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# Comparison of Serum and Plasma Aluminum Concentrations in Sheep

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**ABSTRACT**

Although Aluminum (Al) is a non-essential toxic (neurotoxic, immunotoxic, cardiotoxic, etc.) element, it is widely used in several industries (pharmaceuticals, cosmetics, food, etc.). Al-containing vaccines, medicines, contaminated food, and polluted air are the main routes of exposure (inhalation, dermal or oral). Determination and monitoring of blood element concentrations is an important tool for the clinical diagnosis of toxicity in animal health and sheep is one of the indicator species for assessing environmental health status. Aluminum is highly (>80) bound to protein in blood. Inductively coupled plasma mass spectrometry (ICP-MS) is a current analytical method with high potential to contribute to clinical diagnosis in the field of animal health, where elemental concentrations in biological samples can be measured at multiple and parts per trillion (ppt) concentrations. The aim of this study was to determine, statistically analyze, and compare Al concentrations in sheep (n=12) serum and plasma by ICP-MS. The concentrations (mean±SE) were 56.34±1.82 and 62.22±2.30 for serum and plasma, respectively. The mean plasma concentration was higher than the mean serum concentration, but the differences were not statistically significant (P=0.06). While toxic concentrations in the liver and kidney have been reported, there are no blood data for Al in sheep and the serum reference concentration is reported for humans only. Owing to the lack of information on Al toxicity in sheep, experimental acute toxic concentration studies of blood (whole blood, plasma, and serum) are required for Al in sheep. In conclusion, no statistical difference was found between serum and plasma concentrations, and no clinical signs were observed at these concentrations.

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## INTRODUCTION

Aluminum (Al) is the third most abundant element in the Earth's crust and is used in the pharmaceutical, cosmetics and food industries. It is not essential for animals and has toxic effects above tolerable concentrations (Altınok-Yipel, 2022a; Bengt, 2015; Gupta, 2012; Klein, 2019; Klotz, 2017; Lavanya, 2021; Rahimzadeh, 2022; San Martín, 2022; Uysal, 1990). Al is neurotoxic for humans and animals because it can cross the blood-brain barrier and the nervous system is the target system (Altınok-Yipel, 2022a; Bengt, 2015; Klein, 2019; Klotz, 2017; Yokel, 2000). It is considered a major risk factor for Alzheimer's and other neurodegenerative diseases (Ali and Khan, 2018; Lavanya, 2021; Yokel, 2000). It is also associated with skeletal, respiratory, immune, cardiac, and hematological disorders (Badea, 2019; Carbonara, 2023; Gupta, 2012; Klein, 2019). Most of the Al is stored in the bones and lungs, but small amounts can also be stored in the muscles and liver. It can be found in the blood (as bounded to the transferrin protein as 80-90%) and other body fluids (lymph, urine, cerebrospinal fluid, etc.) (Rahimzadeh, 2022; San Martín, 2022; Skibniewska and Skibniewski, 2019). Al is excreted by the kidneys. Normally tolerable concentrations may be toxic in patients with renal disease. It can accumulate in bones in patients on peritoneal or hemodialysis (Klein, 2019; San Martín, 2022). In addition, Al exposure in pregnant rats has been reported to increase tissue (liver, kidney, bone, and brain) concentrations of some essential trace elements (Cu, Fe, Mg, Mn and Zn), and this change may alter the level of oxidative stress (Bellés, 2001; Guo and Wang, 2011). However, a significant positive correlation has been reported between Al and body composition such as body weight, body mass index, fat-free mass, muscle and fat mass, fat percentage and internal adiposity, mineral and protein weight, body density and basal metabolic rate (Çetin, 2016).

Inhalation, dermal and oral exposure can occur from contaminated food, Al containers, and additives (Badea, 2019; Bengt, 2015; Rahimzadeh, 2022). Another important route of exposure is the administration of Al-containing vaccines (adjuvants), drugs and fluids (hemodialysis solutions and parenteral nutrition solutions) (Altınok-Yipel, 2019; Bengt, 2015; Gupta, 2012; Klein, 2019; Rahimzadeh, 2022; San Martín, 2022). Determination and monitoring of non-essential elements such as Al are clinically important because they can cause toxic effects above tolerable concentrations and can alter essential macro and microelement concentrations (Cu, Fe, Se and Zn) (Altınok-Yipel, 2022a; Altınok-Yipel, 2022b; Altınok-Yipel, 2022c; Gupta, 2012; Nordberg, 2015; Prashanth, 2015; Tiwari, 2010).

Laboratory tests that determine hematological and biochemical parameters in plasma or serum samples are important tools for clinical diagnosis in human and animal health (Akdag and Kader, 2021; Altınok-Yipel, 2022b; Kara, 2024). Absorption, accumulation, and circulation processes of elements associated with potential toxicity can be monitored in the blood. Blood and other fluids, tissues, or organs (urine, hair, liver, etc.) are often used as bioindicators to assess health status for elemental toxicity (Altınok-Yipel, 2022b). It has also been reported that sheep and some other animal species (dogs, cats, etc.) are used as indicator species to assess environmental pollution (Altınok-Yipel, 2022a).

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a spectroscopic method of analysis used in the determination of toxic trace elements such as Al, including low concentrations at the ppt level in the blood (serum and plasma), capable of multi-element analysis, aiding precise and accurate medical diagnosis (Cedeño, 2020; Kricka and Park, 2018; Martín, 2022; Triantafillidis, 2023). It is suitable for determining trace element concentrations in small-volume samples such as blood and has become the method of choice in recent years (Komarova et al., 2021). According to the literature review, no reference concentrations have been reported for Al in sheep serum or plasma (Durak et al., 2024).

The aim of this study was to determine and compare Al concentrations in sheep serum and plasma using the ICP-MS method.

## MATERIAL and METHODS

### Blood Samples

As part of the study, blood samples (without anticoagulant and with heparin) were collected in tubes from the jugular vein of 12 healthy (according to clinical examine of body temperature, heart rate, respiratory rate, capillary refill time, physical condition, appetite, etc.) sheep (1-4 years old Awassi) fed and watered ad libitum under the approval of the local animal experimentation ethics committee (no: 2022/03-03), serum and plasma were then obtained by centrifugation at 4000 rpm for 10 min and stored at -80 °C.

### Aluminum Analysis

The concentrations of Al in the serum and plasma samples were determined by ICP-MS under the conditions given in Table 1. The method was validated according to the parameters of limit of detection (LOD) and limit of quantification (LOQ) (μg $L^{-1}$), recovery (%), correlation coefficients ($r^2$) and relative standard deviation (%RSD) (Table 2).

**Table 1**

*ICP-MS Operating Conditions*

| Plasma power | Pump speed | Plasma flow | Auxilary flow | Nebulizer flow |
|---|---|---|---|---|
| 1300 W | 20 rpm | 9.0 L $min^{-1}$ | 1.65 L $min^{-1}$ | 0.93 L $min^{-1}$ |

**Table 2**

*Validation Results of Al Analysis Method in ICP-MS*

| LOD (μg $L^{-1}$) | LOQ (μg $L^{-1}$) | Recovery (%) | $r^2$ | RSD |
|---|---|---|---|---|
| 2.14 | 8.08 | 93.8 | 0.999 | 1.9 |

### Statistical analysis

After homogeneity (Levene) and normality (Shapiro-Wilk) distribution tests of the data obtained, the statistical difference between the groups was tested by independent samples t-test with SPSS software version 27.0 (IBM Corp., USA). The significance level was set at. 05 (Selvi, 2024).

## RESULTS

Serum and plasma Al concentrations (μg $L^{-1}$) of sheep blood samples determined by ICP-MS are presented in Table 3 as the arithmetic mean, standard error, standard deviation, median, geometric mean, minimum and maximum.

**Table 3**

*Serum (n=12), Plasma (n=12) and Total (n=24) Arithmetic Mean, Geometric Mean, Minimum and Maximum Al Concentrations (μg $L^{-1}$)*

| | Serum | Plasma | *P* | Total |
|---|---|---|---|---|
| Arithmetic mean | 56.34 | 62.22 | 0.06 | 59.28 |
| Standard error | 1.82 | 2.30 | | 1.56 |
| Standard deviation | 6.30 | 7.97 | | 7.64 |
| Median | 57.97 | 60.81 | | 59.44 |
| Geometric mean | 56.01 | 61.74 | | 58.80 |
| Minimum | 45.31 | 45.96 | | 45.31 |
| Maximum | 66.06 | 75.49 | | 75.49 |

Although the mean plasma Al concentration was higher than the mean serum concentration, the difference was not statistically significant.

## DISCUSSION

Aluminum concentrations can be measured in tissues, hair, urine, feces and blood (Gupta, 2012). Although hair or bone analysis is the predominant means of determining Al toxic exposure, sample preparation procedures and the need for standardization should be considered (Badea, 2019; Wołowiec, 2013). The preference for blood samples in Al analysis includes clinically simpler and more applicable methods. In addition, blood samples are often used in routine clinical practice (Carbonara, 2023; Komarova, 2021; Martín, 2022). When assessing the toxicological accumulation of elements, it may be a good approach to priorities the target organ, especially in the case of low long-term exposure to Al, where the measurement of Al concentrations in the serum may not reflect accumulation in tissues (Carbonara, 2023; Van Landeghem, 1998). However, serum is useful for measuring acute exposure (Carbonara, 2023; Martín, 2022; Tomza-Marciniak, 2012). Therefore, it is considered a more accurate approach to prefer serum and plasma samples for the investigation of acute Al exposure and target tissues for chronic exposure. Carbonara, (2023) stated that the definitions of 'Al toxicity' and 'Al deposition in bone' should be distinguished (Carbonara, 2023). Al concentrations above a certain level in the diet (1200 ppm) have been suggested to be toxic to sheep (Gupta, 2012).

Although toxic concentrations of Al have been reported in the liver (6-11 ppm) and kidney (4-5 ppm) of sheep, there are insufficient data on serum and plasma concentrations (Durak, 2024; Gupta, 2012). However, as with some elements, there is insufficient information on the ideal matrix (whole blood, plasma, serum) for measuring Al blood concentrations. In the present study, although there was no statistical difference between serum and plasma, the mean plasma Al concentration was higher than the serum concentration. The higher Al concentration in plasma than in serum can be explained by the fact that most of the Al in plasma is bound to proteins (Greger, 1997; San Martín, 2022).

Durak (2024) found the mean serum Al concentration to be 2.7 ppm in a study of 313 healthy sheep (Durak, 2024). This result is higher (~45 times) than the mean concentration of our study. Although not possible to determine whether the determined mean concentration was within

physiological limits, were can within the acceptable range because the animals appeared healthy was stated by authors (Durak, 2024).

Komarova (2021) reported a plasma Al concentration of 6.9 μg $L^{-1}$ measured by ICP-MS in a human study. (Komarova, 2021). Reference values for serum Al concentration in humans have been established <5-7 μg $L^{-1}$ in serum, 16 μg $L^{-1}$ in urine and <20-60 μg $L^{-1}$ in dialysis patients. (Bengt, 2015; Martín, 2022). The critical concentration at which neurological symptoms occur in humans is 100 μg $L^{-1}$ in urine. (Rahimzadeh, 2022). No toxic reference concentration for Al in sheep has been reported. Serum Al concentrations are lower than those in other tissues (Greger, 1997).

There is insufficient information on the acute toxic effect of Al in ruminants leading to mortality (Thompson, 1991). Studies have shown that some of the orally ingested Al is excreted in the urine, whereas some accumulates in the tissues. This accumulation varies depending on various factors (age, disease, kidney function, etc.) (Greger, 1997). When studies on species-specific toxic Al blood concentrations are reviewed, studies on sheep are quite insufficient (Durak, 2024).

In toxicity studies of Al (neurotoxicity, embryotoxicity, etc.) in mice, rats, and dogs, the lowest concentrations of adverse effects were 52, 75, and 100 mg $kg^{-1}$ BW $day^{-1}$ respectively. Acute oral toxicity concentrations for Al compounds in mice and rats (mouse $LD_{50}$: 164- >730; rat $LD_{50}$: 162- >730) have been reported over a wide range (Aguilar, 2008).

Al accumulation may be observed in patients with chronic kidney disease or hemodialysis patients (dialysis solutions containing Al). It has been reported that blood Al concentrations in hemodialysis patients should not exceed 20 μg $L^{-1}$. Monitoring of Al in at-risk dialysis patients recommended (Martín, 2022).

## CONCLUSION

Al is an important toxic element that has adverse effects (especially neurological) on many systems and organs. It has a potential for exposure to humans and animals through environmental and food contamination. Also, Al toxicity has been observed in some diseases such as renal diseases. However, there is a lack of information on Al toxicity in animal species. In particular, studies on blood concentrations and reference ranges of Al in sheep are inadequate. Furthermore, the choice of sample (whole blood, serum, plasma) according to the toxic dynamic and kinetic properties of the elements (binding to proteins, etc.) is essential for the accurate determination of blood concentrations, which play an important role in toxic element analysis.

In conclusion, no statistical difference was found between serum and plasma concentrations in terms of sample prefered for Al analysis. In addition, no clinical signs of toxicosis or diseases were observed at the determined plasma and serum concentrations. Therefore, experimental acute toxic concentration studies in sheep, including whole blood, plasma and serum concentrations are required.

## Ethics Committee Approval

22/03/2022 dated and 2022/03-03 numbered was given by Hatay Mustafa Kemal University, local animal experimentation ethics committee.

## Author Contributions

All authors contributed to the study conception and design.; Methodology: Fulya ALTINOK-YİPEL; Sampling and laboratory analysis: Fulya ALTINOK-YİPEL, Mustafa YİPEL Statistical

analysis and interpretation of the data: Mustafa YİPEL; Drafting: Fulya ALTINOK-YİPEL; all authors commented on previous versions of the manuscript; Reviewing and editing: Fulya ALTINOK-YİPEL, Mustafa YİPEL.

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**Conflict of Interest**

Please indicate whether there is a conflict of interest.

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