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Research Article

Biochemical Alterations in Chickens (*Hisex brown*) Exposed to Lignite Fly Ash and Heavy Metal Mixtures from Kosovo Thermoelectric Power Plants

Rrahman Ferizi[1], Ahmet Karataş[2], Mohamed Fawzy Ramadan[3], Qenan Maxhuni[4*]

[1]Premedical Department, Faculty of Medicine, University of Prishtina, 10000 Prishtina, Kosovo; [2]Department of Biology, Faculty of Science-Arts, Niğde Ömer Halisdemir University, Niğde, Türkiye; [3]Department of Biochemistry, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt; [4]Department of Laboratory Biochemistry, Alma Mater Europaea, Campus College "Rezonanca", 10000 Prishtina, Kosovo.

Abstract | In this experimental study, the toxic effects of exposure to lignite ash and heavy metals on selected biochemical parameters in chickens (*Hisex brown*) were analyzed. A total of 14 Hisex Brown chickens were assigned to three experimental groups: a control group (n = 5), an ash-treated group (n = 5), and a heavy metal complex-treated group (n = 4). After a total exposure period of 30 days, plasma biochemical parameters, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, and alkaline phosphatase (ALP), were analyzed, applying standard enzymatic and spectrophotometric methods. AST activity differed significantly among the experimental groups ($p < 0.001$), with the highest mean activity observed in the ash-treated group and the lowest in the metal complex-treated group. ALT activity was numerically higher in the treated groups than in the control group; however, the overall difference was not statistically significant ($p = 0.1696$). Plasma glucose concentration ($p = 0.4449$) and ALP activity ($p = 0.9715$) also showed no statistically significant differences among the experimental groups. These findings suggest that exposure to lignite ash and the heavy metal complex may affect plasma AST activity under the experimental conditions applied, whereas the effects on ALT, glucose, and ALP remain inconclusive.

Keywords | Alkaline phosphatase, Poultry, Biomonitoring, Oxidative stress, Kosovo, Glucose

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***Correspondence** | Qenan Maxhuni, Department of Laboratory Biochemistry, Alma Mater Europaea, Campus College "Rezonanca", 10000 Prishtina, Kosovo; **Email:** qenan.maxhuni@rezonanca-rks.com
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INTRODUCTION

Environmental pollution represents one of the most serious ecological and health problems in Kosovo (World Health Organization, 2021; United Nations Environment Programme, 2022). In addition to traditional biological pollution, urban and industrial development has led to a significant increase in air, water, and soil pollution. Pollutants released by the energy industry, metallurgy, transport, and the inappropriate use of agrochemicals spread through the air, water, and food chain, negatively affecting ecosystems and the health of the human population (World Health Organization, 2021; United Nations Environment Programme, 2022).

Kosovo is considered one of the most contaminated areas in Europe due to its large reserves of lignite, intensive exploitation of mineral resources, and the use of outdated industrial technologies (United Nations Environment Programme, 2022; Adrovic *et al*., 2020). One of the main sources of pollution remains the Kosovo thermal power plants in Kastriot (Obiliq), which, during the combustion of lignite, release large amounts of sulfur dioxide (SO_2), nitrogen oxides (NO_x), carbon monoxide (CO), dust particles, and fly ash (United Nations Environment Programme, 2022; Adrovic *et al*., 2020). In addition to toxic gases, ash from thermal power plants also contains heavy metals and radionuclides that pose a long-term risk to the environment and living organisms (Rozhaja *et al*., 1980).

Spectrochemical and radiological analyses have shown that Kosovo lignite is characterized by high sulfur contents and high ash production during combustion (Rozhaja *et al*., 1980). Ash contains toxic elements such as lead (Pb), cadmium (Cd), arsenic (As), nickel (Ni), and fluorine (F), all of which can contaminate soil and groundwater (United Nations Environment Programme, 2022; Rozhaja *et al*., 1980). Industrial effluents discharged into the Sitnica River have contributed to its severe ecological degradation, leading to a decrease in dissolved oxygen and an increase in the concentration of phenols and ammonia (World Health Organization, 2021; Adrovic *et al*., 2020).

In addition to Kastriot (Obiliq), the Mitrovica and Zveçan regions represent one of the most polluted industrial areas in Kosovo (World Health Organization, 2021; Adrovic *et al*., 2020). The long-term activity of the "Trepça" metallurgical complex has resulted in significant pollution of the air, soil, and food products with lead, zinc, and cadmium (World Health Organization, 2021; United Nations Environment Programme, 2022; Adrovic *et al*., 2020). High concentrations of lead have been reported in the soil, vegetables, milk, and blood of the local population, especially in children and pregnant women (United Nations Environment Programme, 2022). Various studies have also evidenced bioaccumulation of heavy metals in animal organisms exposed in these regions (World Health Organization, 2021; Aljohani, 2023).

Radioactive pollution constitutes another aspect of ecological and toxicological importance. Natural radionuclides are present in lignite concentrate in ash and slag during coal combustion (Mihalj *et al*., 1996; Rozhaja *et al*., 1980). Radiochemical analyses of the ash from the thermal power plants in Kastriot have identified isotopes such as Ra226, U235, and Pb214, which may contribute to increased radiation doses in the population living near the ash dumps (Table 1) (Rozhaja *et al*., 1980).

After the 1998-1999 conflict, depleted uranium was also reported in some areas of Kosovo. Although official data are environmental, the potential impact of radionuclides and industrial pollution remains an issue of interest for environmental and public health monitoring (Mihalj *et al*., 1996; Rozhaja *et al*., 1980). In addition to industrial pollution, environmental degradation is also aggravated by the inappropriate use of pesticides and artificial fertilizers, urban pollution, and low levels of ecological awareness (World Health Organization, 2021; United Nations Environment Programme, 2022; Adrovic *et al*., 2020; Christensen, 1988). The consequences of this pollution are reflected in the biodiversity of the region, which includes the reduction of birds, changes in the organization of aquatic organisms, and the biological degradation of rivers (World Health Organization, 2021; Aljohani, 2023; Christensen *et al*., 1988).

Table 1: Spectrochemical analysis of lignite ash from Kosovo.

Elements	Concentration	Elements	Concentration
As (Arsenic)	2	La (Lanthanum)	2
Au (Gold)	-	Cd (Cadmium)	0.4
B (Boron)	21	Br (Bromine)	2
Ba (Barium)	110	Cl (Chlorine)	32
Bi (Bismuth)	3	P (Phosphorus)	780
Mn (Manganese)	230	Ge (Germanium)	0.1
Sb (Antimony)	-	Se (Selenium)	0.6
Pb (Lead)	2	U (Uranium)	2
Cr (Chromium)	11	Th (Thorium)	2
Ga (Gallium)	2	Li (Lithium)	1
Na (Sodium)	23	Nb (Niobium)	3
Mo (Molybdenum)	6	Rb (Rubidium)	
Sn (Tin)	0.5	Sm (Samarium)	1
V (Vanadium)	8	Pr (Praseodymium)	0.9
Cu (Copper)	8	Ce (Cerium)	3
F (Fluorine)	2	I (Iodine)	0.5
Y (Yttrium)	3	Nd (Neodymium)	0.8
Zn (Zinc)	1	Te (Tellurium)	0.4
Zr (Zirconium)	6	Be (Beryllium)	Not detected
Co (Cobalt)	0.4	Eu (Europium)	0.3
Sr (Strontium)	91	Pd (Palladium)	-
Ti (Titanium)	660		

Values below detection limits 0.2 ppm value = g/t: 1000 ppm = 0.1 ‰/ All elements at concentrations <0.2 ppm were not detected.

Toxicological and ecotoxicological studies have shown that pollutants initially affect cellular metabolism and enzymatic activity, leading to physiological and histopathological changes (Aljohani, 2023; Christensen *et al*., 1988). For this reason, biomonitoring is of particular importance for the effect of effects on living organisms (World Health Organization, 2021; Aljohani, 2023). Biologically active monitoring is given special importance, because it allows for the control of experimental conditions and a more accurate interpretation of the effects of toxicants (World Health Organization, 2021; Aljohani, 2023). In this context, biochemical parameters such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and plasma glucose are widely used as indicators of metabolic status and toxic effects of heavy metals and industrial pollutants (Aljohani, 2023; Christensen *et al*., 1988; Chowdhury and Saikia, 2020). Changes in the activity of some enzymes are associated with diseases of protein and carbohydrate metabolism, hepatic damage, necrosis, and other harmful health processes from exposure to pollutants (Christensen *et al*., 1988; Chowdhury and Saikia, 2020). Therefore, monitoring biochemical parameters in the organization of experiments does not represent in a total an important approach to the effect of heavy metals and radionuclides on biological systems, as well as for a better understanding of the impact of industrial pollution on the environment and the public (Aljohani, 2023; Christensen *et al*., 1988; Chowdhury and Saikia, 2020).

The aim of this study was to evaluate the effects of dietary exposure to lignite ash from Kosovo thermal power plants and a heavy metal complex on selected plasma biochemical parameters AST, ALT, glucose, and ALP in Hisex Brown chickens used as an experimental biomonitoring model.

MATERIALS AND METHODS

Experimental animals and study design

For this study, 14 Hisex Brown chickens, approximately 7 weeks of age, were included. The chickens were assigned to three experimental groups: A control group consisting of five chickens (n = 5), an ash-treated group consisting of five chickens (n = 5), and a heavy metal complex-treated group consisting of four chickens (n = 4). The groups were housed separately under the experimental conditions described below. The control group received commercial feed and drinking water ad libitum. The feed of the second group was supplemented with lignite ash from the Kosovo Thermal Power Plants at a concentration of 50 g/kg feed. The feed of the third group was supplemented with a metal complex containing Pb, Co, Ni, Cr, Zn, Cu, and Cd at a combined total concentration of 373 μg metals/g feed. The animals were maintained under standard laboratory and husbandry conditions with controlled environmental temperature, humidity, and natural light–dark cycle conditions throughout the experimental period. All chickens had free access to feed and drinking water during the experiment.

Since the literature data show that external factors depending on the intensity and duration, especially transport conditions, can affect the vitality of the animals, the chickens were not treated with the experimental ingredients for seven consecutive days in order to allow adaptation and acclimatization to the new environmental conditions.

Following the acclimatization period, the experimental treatment was initiated on the eighth day after transportation to the research laboratory at the Faculty of Medicine.

The treatment of the chickens of the second and third groups with the ingredients described above was continued for 30 days. After 30 days of dietary exposure, the chickens were weighed and subsequently decapitated.

Blood sampling was performed immediately after sacrifice under standardized laboratory procedures. Blood was collected in heparinized test tubes. To separate the plasma from the corpuscular elements of the blood, the blood samples were centrifuged at 3000 rpm for 10 min. The plasma was then transferred into clean test tubes and stored at 4 °C until biochemical analyses.

Feed composition

Table 2 presents the guaranteed nutritional composition of the commercial feed used during the experimental period, while Table 3 presents its vitamin and micronutrient composition per kilogram of feed.

Table 2: Feed composition.

Parameter	**Composition**
Crude protein (min.)	18%
Moisture (max.)	30.5%
Cellulose (max.)	6%
Ash (max.)	8%
Calcium (max.)	11%
Phosphorus (min.)	0.5%
Vitamin B6	0.75 mg
Vitamin B12	0.07 mg
Biotin	0.05 mg

Table 3: Vitamin and micronutrient composition per 1 kg of feed mixture.

Component	**Amount/kg feed**
Vitamin A	3500 IU
Vitamin D3	500 IU
Vitamin K3	0.5 mg
Vitamin E	6 mg
Vitamin B1	1 mg
Vitamin B2	1.25 mg
Vitamin B6	0.75 mg
Vitamin B12	0.07 mg
Biotin	0.05 mg
Ca-Pantothenate	6.0 mg

Biochemical analyses

The activity of AST transaminase in plasma was determined by the kinetic method recommended by the Expert Panel of the IFCC (International Federation of Clinical Chemistry) for ASAT activity determination. The activity of ALT transaminase in plasma was determined by the kinetic method recommended by the Expert Panel of the IFCC for ALAT activity determination. The level of alkaline phosphatase was determined using the optimal standard method with ready-made reagents supplied by HUMAN, according to the recommendations of the German Society for Clinical Chemistry (Deutsche Gesellschaft für Klinische Chemie). The concentration of glucose (glycemia) in plasma was determined by the enzymatic GOD-PAP method (enzymatic colorimetric test) according to Barham and Trinder. The above biochemical parameters (AST, ALT, alkaline phosphatase, and glucose) were analyzed using commercial ready-made reagents from HUMAN GmbH and measured using a PU 8720 UV/VIS scanning spectrophotometer (PHILIPS) connected to a computerized monitoring system. All biochemical analyses were performed according to the manufacturer's instructions and standardized laboratory protocols in order to ensure analytical accuracy and reproducibility of the obtained results.

Statistical analysis

The individual chicken was considered the experimental unit. Statistical analyses were based on 14 independent biological observations: five chickens in the control group, five in the ash-treated group, and four in the heavy metal complex-treated group. All data obtained during the evaluation of the biochemical parameters are presented as mean ± standard deviation (SD). Normality and homogeneity of variance were assessed before inferential analysis. Differences among the experimental groups were evaluated using one-way analysis of variance (ANOVA). When the overall ANOVA was statistically significant, Tukey's HSD post hoc test was used to identify pairwise differences between groups. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using IBM SPSS Statistics software (IBM Corp., Armonk, NY, USA).

RESULTS

Plasma AST and ALT activities and glucose concentration

The one-way ANOVA showed a statistically significant effect of treatment on plasma AST activity, $F(2,11) = 37.53$, $p < 0.001$. In contrast, no statistically significant differences were observed in ALT activity, $F(2,11) = 2.09$, $p = 0.1696$, plasma glucose concentration, $F(2,11) = 0.87$, $p = 0.4449$, or ALP activity, $F(2,11) = 0.03$, $p = 0.9715$.

Table 4: One-way ANOVA analysis of biochemical parameters in experimental chicken groups.

Parameter	F-value (df=2,11)	p-value
AST	37.53	p < 0.001
ALT	2.09	0.1696
Glucose	0.87	0.4449
ALP	0.03	0.9715

Table 5: Plasma AST activity in experimental groups presented as mean ± SD.

Group	Mean (IU/L)	SD (IU/L)	n
Control	20.4	7.8	5
Ash	46.41	8	5
Metal complex	6.97	3.7	4

Table 6: Tukey HSD post hoc analysis for AST activity between experimental groups.

Group 1	Group 2	Mean difference	Adjusted p value	Lower 95% CI	Upper 95%	Significant
Ash	Control	-26.01	0.0003	-37.98	-14.04	Yes
Ash	Metal complex	-39.44	<0.001	-52.14	-26.74	Yes
Control	Metal complex	-13.43	0.0383	-26.13	-0.73	Yes

AST activity showed marked differences among the experimental groups. As presented in Table 5, the highest AST activity was recorded in chickens treated with lignite ash (46.41 ± 8.00 IU), followed by the control group (20.40 ± 7.80 IU), whereas the lowest AST activity was observed in the metal complex-treated group (6.97 ± 3.70 IU).

Because the overall ANOVA for AST activity was statistically significant, Tukey's HSD post hoc test was performed. Significant pairwise differences were observed between the ash-treated and control groups, the ash-treated and metal complex-treated groups, and the control and metal complex-treated groups (Table 6). These differences are illustrated in Figure 1.

ALT activity showed numerical variation among the experimental groups. The lowest mean activity was recorded in the control group (11.86 ± 5.70), while higher mean values were observed in the ash-treated group (18.49 ± 6.30) and the metal complex-treated group (17.01 ± 2.90). The highest mean ALT activity was recorded in the ash-treated group. However, the overall difference among groups was not statistically significant, $F(2,11) = 2.09$, $p = 0.1696$. This pattern is illustrated in Figure 2.

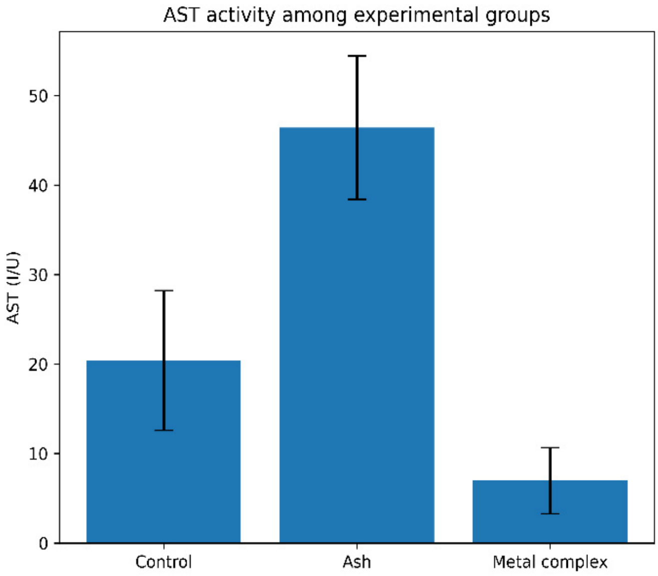

Figure 1: Plasma AST activity in control, ash-treated and metal complex-treated chickens.

Table 7: Plasma ALT activity in experimental groups presented as mean ± SD.

Group	Mean (IU/L)	SD (IU/L)	n
Control	11.86	5.70	5
Ash	18.49	6.30	5
Metal complex	17.01	2.90	4

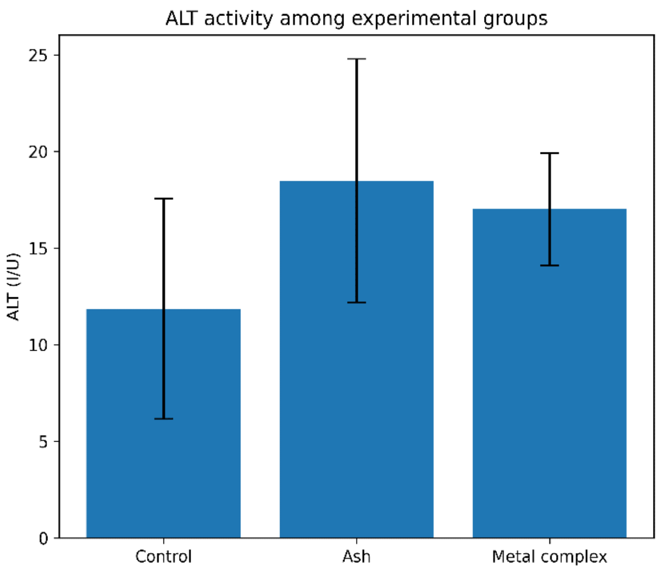

Figure 2: Plasma ALT activity in control, ash-treated and metal complex-treated chickens.

Table 8: Plasma glucose concentrations in experimental groups presented as mean ± SD.

Group	Mean (mg/dL)	SD (mg/dL)	n
Control	80.10	44.00	5
Ash	99.10	29.10	5
Metal complex	111.10	31.00	4

Plasma glucose concentration did not differ significantly among the experimental groups, $F(2,11) = 0.87$, $p = 0.4449$. As shown in Table 8, the mean glucose concentration was 80.10 ± 44.00 mg/dL in the control group, 99.10 ± 29.10 mg/dL in the ash-treated group, and 111.10 ± 31.00 mg/dL in the metal complex-treated group. Although the treated groups showed numerically higher mean values than the control group, the overall difference was not statistically significant. These findings are illustrated in Figure 3.

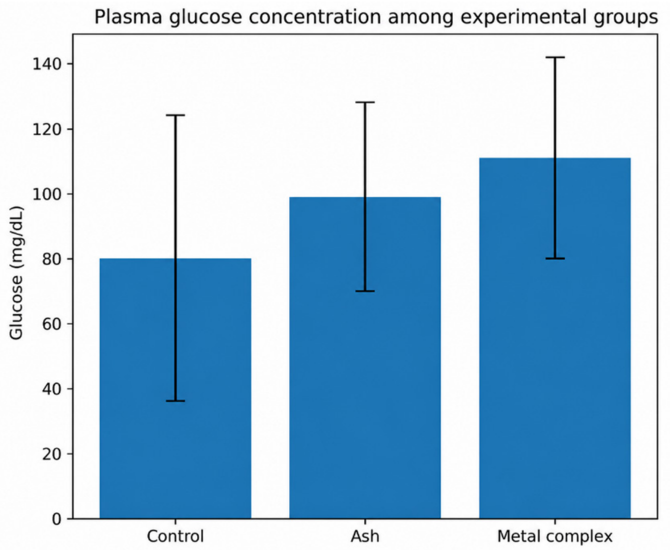

Figure 3: Plasma glucose concentrations in control, ash-treated and metal complex-treated chickens.

Plasma alkaline phosphatase activity

The plasma alkaline phosphatase activity did not differ significantly among experimental groups. During this research, it was observed that chickens treated with ash untouched by heavy metals resulted in ALP activity values close to the control group, while birds treated with heavy metal complexes resulted in slightly increased ALP values (Table 9).

Table 9: Plasma alkaline phosphatase (ALP) activity in experimental groups presented as mean ± SD.

Group	Mean (IU/L)	SD (IU/L)	n
Control	779.6	259.2	5
Ash	773.02	261.12	5
Metal complex	813.25	270.67	4

Plasma alkaline phosphatase (ALP) activity did not differ significantly among the experimental groups, $F(2,11) = 0.03$, $p = 0.9715$. As shown in Table 9, ALP activity was similar in the control group (779.60 ± 259.20 IU/L) and the ash-treated group (773.02 ± 261.12 IU/L), while a small numerical increase was observed in the metal complex-treated group (813.25 ± 270.67 IU/L). These findings are graphically presented in Figure 4.

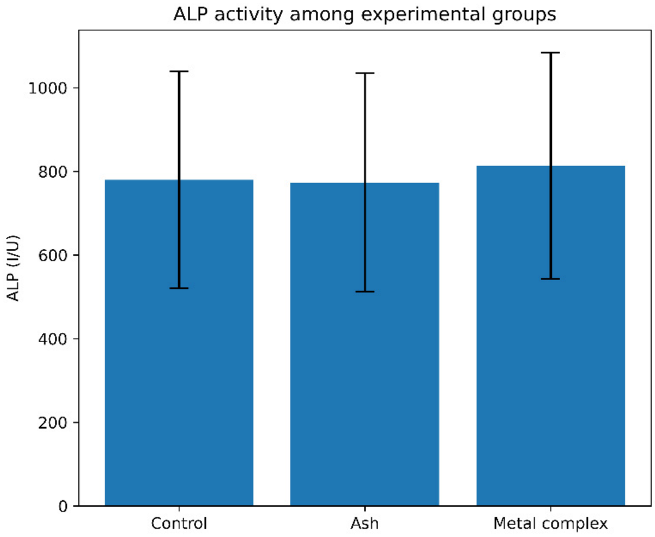

Figure 4: Plasma alkaline phosphatase (ALP) activity in control, ash-treated and metal complex-treated chickens.

DISCUSSION

AST and ALT are commonly used as biochemical indicators of tissue injury in toxicological studies. However, their interpretation in birds requires caution because changes in plasma enzyme activity may reflect effects on hepatic, muscular, or other tissues and should not be considered definitive evidence of hepatocellular damage when used in isolation (Aljohani, 2023). In the present study, AST and ALT showed different response patterns following exposure to lignite ash and the heavy metal complex. AST activity differed significantly among the experimental groups, whereas ALT activity showed only a numerical increase in the treated groups. Mean ALT activity was 11.86 ± 5.70 IU/L in the control group, 18.49 ± 6.30 IU/L in the ash-treated group, and 17.01 ± 2.90 IU/L in the metal complex-treated group. However, the overall difference in ALT activity was not statistically significant, $F(2,11) = 2.09$, $p = 0.1696$. Therefore, the observed ALT pattern should be interpreted as a numerical trend rather than as evidence of a statistically confirmed treatment effect. The different responses of AST and ALT may reflect differences in their tissue distribution and sensitivity to toxicant exposure; however, the underlying mechanisms cannot be established from the present biochemical measurements alone.

Plasma glucose concentrations were numerically higher in the ash-treated and metal complex-treated groups than in the control group; however, these differences were not statistically significant. Therefore, the present findings should not be interpreted as evidence of treatment-induced hyperglycemia. The observed numerical variation may reflect biological and pre-analytical variability, including individual differences, nutritional status, handling stress, and sample-processing conditions. Although exposure to certain heavy metals may influence carbohydrate metabolism through stress-related and endocrine mechanisms, the present results do not demonstrate such an effect. Further studies with larger experimental groups and standardized sampling conditions are required to determine whether lignite ash or heavy metal exposure affects glucose metabolism in chickens.

Alkaline phosphatase is a metalloenzyme involved in several physiological processes, including bone mineralization, calcium and phosphate metabolism, membrane transport, cellular growth, and hepatocellular function. Because ALP activity may respond to changes in tissue metabolism, cellular membrane integrity, nutritional status, and exposure to environmental contaminants, it has been used as a biochemical biomarker in toxicological studies. Nevertheless, plasma ALP is not specific to a single organ, and its interpretation should take into account the age, physiological condition, skeletal development, and nutritional status of the animals.

In the present study, plasma ALP activity was slightly lower in the ash-treated group (773.02 ± 261.12 IU/L) than in the control group (779.60 ± 259.20 IU/L), whereas a small numerical increase was observed in the metal complex-treated group (813.25 ± 270.67 IU/L). However, the differences among the experimental groups were not statistically significant, $F(2,11) = 0.03$, $p = 0.9715$. Therefore, the observed numerical variation should not be interpreted as evidence of a confirmed treatment-related effect on plasma ALP activity.

Previous toxicological studies have shown that exposure to cadmium, lead, and other metals may alter biochemical enzyme activities and tissue function in exposed animals (Khalesi *et al*., 2017; Rani and Singh, 2019). However, responses may vary according to the animal species, metal composition, exposure dose, exposure duration, tissue examined, and route of administration. Because much of the available evidence originates from aquatic organisms, direct comparisons with chickens should be made cautiously.

Heavy metals may influence biochemical processes through oxidative stress, interaction with enzyme systems, alterations in mineral homeostasis, and effects on hepatic, renal, or skeletal tissues (Aljohani, 2023). Nevertheless, the present study did not measure oxidative stress markers, tissue-specific ALP isoenzymes, mineral balance, or histopathological alterations. Consequently, the mechanisms underlying the small numerical differences observed in ALP activity cannot be determined from the present results. The absence of a statistically significant effect may reflect a limited biological response under the applied experimental conditions, although the small sample size may also have reduced the ability to detect subtle treatment-related changes. Further studies involving larger experimental groups, additional biochemical markers, mineral measurements, and histopathological analyses are required to clarify whether lignite ash or heavy metal exposure influences ALP-related physiological processes in chickens.

CONCLUSIONS

This study showed that exposure of Hisex Brown chickens to Kosovo lignite ash and a heavy metal complex was associated with significant differences in plasma AST activity among the experimental groups. ALT activity was numerically higher in the ash-treated and metal complex-treated groups than in the control group; however, the overall difference was not statistically significant. Plasma glucose concentration and ALP activity also did not differ significantly among the groups. These findings suggest that AST may represent a sensitive biochemical response under the experimental conditions applied, whereas the results for ALT, glucose, and ALP remain inconclusive. Given the limited sample size, the findings should be interpreted cautiously and confirmed through studies involving larger experimental groups, additional hepatic and oxidative stress biomarkers, and histopathological evaluation.

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AUTHOR'S CONTRIBUTION

RF conceived and designed the study. QM and RF contributed to field sampling, data collection, laboratory analyses and interpretation of biochemical parameters. MFR and AK contributed to scientific interpretation, manuscript revision and critical review of the article. All authors have read and agreed to the published version of the manuscript.

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IRB statement

The collection and handling of chickens were carried out with attention to animal welfare and with efforts to minimize stress and harm to the animals during sampling. All procedures were conducted in accordance with generally accepted principles for the ethical use of animals in biological and environmental research.

Ethical approval

All experimental procedures involving animals were conducted in accordance with institutional guidelines for animal welfare and experimental procedures.

Informed consent statement

Not applicable.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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