

# Dietary Zinc Status in Offspring of Pregnant Rats Fed on a Zinc-Deficient Diet Is Associated with Serum Albumin, Ast, and Alt Levels

Meltem Gumus<sup>1</sup>, Saltuk Bugra Baltaci<sup>2</sup>

<sup>1</sup>Department of Child Health and Diseases, Selcuk University, Medical Faculty, Konya-Turkey

<sup>2</sup>Department of Physiology, Regenerative and Restorative Medical Research Center, Istanbul Medipol University, Istanbul, Turkey

Received: 2023-06-15 / Accepted: 2023-07-21 / Published Online: 2023-07-22

## Correspondence

Meltem Gumus, MD

Address: Department of Child Health and Diseases, Faculty of Medicine, Selcuk University, Konya, Turkey

e-mail: [meltem.dorum@selcuk.edu.tr](mailto:meltem.dorum@selcuk.edu.tr)



This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

## ABSTRACT

**Objective:** This study was carried out to investigate whether dietary zinc status is associated with serum albumin, AST and ALT levels in male offspring of mother rats fed a zinc deficient diet.

**Methods:** The study was carried out on male offspring (Groups 1, 2, 3) born to rats fed a zinc deficient diet and on male offspring (Group 4) born to mothers fed a standard diet. Group 1: Zinc deficient, Group 2; standard rat chow, Group 3: Zinc supplemented diet. Animals of group 4 were used as control group. After the completion of the experimental stages of the study, albumin, AST, ALT, free and total bilirubin levels in serum samples taken from animals were determined by spectrophotometric method.

**Results:** Dietary zinc deficiency (group 1) significantly decreased serum albumin values ( $p < 0.004$ ). Animals in both the zinc deficient (Group 1) group and the Group 2 animals born to mothers fed a zinc deficient diet and fed standard rat chow had the highest AST and ALT levels ( $p < 0.001$ ).

**Conclusion:** The present study is the first to show that dietary zinc status can directly affect liver function in rats born to zinc deficient mothers by causing changes in serum albumin, AST and ALT levels.

**Keywords:** Maternal zinc deficiency, zinc, Albumin, AST, ALT, male offspring rat

## INTRODUCTION

The liver, which is the main organ of zinc metabolism, is also critical in regulating and maintaining the zinc balance of the body [1]. Zinc plays a role in many enzymatic reactions of the liver, especially in the urea cycle [2]. Low zinc levels in liver patients were first reported by Vikbladh [3] in 1951. In later studies, it has been shown that both liver and serum zinc decrease in chronic liver diseases [4, 5].

Patients with liver cirrhosis have widespread zinc deficiency [6]. The levels of this deficiency are closely related to the severity of the disease [7]. Zinc deficiency in liver patients is also affected by changes in carbohydrate and protein metabolism. Another factor that can lead to zinc deficiency in liver diseases is changes

in albumin concentration. Albumin is the main circulating transporter of zinc [8].

Decreased albumin levels in liver diseases also lead to a decrease in zinc absorption, leading to the progression of liver disease [9]. Zinc deficiency is seen in 85.6% of liver patients [10]. Zinc deficiency in these patients may also be caused by metabolic disorders such as hepatic steatosis, iron overload, insulin resistance [11]. Zinc supplementation for liver patients improves these disorders [10, 11].

Aminotransferases; Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most sensitive tests showing liver tissue damage [12]. They are released from the

damaged liver cell. In cholestatic diseases, mild elevations can be seen due to hepatocyte damage caused by bile stasis. Since ALT is found in low concentrations in other tissues such as muscle, it is more specific to liver diseases [12, 13].

The aim of this study is to investigate how zinc deficiency and its administration affect circulating albumin, AST , ALT, direct and total bilirubin levels in male offspring rats born to mothers fed a zinc-deficient diet during pregnancy.

**MATERIALS AND METHODS**

The study was carried out at Selcuk University Experimental Medicine Research and Application Center. The study protocol was approved by the animal ethics committee of the same center (decision dated 27.03.2020 and numbered 2020-15).

**Animal Material and Groups**

The juveniles used in the study were obtained from 20 adult female rats, 15 of which were fed a zinc-deficient diet during their pregnancies and 5 of which were fed with standard rat chow during their pregnancies.

**Feeding of Animals**

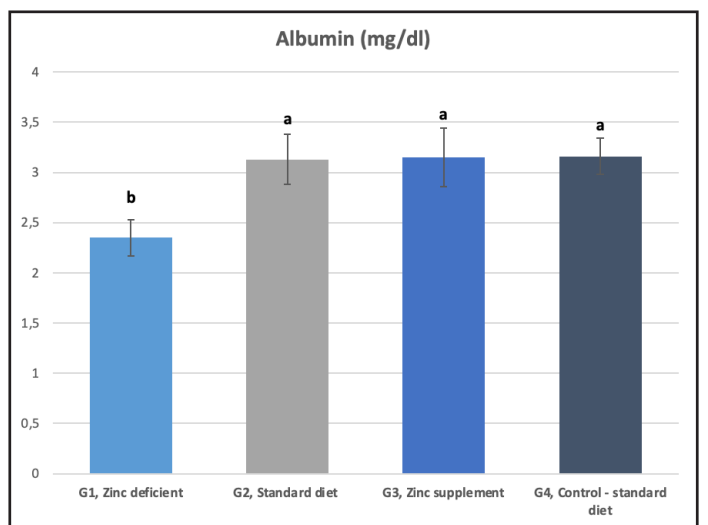
The rats were fed ad libitum and kept in 12-h light/dark cycle. Animal feed were obtained from Selcuk University Experimental Medicine Research and Application Center as normal standard rat feed (in pellets) (Table 1).

Energy content was 367 (11%) kcal / kg for Fat, 768 (24%) kcal / kg for Protein and 2,091 (65%) kcal / kg for Carbohydrates.

Vitamin mix: The vitamin mix of feed given to experimental animals contains vitamins A, D3, E, K, B1, B2, B6, B12 and nicotinamide, folic acid, D-biotin and choline chloride.

**Table 1.** Content of some trace elements and minerals in standard rat feed

Content	Value	Unit
Aluminium	79.37	mg/kg
Iron	3,484.07	mg/kg
Iodine	1.66	mg/kg
Cobalt	0.34	mg/kg
Copper	12.81	mg/kg
Manganese	95.06	mg/kg
Molybdenum	1.10	mg/kg
Sulfur	1,141.22	mg/kg
Selenium	0.25	mg/kg
Zinc	95.18	mg/kg
Calcium	7,012	mg/kg
Potassium	8,797	mg/kg
Magnesium	2,220	mg/kg
Sodium	2,128	mg/kg
Phosphorus	5,014	mg/kg



**Figure 1.** Serum Albumin Levels of Study Groups

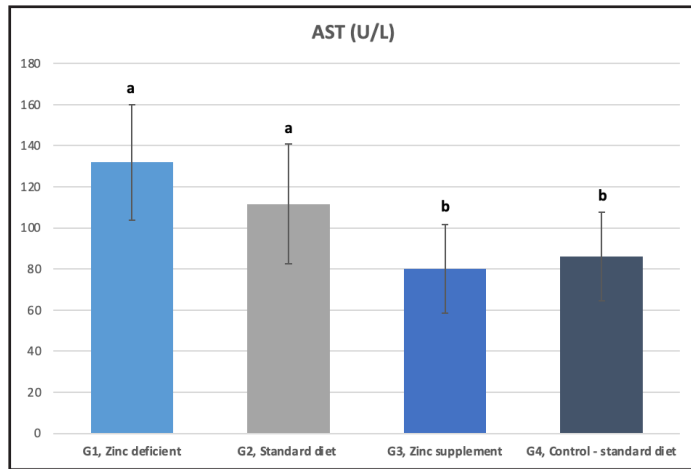
Means with different superscripted letters in the same column are statistically significant a>b (P<0.05).

Pairwise Comparison P Values of the Groups According to the Mann-Whitney U Test Results:

Albumin:G1-G2:0,004; G1-G3:0,000; G1-G4:0,000; G2-G3:0,989; G2-G4:0,939; G3-G4:0,915

**Main Points;**

- This study shows that dietary zinc status causes changes in serum albumin, AST and ALT levels in male offspring born to mothers fed a zinc deficient diet during pregnancy.
- This study is the first to show that dietary zinc status can directly affect liver function in rats born to zinc-deficient mothers.

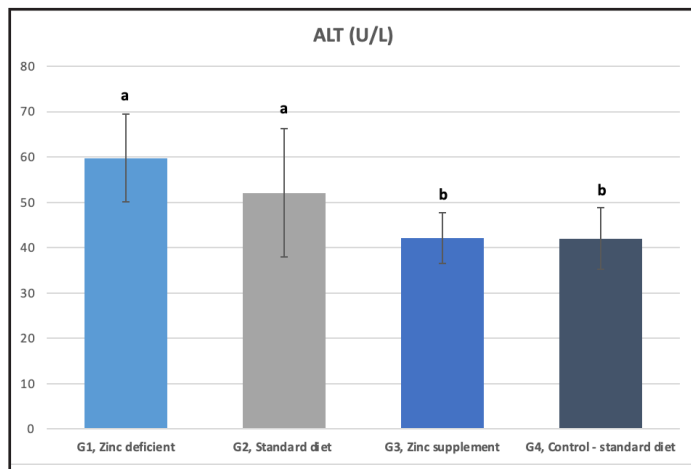


**Figure 2.** Serum AST Levels of Study Groups

\*Means with different superscripted letters in the same column are statistically significant a>b (P<0.05).

Pairwise Comparison P Values of the Groups According to the Mann-Whitney U Test Results:

AST=G1-G2:0,294; G1-G3:0,000; G1-G4:0,001; G2-G3:0,041; G2-G4:0,030; G3-G4:0,951

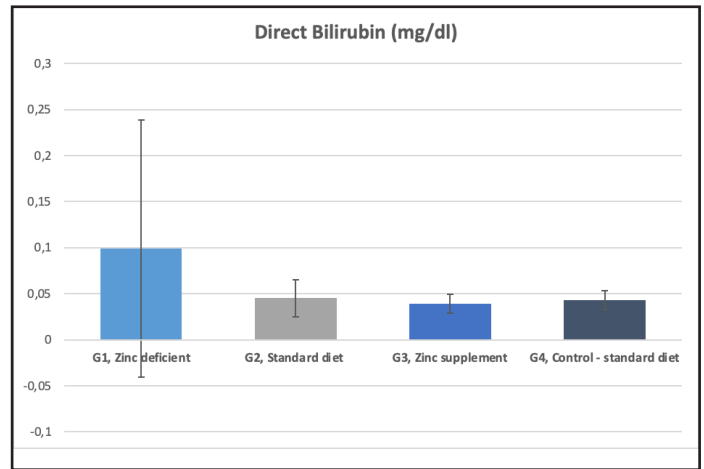


**Figure 3.** Serum ALT Levels of Study Groups

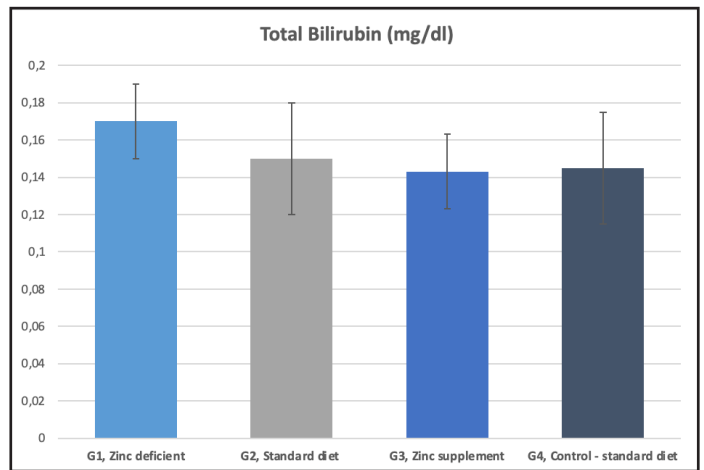
\*Means with different superscripted letters in the same column are statistically significant a>b (P<0.05).

Pairwise Comparison P Values of the Groups According to the Mann-Whitney U Test Results:

ALT.G1-G2:0,297; G1-G3:0,001; G1-G4:0,001; G2-G3:0,113; G2-G4:0,040; G3-G4:1,000



**Figure 4.** Direct Bilirubin Levels of Study Groups



**Figure 5.** Direct Bilirubin Levels of Study Groups

**Experimental Groups**

Offspring rats (Groups 1, 2, 3) born to mothers fed a zinc deficient diet (2.8 mg/kg zinc) and offspring rats born to mothers fed standard rat chow (Group 4) were separated from their mothers at 21 days of age. Male offspring rats were divided into groups as follows:

Group 1, Zinc deficient: Offspring rats in this group were fed a zinc deficient diet (2.8 mg/kg zinc) for 70 days [14].

Group 2, Standard Diet: Animals in this group were fed standard rat chow for 70 days.

Group 3, Zinc supplemented: Animals in this group received zinc supplementation (5 mg/kg/day ip zinc sulfate) until the end of the study (70 days) in addition to standard rat chow.

Group 4, Control: Control animals in this group were fed with standard rat chow.

### Informed Consent

After the completion of the experimental stages of the study, all animals were sacrificed under general anesthesia and serum samples were taken. General anesthesia was administered to all animals (with intramuscular administration of a combination of Ketalar (60 mg/kg), Parke-Davis and xylazine (5 mg/kg) “Rompun, Bayer”) to avoid animal suffering.

### Biochemical Analyzes

Biochemical analysis were carried out on 3 ml serum samples obtained from blood from animals. Albumin (mg/dl), AST (U/L), ALT (U/L), free and total bilirubin levels (mg/dl) in the obtained serum samples were determined by spectrophotometric method in the Abbott Architect c8000 device (Abbott Architect c8000 Chemistry Analyzer).

### Statistical Analysis

The mean and standard errors of the data in the study were calculated. Kruskal-Wallis H test was used for the difference between the groups. Mann-Whitney U test was used to determine which group the difference consisted of. A  $p < 0.05$  level was considered significant.

### RESULTS

The lowest serum albumin values in our study were obtained in group 1 fed a zinc deficient diet ( $p < 0.004$ ). Serum albumin values of groups 2, 3 and 4 were not different from each other (Graphic 1). In our study, the highest AST and ALT levels were obtained in Group 1, which was fed with a zinc-deficient diet after maternal zinc deficiency was created, and in Group 2, which was fed with a normal diet after maternal zinc deficiency was created ( $p < 0,001$ ). After the maternal zinc deficiency was established, the AST and ALT levels of Group 3, which was supplemented with zinc in addition to the standard rat diet, were higher than Groups 1 and 2 ( $p < 0,001$ ), and were not different from the control group (Group 4) (Graphics 2, 3). Direct and total bilirubin values did not differ between the groups (Graphics 4, 5).

### DISCUSSION

An important trace element, zinc is associated with many events from growth, reproduction, immune functions to aging [15]. The liver can increase the bioavailability of many elements, especially zinc [16]. In this respect, a relationship between liver and zinc is inevitable. At the same time, the deficiency of zinc, which is a powerful antioxidant element, in the body

may contribute to deterioration in liver functions and/or the progression of chronic liver diseases [16, 17]. Yang et al. [17] reported a significant decrease in serum albumin levels of the patient group whose zinc was found to be significantly lower than the controls in a study performed on patients with cirrhosis. Similarly, it has been reported that serum zinc and albumin levels are significantly lower in patients with infections caused by acute phase response activation [18]. Morisaku et al. [19] found low serum zinc and albumin levels in patients with malignant lymphoma. The same researchers reported that their findings were the first to show that albumin was correlated with zinc in malignant lymphoma [19]. In our study, the lowest serum albumin values were obtained in group 1 fed a zinc-deficient diet. This finding shows that dietary zinc deficiency suppresses albumin concentration in rats with maternal zinc deficiency. Reporting that albumin levels decrease in parallel with low zinc levels in various diseases, especially liver diseases [17-19] is in strong agreement with the low zinc and albumin levels we obtained in our study.

Elevated AST levels have been demonstrated in patients with hepatitis B liver cirrhosis, with decreased serum zinc levels. In the same study, it was reported that as the disease progresses, the decrease in zinc levels and the increase in AST levels occur more severely [20]. Reporting that increased AST and ALT levels in protein-deficient rats are prevented by zinc supplementation is a critical finding for the relationship between zinc and AST and ALT [21]. Similarly, Yousef et al. [22] reported that zinc deficiency causes an increase in AST and ALT levels in growing rats. In conclusion, zinc has a critical relationship with AST and ALT levels, which are used as markers for liver dysfunction.

In the current study, the highest AST and ALT levels were obtained in Group 1 fed with zinc deficient diet after maternal zinc deficiency was established and Group 2 fed with normal diet after maternal zinc deficiency was established. High AST and ALT levels, especially in Group 2, are a very important finding. Because the animals in this group with maternal zinc deficiency were fed with a normal diet, but there was no suppression in AST and ALT levels. However, in our study, AST and ALT levels of Group 3, which was supplemented with zinc in addition to the standard rat feed after maternal zinc deficiency was created, were lower than Group 1 and 2, and were not different from the control group (Group 4).

In our study, direct and total bilirubin values did not differ

between the groups. We could not find a study that directly deals with the relationship between bilirubin and zinc in med-line scans.

A report was published in 2013, stating that severe cholestasis seen in 3 premature babies in a healthcare facility in the United States can be prevented with zinc supplementation (Centers for Disease Control and Prevention “CDC” 2013) [23]. However, there is no detailed breakdown of the said report. We did not find an association between maternal zinc deficiency or zinc status and bilirubin levels in the current study.

In the current study; Albumin levels, which were significantly suppressed in zinc deficiency, reached control values with standard feed or zinc application. However, serum AST and ALT levels were significantly higher both in the group fed with a zinc deficient diet after maternal zinc deficiency was established (group 1) and in the group fed with a normal diet after maternal zinc deficiency was established (group 2). In our study, it is an important finding that feeding the pups born to mothers fed a zinc-deficient diet during pregnancy with standard rat chow for 70 days did not abolish the increase in AST and ALT levels. This critical finding shows that liver development and therefore liver functions of offspring born to mothers with zinc deficiency during pregnancy may be adversely affected.

## CONCLUSION

The present study is the first to show that dietary zinc status can directly affect liver functions by causing changes in serum albumin, AST and ALT levels in rats with maternal zinc deficiency.

In possible future studies, revealing the effects of different doses of zinc in the diet may provide us with more concrete information.

**Author Contributions:** MG and SBB made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; MG and SBB drafted the work or revised it critically for important intellectual content, approved the version to be published. The authors declare that all data were generated in-house and that no paper mill was used.

**Conflict of interest:** The authors declare that they have no potential conflicts of interest to disclose.

## Research Data Policy and Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**Funding:** This study was supported by the Scientific Research Projects Coordinatorship of Selcuk University (SUBAPK; project no. 20401093).

**Ethical Approval:** This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Experimental Animals Ethics Board of Selcuk University Experimental Medicine Research and Application Center (2020-15). This research was performed on the animals (rat).

## REFERENCES

- [1] Bloom A, Bloom S, Silva H, Nicoll AJ, Sawhney R (2021) Zinc supplementation and its benefits in the management of chronic liver disease: An in-depth literature review. *Ann Hepatol.* 100549. <https://doi.org/10.1016/j.aohep.2021.100549>
- [2] Riggio O, Merli M, Capocaccia L, Caschera M, Zullo A, Pinto G, Gaudio E, Franchitto A, Spagnoli R, D'Aquilino E (1992) Zinc supplementation reduces blood ammonia and increases liver ornithine transcarbamylase activity in experimental cirrhosis. *Hepatology* 16(3):785-789. <https://doi.org/10.1002/hep.1840160326>
- [3] Vikbladh I (1951) Studies on zinc in blood II. *Scand J Clin Lab Invest.* 3(2):1-74.
- [4] Ebara M, Fukuda H, Hatano R, Yoshikawa M, Sugiura N, Saisho H, Kondo F, Yukawa M (2003) Metal contents in the liver of patients with chronic liver disease caused by hepatitis C virus. Reference to hepatocellular carcinoma. *Oncology* 65 (4):323-330. <https://doi.org/10.1159/000074645>
- [5] Omran DA, Darweesh SK, Fouad H, Mahmoud M, Saif S, Fared A, Hassany M, Mobarak L, El-Tahawy MA, Yosry A (2017) Serum zinc deficiency and its relation to liver fibrosis in chronic HCV: a real-life Egyptian study. *Biol Trace Elem Res.* 179 (1):1-7. <https://doi.org/10.1007/s12011-017-0938-x>
- [6] Sengupta S, Wroblewski K, Aronsohn A, Reau N, Reddy KG, Jensen D, Te H (2015) Screening for zinc deficiency in patients with cirrhosis: When should we start? *Dig Dis Sci.* 60

- (10):3130-3135. <https://doi.org/10.1007/s10620-015-3613-0>
- [7] Tan HK, Streeter A, Cramp ME, Dhanda AD (2020) Effect of zinc treatment on clinical outcomes in patients with liver cirrhosis: A systematic review and meta-analysis. *World J Hepatol.* 12 (7):389-398. <https://doi.org/10.4254/wjh.v12.i7.389>
- [8] Grüngreiff K, Gottstein T, Reinhold D, Blindauer CA (2021) Albumin substitution in decompensated liver cirrhosis: Don't forget zinc. *Nutrients* 13 (11):4011. <https://doi.org/10.3390/nu13114011>
- [9] Grüngreiff K, Reinhold D, Wedemeyer H (2016) The role of zinc in liver cirrhosis. *Ann Hepatol.* 15(1):7-16. <https://doi.org/10.5604/16652681.1184191>
- [10] Llibre-Nieto G, Lira A, Vergara M, Solé C, Casas M, Puig-Diví V, Solé G, Humanes A, Grau L, Barradas JM, Miquel M, Sánchez-Delgado J (2021) Micronutrient deficiencies in patients with decompensated liver cirrhosis. *Nutrients* 13(4):1249. <https://doi.org/10.3390/nu13041249>
- [11] Himoto T, Masaki T (2018) Associations between zinc deficiency and metabolic abnormalities in patients with chronic liver disease. *Nutrients* 10(1):88. <https://doi.org/10.3390/nu10010088>
- [12] Celik D, Tatar B, Köse S, Odemis I (2020) Evaluation of the diagnostic validity of noninvasive tests for predicting liver fibrosis stage in chronic hepatitis B patients. *Acta Gastroenterol Belg.* 83(3):419-425.
- [13] Dalgıç B (2002) Çocuklarda Karaciğer Hastalıklarının Değerlendirilmesi. *STED* 11(5):173-176.
- [14] Goktepe E, Baltaci SB, Unal O, Unlukal N, Mogulkoc R, Baltaci AK (2023) The relationship between beta cell activation and SLC30A8/ZnT8 levels of the endocrine pancreas and maternal zinc deficiency in rats. *J Trace Elem Med Biol.* 2023 May 19;79:127217. <https://doi.org/10.1016/j.jtemb.2023.127217>
- [15] Baltaci AK, Mogulkoc R, Baltaci SB (2019) Review: The role of zinc in the endocrine system. *Pak J Pharm Sci.* 32(1):231-239.
- [16] Katayama K (2020) Zinc and protein metabolism in chronic liver diseases. *Nutr Res.* 74:1–9. <https://doi.org/10.1016/j.nutres.2019.11.009>
- [17] Yang W, Wang X, Yu Z, Li C, Sun M, Li Y, Hui Y, Guo G, Fan X, Jiang K, Sun C (2022) Low levels of serum zinc associate with malnutrition risk assessed by the Royal Free Hospital-Nutritional Prioritizing tool in cirrhosis. *Biol Trace Elem Res.* 200(10):4289-4296. <https://doi.org/10.1007/s12011-021-03033-1>
- [18] Hedegaard CV, Soerensen MD, Jørgensen LH, Schaffalitzky de Muckadell OB (2022) Investigating hypozincemia and validity of plasma zinc measurements in infected patients. *Scand J Clin Lab Invest.* 82(5):371-377. <https://doi.org/10.1007/s12011-021-03033-1>
- [19] Morisaku M, Ito K, Ogiso A, Imai M, Hiraoka Y, Zennami M, Tsuge M, Mori M, Toyosato S, Matsuda H, Ando Y, Tokuda M, Tomita A, Yamada S (2022) Correlation between serum albumin and serum zinc in malignant lymphoma. *Fujita Med J.* 8(2):59-64. <https://doi.org/10.20407/fmj.2021-006>
- [20] Wang S, Fan X, Gao Y, Zuo L, Hong M, Xu Y (2023) The relationship between zinc deficiency and hepatocellular carcinoma associated with Hepatitis B liver cirrhosis: A 10-year follow-up study. *Biol Trace Elem Res.* 201(1):114-120. <https://doi.org/10.1007/s12011-022-03156-z>
- [21] Sidhu P, Garg ML, Dhawan DK (2005) Time dependent study to evaluate the efficacy of zinc on hepatic marker enzymes and elemental profile in serum and liver of protein deficient rats. *Biometals* 18(1):97-106. <https://doi.org/10.1007/s10534-004-1960-y>
- [22] Yousef MI, El-Hendy HA, El-Demerdash FM, Elagamy EI (2002) Dietary zinc deficiency induced-changes in the activity of enzymes and the levels of free radicals, lipids and protein electrophoretic behavior in growing rats. *Toxicology* 175(1-3):223-234. [https://doi.org/10.1016/s0300-483x\(02\)00049-5](https://doi.org/10.1016/s0300-483x(02)00049-5)
- [23] Centers for Disease Control and Prevention (CDC) (2013) Notes from the field: Zinc deficiency dermatitis in cholestatic extremely premature infants after a nationwide shortage of injectable zinc - Washington, DC, December 2012. *MMWR Morb Mortal Wkly Rep.* 62(7):136-137.

**How to Cite;**

Gumus M, Baltaci SB. (2023) Dietary Zinc Status in Offspring of Pregnant Rats Fed on a Zinc-Deficient Diet Is Associated with Serum Albumin, Ast, and Alt Levels. *Eur J Ther.* 29(3):552-557. <https://doi.org/10.58600/eurjther1659>