The effects of clinoptilolite supplementation to diet on serum enzyme activities in laying hens

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SUMMARY

This experiment was conducted to determine the effects of a dietary clinoptilolite (CPL) supplementation on some serum enzyme (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), creatine kinase (CK), and lactate dehydrogenase (LDH)) activities in laying hens. For that, 96 White Lohman LSL laying hens aged of 70 weeks were randomly divided into 4 equal groups (n = 24) according to the CPL content added to the basal diet (2 650 kcal/kg ME and 16% crude protein) during 8 weeks: 0% in the group I (controls), 1% in the group II, 2% in the group III and 3% in the group IV. The AST, ALT, ALP, GGT, CK and LDH activities were measured using a semi-automated Chemistry Analyzer. Supplemental CPL did not affect serum AST, ALT, CK, GGT, but it significantly (P<0.05) increased the serum ALP and LDH activities. Moreover, a clinoptilolite dose – effect relationship was evidenced, birds receiving the highest CPL dose exhibiting the more marked alterations of serum ALP and LDH activities. Because of the roles of ALP in the formation of bones and LDH in the metabolism of carbohydrate, these results suggest that CPL supplementation may be harmful in laying hens.

Keywords: Clinoptilolite, laying hens, serum, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transpeptidase, creatine kinase, lactate dehydrogenase.

INTRODUCTION

Zeolites are crystalline, hydrated aluminosilicates of alkali (i.e. Na+, K+) and alkaline-earth cations (i.e. Mg2+, Ca2+) having infinite three-dimensional structures. Their ability to gain and lose water induces structural major changes. The Ca2+ cations are exchangeable with other cations such as NH4+, Mg2+, Na+ and K+ [19]. The wide ranges of Zeolite applications are based on their physicochemical properties. Zeolites present high ion selectivity for Ca and P, a high adsorption capacity for high molecular compounds, an efficient cation exchange capacity, as well hydration, dehydration and deodorising properties and acid resistance [22,26]. Consequently, they have found various applications as adsorbents, ion exchanges and catalysts in industry, agriculture, veterinary medicine, sanitation and environmental production. Biological applications include the removal of ammonia from waste water, air filtration and deodoration, soil amelioration and fertilization [4,14,15]. The effects of dietary Zeolites in poultry have been investigated extensively and a growth promoting effect of clinoptilolites used as additives in poultry nutrition was reported [9].

Certain tissue cells contain characteristic enzymes which enter the blood only when the cells to which they are confined are damaged or destroyed. The presence in the blood of significant quantities of these specific enzymes indicates the probable site of tissue damage. Among the most sensitive and widely used of these liver enzymes are the aminotransferases. They include AST and ALT. These enzymes are normally contained within liver cells. If the liver is injured, the liver cells spill the enzymes into blood, raising the enzyme levels in the blood and signaling the liver damage. ALT test measures the amount of the enzyme ALP in the blood. ALP is made mostly in the liver and in bone with some made in the intestines and kidneys. The liver makes more ALP than the other organs or the bones. Some conditions cause large

RÉSUMÉ

Effets d’une supplémentation en clinoptilolite sur les activités des enzymes sériques chez les poules pondesues

Cette expérimentation étudie les effets d’une supplémentation en Clinoptilolite (CPL) sur les activités de certaines enzymes sériques (aspartate aminotransferase (AST), alanine aminotransferase (ALT), phosphatase alkaline (ALP), gamma-glutamyl transpeptidase (GGT), creatine kinase (CK), and lactate dehydrogenase (LDH)) chez les poules pondesues. A cette fin, 96 poules pondesues White Lohman LSL âgées de 70 semaines ont été aléatoirement réparties en 4 groupes égaux selon la teneur en CPL ajoutée à la ration de base pendant 8 semaines : 0 % dans le groupe I (témoins), 1 % dans le groupe II, 2 % dans le groupe III et 3 % dans le groupe IV. Les activités enzymatiques de AST, ALT, ALP, GGT, CK et LDH ont été mesurées en utilisant un analyseur chimique semi-automatique. La supplémentation en CLP n’est pas eu d’effet significatif sur les activités sériques de AST, ALT, CK, GGT mais elle a augmenté de façon significative celles de ALP et LDH. Une relation dose-dépendante a été montrée : les activités ALP et LDH des oiseaux recevant la dose la plus élevée de CPL ont été les plus altérées. Étant donné le rôle de l’ALP dans la formation de l’os et celui de la LDH dans le métabolisme glucidique, l’addition de CPL dans la ration des poules pondesues pourrait s’avérer dangereuse.

Mots clés : Clinoptilolite, poules pondesues, serum, aspartate aminotransferase, alanine aminotransferase, phosphatase alkaline, gamma-glutamyl transpeptidase, creatine kinase, lactate dehydrogenase.
amounts of ALP in the blood. These conditions include rapid bone growth, bone disease or a disease that affects how much calcium is in the blood, or damaged liver cells. Clinically, CK is assayed in blood tests as a marker of myocardial infarction, rhabdomyolysis, muscular dystrophy, myositis, myocarditis, malignant hyperthermia and neuroleptic malignant syndrome. GGT catalyzes the transfer of the glutamyl groups among different polypeptides and amino acids. Clinically significant GGT found in the blood comes from cells that line the biliary tract. GGT levels rise dramatically with obstructive diseases of the biliary tract and liver cancers. GGT is especially useful in assessing liver function associated with alcohol-induced liver disease. This enzyme catalyzes the reversible reaction between pyruvic and lactic acids. LDH is present in nearly all types of metabolizing cells, but different cells have different forms of the enzyme which can be distinguished. The enzyme is especially concentrated in the heart, liver, red blood cells, kidneys, muscles, brain, and lungs. Certain diseases have classic patterns of elevated LDH isoenzyme levels. For example, an LDH-1 level higher than that of LDH-2 is indicative of a heart attack or injury; elevations of LDH-2 and LDH-3 indicate lung injury or disease; elevations of LDH-4 and LDH-5 indicate liver or muscle disease or both. A rise of all LDH isoenzymes at the same time is diagnostic of injury to multiple organs [16,24].

The purpose of this experiment was to measure some serum enzyme (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), creatine kinase (CK), and lactate dehydrogenase (LDH)) activities in laying hens dietary supplemented with clinoptilolite.

### Materials and Methods

#### ANIMALS AND PROTOCOL DESIGN

The purpose of this experiment was to measure some serum enzyme (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), creatine kinase (CK), and lactate dehydrogenase (LDH)) activities in laying hens dietary supplemented with clinoptilolite.

#### ANIMALS AND PROTOCOL DESIGN

The Research Animal Ethic Committee of Atatürk University approved all procedures under this experimental protocol. Ninety-six 70 week-old white Lohman LSL hens with a mean body weight of 1.5 kg were randomly assigned to one of the 4 experimental diets for a period of 8 weeks (n = 24). Within a given group, 6 subgroups of 4 hens each were constituted and hens were housed in 50 x 46 x 46 cm³ cages. The room temperature was maintained at 20°C, and lighting is supplied 10 h natural light and 6 h artificial to 8 h darkness was provided and hens were equally distributed in upper and lower cages in order to minimize the cage level effect. Hens of the group I served as controls and received only the basal diet (Metabolisable Energy: 2650 kcal / kg - Crude protein: 16%) whereas birds of the 3 other groups (II, III and IV) were supplemented with various dosages of Clinoptilolite (CPL) in the ration (1, 2 and 3% respectively) during 8 weeks (Table I). The mineralogy of CPL can be summarized as follows: 70% clinoptilolite and 30% illite, calcite, apatite, rutile and feldspar. CPL chemical characteristics were: 4.35%

<table>
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<tr>
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<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
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<tr>
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<tr>
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#### Nutritional values of diets

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<tr>
<td>Crude protein (%)</td>
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<td>16</td>
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<tr>
<td>Crude fiber (%)</td>
<td>3.85</td>
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<tr>
<td>Ether extract (%)</td>
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<tr>
<td>Ca (%)</td>
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<tr>
<td>P (%)</td>
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</table>

¹Per kg contains: Vitamine A 1,500,000 IU, Cholecalciferol 150,000 ICU, Vitamin E (di-tocopherol acetate) 3,000 IU, menadione 50 mg, thiamine 30 mg, riboflavin 60 mg, niacin 200 mg, pantothenic acid 80 mg, pyridoxine 50 mg, folic acid 10 mg, Vitamin B12 150 mg, Mn 800 mg, Zn 600 mg, Fe 300 mg, Cu 50 mg, I 20 mg and Se 1.5 mg. In the text.

²ME: Metabolisable Energy.

Table I: Ingredients and chemical compositions of the experiment diets given to the laying hens.

H2O, 68.70% SiO2, 10.20% Al2O3, 4.20% CaO, 2.10% K2O, 1.31% Fe2O3, 0.85% MgO, 0.40% Na2O, 0.18% MnO, 0.14% TiO2, 0.10% P2O5. CPL ion selectivity gradient was: Cs > NH4+ > Pb > K > Na > Ca > Mg > Ba > Cu > Zn > Mn. Cationic exchange capacity (CEC) was 2.16 meq/100g. The distribution of CPL particle size (%w/w) was determined using a separator equipped with 5 screens: 23.49% bigger than 2.000 mm, 66.78% between 1.000 and 2.000 mm, 8.46% between 0.500 and 1.000 mm, 0.85% between 0.250 and 0.500 mm, 0.23% between 0.125 and 0.250 mm and 0.19% below 0.125 mm. Feed was supplied at 8 hours am and water was available ad libitum.

BLOOD COLLECTION AND BIOCHEMICAL ANALYSIS

At the end of the experiment, blood samples were collected from all birds from each group by puncture of the cutaneous ulnaris vein into sterile tubes. After clotting at room temperature during 30 minutes, blood was centrifuged at 3000 g for 10 minutes at room temperature. Serum was carefully harvested and stored at -20°C until analysis. Serum aspartate aminotransferase [3], alanine aminotransferase [3], alkaline phosphatase [24], gamma-glutamyl transpeptidase [20], creatine kinase [13] and lactate dehydrogenase [2] activities by semi-automated Chemistry Analyzer (Chem-Pro, PMCH-703, Ataturk University, College of Health Service Laboratory).

STATISTICAL ANALYSIS

Statistical analysis was performed by the statistical package SPSS, version 6.0. Multiple comparisons of the data were done using the Duncan test after one-way analysis of variance (ANOVA) and simple correlation analysis was performed between serum enzyme activities in laying hens supplemented with Clinoptilolite (1 to 3%). A P < 0.05 was considered as statistically significant. The data are presented as mean ± standard error of mean (SEM).

Results

The results of serum enzyme activities obtained in laying hens supplemented or not with Clinoptilolite were presented in Table II. Whereas aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transeptidase and creatine kinase activities remained relatively similar in all groups, alkaline phosphatase and lactate dehydrogenase activities were significantly modified in the experimental groups compared to the controls (P <0.01). Dietary clinoptilolite supplementation has significantly increased serum alkaline phosphatase and lactate dehydrogenase activities. In this way, LDH activity positively correlated with ALP and CK activities (r = 0.932, p < 0.01) and (r = 0.336, p < 0.05) respectively and a significant positive correlation was also evidenced between ALP and CK activities (r = 0.347, P < 0.05) in laying hens supplemented with clinoptilolite.

Discussion

OGUZ H. et al. [18] have also reported that the dietary clinoptilolite supplementation (1.5%) significantly reduced serum AST activity and increased ALT activity in broiler chickens. DWYER et al. [8] found no changes in biochemical indicators of kidney and liver functions (albumin, blood urea nitrogen, alkaline phosphatase, γ-glutamyl transferase, alanine aminotransferase, glucose) in broiler chickens supplied with clinoptilolite. YOUSEF [28] reported that there were no effects in the activities of ALT, AST, and ALP but, LDH activity was significantly increased in plasma due to AlCl3 administration. WILHELM et al. [27] also showed that Al exposure can result in Al accumulation in the liver and this metal can be toxic to the hepatic tissue at high concentrations. Salts of Al may bind to DNA, RNA; inhibit such enzymes as alkaline phosphatases, phosphodiesterase and phosphoxygenase [17]. ANANE and CREPPY [1] determined that the activities of LDH were significantly increased during Al exposure and this was used as a marker of aluminium toxicity. This was further confirmed when Al treatment was found to have a significant effect on the various membrane-bound enzymes in terms of decreased activities of AST, ALT, ALP. Aluminum citrate had no significant effect on serum ALP activity. The concomitant localization of Al and the early cal-
cification defect in the region of tibia malformation implicate aluminum in the pathogenesis of the skeletal abnormality [11]. Although several studies have confirmed the positive role of Al in various pathological disorders in mammals [22], aluminum toxicity in other species particularly in avis needs attention. Similarly when chicks were fed with AlCl₃ for long time resulted in alteration of serum ALP suggesting involvement of Al with metabolism of bone and renal tissues of chicks [23]. SZILAGYI et al [23] also showed that from the beginning of the third week Al was added to the diet as AlCl₃. Treatments included supplemental Al content of 0, 200, 1000 and 3000 mg/kg ration. The treated groups showed significantly elevated ALP activities and this change was dose-dependent. High levels of ALP are due to increased osteoblastic activity, provoked by the disturbance of bone formation, caused in turn by aluminium. In the treated groups, the activities of AST, GGT, and CK were similar in the controls and treated animals. There are some reports which indicate a relationship between death of fish and birds with increasing levels of Al in the water due to elevation of environmental acid content [7, 12]. In recent years, several workers have demonstrated that facilitation of gastrointestinal absorption of Al and its preferable accumulation in brain, bone, liver and kidney occur by dietary organic constituents [5, 6]. Aluminium enters the organism via gastrointestinal tract and lungs. In blood plasma, Al is bound to nondialyzable components. It is stored in lung and hilar lymph nodes from inhaled air. Increased doses of Al give rise to its concentrations mainly in the brain, liver, and blood. Al absorption and accumulation in blood and tissues occurs in patients with chronic renal failure on dialysis [10, 25]. Aluminium is a ubiquitous element found in every food product. The sources of Al are medicines and is also added to drinking water for purification purposes.

Consequently, clinoptilolite supplementation whereas aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase and creatine kinase activities remained relatively similar in groups, alkaline phosphatase and lactate dehydrogenase activities were significantly modified in the experimental groups compared to the controls. Abnormally high blood levels of alkaline phosphatase may indicate disease in bone or liver, bile duct obstruction, or certain malignancies. An increased amount of lactate dehydrogenase in the blood may be a sign of tissue damage and some types of cancer or other diseases. It was concluded that dietary Clinoptilolite supplementation would be deleterious for laying hens.

References


