0.67±0.11	0.08±0.01^{b}	0.59±0.13
P	0.58	0.50	0.02	0.07
Reference ranges (Puls 1994, Radostits et al. 2006, Kaneko et al. 2008)	1.66-2.22	0.58-1.6	0.08-0.5	0.8-1.5

Differences in letters within the same column are statistically significant ($p \leq 0.05$).

The findings revealed statistically significant differences in Ca, K, and Se concentrations between the groups ($p \leq 0.05$) in the study. Ca concentrations were found to be significantly lower in septicemia groups (septicemia plasma and septicemia serum) than in the control plasma group. The septicemia serum group's K concentrations were also found to be significantly lower than the other groups. Furthermore, when the septicemia groups were compared, it was found that the plasma K concentration (septicemia plasma) increased, which was statistically significant, more than the serum K concentration (septicemia serum). The concentration of selenium (Se) was found to be significantly higher in the healthy groups (control plasma and control serum) than in the septicemia serum group.

Serum Ca, Cu, Se, and Zn concentrations were within normal limits, Na concentrations were low, and Mg, K, Fe, and P concentrations were high when the data from the control group were compared to the reference ranges.

DISCUSSION

Animals require around 30 of the 103 elements in the periodic table to grow, develop, and survive. Of these, 16 are considered essential trace elements (Se, Zn, Cu, Fe, Mo, Mn, etc.). Because trace elements are difficult to detect in biological tissues and require careful measurements, this categorization was created first. Despite the fact that the development of new instruments has made measurements easier, the term "trace elements" is still used (Kaneko et al. 2008). AAS and ICP-MS were used in this study to determine the concentrations of macro and micro (trace) essential elements in sheep plasma and serum samples, which are more sensitive and reliable approaches than the measuring methods frequently used in clinics.

The link between the elements and the risk of infection and nutritional deficits is complicated because of the metabolic changes in this process (Saner et al. 2000).

Although the results for Na concentration in sheep serum samples are lower than the reference values (Underwood 1999, Radostits et al. 2006), Na deficiency is not a serious condition and Fan et al. (2020) is similar to their study findings.

P serum concentration were above the reference range in the all groups of current study while found to be low compared to other studies (843-1910 mg L-1) (Underwood 1999, Radostits et al. 2006, Fan et al. 2020).

In the previous two decades, Zn, Cu, Se, and other key trace elements have been discovered to have a critical role in modifying the immune response and changing the risk of infection (Saner et al. 2000). The concentrations of elements, such as Cu, Se, and Zn, in sheep tissues can be affected by differences in soil (in terms of plants used in diet) and water concentrations in the region where the sheep are raised, the ration contents, genetic factors, climate conditions, pregnancy, and antagonistic interactions of elements with each other (Erdoğan et al. 2003).

Animals require Se as a trace element. The normal functioning of bone and endocrine metabolism, immunological, endocrine, and reproductive processes all require a sufficient concentration of Se (Huo et al. 2020). Se deficiency in sheep is linked to a variety of clinical conditions, including reproductive problems, muscle degeneration, and cell membrane damage, in addition to its toxic effects. As a result, clinical Se status monitoring is critical. The results are evaluated differently depending on the species, age, race, analysis method, and sample type (Pamukçu et al. 2001). Various ranges of values have been reported in studies. Naghadeh et al. (2015) determined plasma Se concentrations to be 0.01-0.29 mg L-1, Humann-Ziehank et al. (2016) reported that that plasma Se concentrations vary between 1.24 and 21.6 mg L-1 in their study. In their study, Yeltekin et al. (2018) discovered very low values in the control group (0.028 mg L-1) that were associated with regional differences and nutrition. It has been reported that Se can be toxic in this context, and caution should be exercised when using it as a supplement in non-deficient areas (Vázquez-Armijo et al. 2011). While Se concentrations were within normal limits in all groups in the current study, it was found to be significantly higher in healthy groups (control plasma and control serum) than in the septicemia serum group. Yeltekin et al. (2018) found in their study that Se concentrations in sheep with contagious ecthyma were lower than in healthy sheep (0.013-0.028 mg L-1). Huo et al. (2020) discovered that lambs with clinically aberrant gait, weakness, anemia, polypnea, and arrhythmia had significantly lower plasma Se concentrations than healthy sheep.

Despite the lack of a reference study on variations in element concentrations in septicemic sheep, the link between diseases and elements in several animal species has attracted interest. However, the roles and interactions of elements with bacterial diseases have yet to be fully understood (Beisel 1976, Middleton et al. 2004).

Essential trace elements such as iron, Zn, and Cu have been linked to infection and sepsis in living organisms (Coşkun et al 2020). In 25 newborn calves with sepsis, Coşkun et al. (2020) found a large increase in K and Cu concentrations and a significant decrease in Na and Ca concentrations. The same study noted that K, Cu, Na, and Ca elements may play a crucial role in functional impairments caused by sepsis. Similar findings were achieved in the current investigation, and Ca, Se, and K concentrations were shown to be considerably lower in the septicemia groups.

In an E. coli-induced intraperitoneal sepsis model, Zn, Cu, and Fe concentrations were found to increase in rats (Konukoğlu et al. 2001). Again, serum Cu concentrations increased significantly after 12, 24, and 72 hours of endotoxin injection in hamsters, although serum Zn concentrations decreased after 4-48 hours (Etzel et al. 1982).

Serum Fe, Zn, and Cu concentrations were found to be lower in infants with sepsis syndrome than in the control group in another investigation, and it was suggested that Zn concentrations, in particular, could be a prognostic indicator of sepsis in infancy. According to the findings of this investigation, the plasma and serum Zn, Fe, and Cu concentrations of sheep with septicemia exhibited no significant changes.

CONCLUSION

In conclusion, significant differences in Ca, K, and Se concentrations were detected in healthy and septicemic sheep in the current study. More comprehensive research is needed to improve diagnosis and treatment, get new biomarkers, and explain the mechanisms involved in light of the information about the state and changes of elements in septic patients. The present study will contribute to the current literature by linking macro and micro (trace) necessary elements with different diseases and determining their amounts using more sensitive and up-to-date analysis methods such as AAS and ICP-MS.

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Authorship Contributions: The authors contributed equally to this work

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