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Effects of Dietary Zeolite on Serum Contents and Feeding Performance in Rats

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ABSTRACT

The objective of the present study was to determine the effects of dietary natural clinoptilolite on serum contents, health status and feeding performance of rats. Adult male Sprague–Dawley rats (n=24) were randomly divided into four groups with three replicates including a control group (without zeolite) and three doses of natural zeolite (2, 4 & 6%) in the diets. All rats were fed the above concentrates during the whole experimental period for 56 days. Blood samples were collected from each animal at the end of the experiment. Dietary clinoptilolite increased in serum albumin, triglyceride and VLDL levels ($P<0.05$). However, the differences among treatment groups were not significant for serum minerals (Ca, P, Mg, K, Na, Cl, Fe), urea, Fe binding, LDL, alkaline phosphatase, glucose, uric acid, total Fe, total protein, globulin, cholesterol, HDL cholesterol, creatinin; metabolizable energy and crude protein consumption for 1 g live weight gain of rats ($P>0.05$). The results showed that the supplementation of clinoptilolite did not have positive effect on serum concentrations of the investigated parameters apart from albumin, triglyceride and VLDL, but they had no negative effect on the health status of animals.

Key Words: Blood; Clinoptilolite; Diet; Mineral content; Rat

INTRODUCTION

The main interest in the biological effects of zeolites concerns one or more of their physical and chemical properties, such as ion exchange capacity, adsorption and related molecular sieve properties. Zeolites are used for various applications including adsorbents, ion exchangers and catalysts in industry, agriculture, veterinary medicine, sanitation and environmental protection (Martin-Kleiner, 2001). Natural zeolite tuffs especially clinoptilolite and analysime are the most abundant species widely distributed on huge deposits in some regions of Turkey (Balevi *et al*., 1999).

Beneficial effects of natural zeolite may be related to the species and the geographical source of the involved zeolite, it's purity and physicochemical properties, as well as the supplemental level used in the diets (Pond *et al.*, 1988). Furthermore, dietary and environmental conditions under which consistent positive responses to zeolite administration are expected should also be considered (Pond & Mumpton, 1984).

Different dietary levels of zeolite were tested in various animal species by many researchers. In veterinary medicine, there is evidence in the literature that the use of natural zeolites have favorable effects on growth and performance of ruminant animals (Walz *et al*., 1998; Ivan *et al.*, 2001). In a research with male Mehraban lambs with 30 and 60 g/kg zeolite inclusion serum K level and animal health were not affected by treatments (Forouzani *et al*., 2004). Besides, dietary zeolites improved weight gain of fattening pigs (Ward *et al.*, 1991), improved feed efficiency and egg production in laying hens (Fethiere *et al*., 1994).

Previous studies in different animal diets indicated that the supplementing of zeolites has no major effect on serum biochemistry. Supplementing broiler diets with hydrate sodium calcium alumino silicate - HSCAS (Dwyer *et al.,* 1997; Başalan *et al.*, 2005; Miles & Henry, 2007) with Na - alumino silicate (Kurtoğlu *et al.,* 1998), comparing with HSCAS, bentonite and PVPP - polyvinylpolyprolidone (Keçeci *et al.*, 1998), with sodium bentonite (Santurio *et al*., 1999; Tauqır & Nawaz, 2001; Tauqır *et al.*, 2001; Eraslan *et al.,* 2005), Novasil PLUS (Bailey *et al.*, 2006); in quail diets, with HSCAS (Eraslan *et al.,* 2004; Şehu *et al.*, 2005) did not effect most of serum parameters. Also in piglet diets, adding sodium bentonite (Schell *et al.*, 1993), clinoptilolite (Malagutti *et al.*, 2002; Prvulović *et al.*, 2007; Alexopoulos *et al.,* 2007); in rat diets, HSCAS (Abdel-Wahhab *et al.*, 1999; Afriyie-Gyawu *et al.*, 2005), clinoptilolite (Martin-

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Keiner *et al.,* 2001; Taş *et al.*, 2007); comparing with montmorillonite and HSCAS (Abdel-Wahhab *et al.,* 2002), Tunus montmorillonit clay - TMC (Abes *et al.*, 2007); in Balb/c mice diet, HSCAS (Abbes *et al.,* 2006b) were used in animal diets.

The aim of the present study was to determine the effects of different levels of dietary zeolite (0, 2, 4 or 6%) on the feeding performance and various serum health parameters (urea, LDL, glucose, uric acid, total Fe, total protein, globulin, triglyceride, cholesterol, HDL cholesterol, albumin, VLDL, creatinine, Ca, P, K, Mg, Na, Cl, Fe levels, Fe binding capacity & alkaline fosfatase activity) in rats.

MATERIALS AND METHODS

Zeolitic material: In this study, zeolite used as the porous template medium was obtained from Manisa Gördes, Turkey. Clinoptilolite was the predominant mineral (95%) in the natural zeolite. The content and rates of clinoptilolite was presented in Table I. All animals accepted and tolerated the zeolite-supplemented diets devoid of problems.

Feeds and feding: Animal feeds were purchased from Elazığ Mixed Feed Mill. Experimental diets were prepared as powder and some feed ingredients were added in order to increase and balance nutritive values of feeds. At the feed unit of Veterinary Faculty, grounded mixed feeds were turned into pellet form with a special apparatus. Pelleted feed was kept in a drying cabin at 70°C for 12 h. In order to keep dry matter contents of all group feeds, water capacity of feeds were lowered to normal levels. When water percentages of feeds were decreased enough, feeds taken out from machine and put back into bags according to their groups after they were cooled and shortened to 2 cm long. Each experimental group received its specific diet throughout the experimental period. All of the experimental diets were prepared as iso-caloric (ME: 3200 kcal/kg) and iso-nitrogenic (crude protein: 22.50%).

Animals and treatments: Eight weeks old, average 307±19 g initial weight, 24 healthy adult male Sprague–Dawley rats were used in the experiments. Animals were obtained from Experimental Research Centre (DUSAM). They were kept individually under standard laboratory conditions (12-h light/dark, 24±3°C). Feed and water were provided as *ad libitum*. The number of rats per group as well as the levels of clinoptilolite (% of concentrates) added in the concentrate feed of each group were as follows:

Group I (control) as standard diet did not contain natural clinoptilolite as feed additive; Group II, III and IV were treatment groups, which contain 2%, 4% and 6% clinoptilolite rates, respectively. Each group comprised of 6 rats.

Blood Sample collection: At the end of the trial, rats were anesthetized by the application of 100 mg/kg dose Ketamin HCL as intra muscular. Blood samples were taken as intra cardiac, prepared and analyzed by Abbott kit and autoanalyser apparatus (Olympus AU 400).

Statistical analyses: The trial was arranged according to Randomized Plots Design with four groups and three replicates. In the statistical comparisons between the groups, one-way analysis of variance (ANOVA) was used. The significance controls of the differences between the groups were determined by the Duncan Multiple Range Test (Duncan, 1955). Averages of the data were calculated. All statistical analyses were carried out using the SPSS program v. 10.0 for windows. Means and standard errors (mean+S.E.M.) were presented in Table II and III. P values <0.05 were accepted as statistically significant.

RESULTS

Metabolizable energy and crude protein consumptions for 1 g live weight gain of rats were presented in Table II. From the results, it is clear that energy and crude protein consumptions were not influenced by the administration of clinoptilolite (P>0.05).

There were significant differences in blood serum albumin, triglyceride and VLDL values among all investigated groups (P<0.05). Addition of dietary clinoptilolite has increased serum albumin, triglyceride and VLDL levels. However, the differences among treatment groups were non-statistically (P>0.05) for serum minerals (Ca, P, Mg, K, Na, Cl, Fe), urea, Fe binding, LDL, alkaline phosphatase, glucose, uric acid, total Fe, total protein, albumin, cholesterol, HDL cholesterol and creatinin (Table III). The highest blood serum urea, Fe binding and LDL values were obtained from control group; alkaline phosphatase, Ca and Na from group II; glucose, uric acid, total Fe, total protein, albumin, globulin, triglyceride, cholesterol, HDL cholesterol, P, Mg and Cl from group III; creatinin, VLDL, K and Fe levels were obtained from group IV (Table III).

Average serum macro mineral contents were ranged between for Ca (10.28 - 10.70 mg/dL), K (5.56 - 6.25 mg/dL), Na (143.93 - 144.74 mg/dL), P (8.48 - 9.87 mg/dL), Mg (2.62 - 2.83 mg/dL) and for Cl (101.87 - 103.73 mEq/L). The lowest serum macro mineral contents were obtained for Ca, Mg, Na and Cl from control group, for P and K from group II. Serum parameters ranged from highest to lowest for urea, Fe binding, LDL, alkaline fosfatase, glucose, uric acid, total Fe, total protein, globulin, triglyceride, cholesterol, HDL cholesterol, albumin, VLDL, creatinine and Fe levels (Table III).

With diets, the lowest levels were obtained for glucose, Fe, total Fe, total protein, albumin, globulin, triglyceride and VLDL from group control; creatinine, uric acid, cholesterol and HDL cholesterol from group II; alkaline fosfatase from group III; urea, Fe binding and LDL levels from the group IV (Table III).

DISCUSSION

Dietary zeolite levels did not effect significantly protein and energy intakes for 1 g live weight gain of rats.

Table I: Chemical analysis of clinoptilolite sample by XRF spectrometer (Anonymous, 2008)

Chemical content	Rate (%)
SiO_2	65 – 72
CaO	2.5 - 3.7
Fe_2O_3	0.8 - 1.9
Al_2O_3	10 – 12
K_2O	2,3 - 3.5
MgO	0.9 - 1.2
Na_2O	0,3 - 0.65
TiO_2	0 - 0.1
MnO	0 - 0.08
LOI*	9 – 12
SiO_2/ Al_3O_2	5.4 - 6.0

*LOI: Lost in burn

Table II: Metabolizable Energy and Crude Protein Consumptions for 1 g Live Weight Gain

Nutrients	GROUPS				Significance level
	Control (I)	2% zeolite (II)	4% zeolite (III)	6% zeolite (IV)	
Energy Consumption (ME, kcal / LWG, g)	59.91 ± 1.88	64.85 ± 2.50	69.24 ± 6.31	69.34 ± 5.90	0.463
Protein Consumption (CP, g / LWG, g)	3.48 ± 0.67	4.54 ± 0.17	4.88 ± 0.45	4.83 ± 0.41	0.195

Within each row, means superscript with the same letter are not significantly different (P<0.05)
ME: metabolizable energy consumption, LWG: live weight gain, CP: crude protein consumption

Table III: Average Blood Serum Parameters

Minerals	GROUPS				Significance
	Control (I)	2% zeolite (II)	4% zeolite (III)	6% zeolite (IV)	
Ca (mg/dL)	10.28 ± 0.33	10.70 ± 0.37	10.43 ± 0.27	10.55 ± 0.34	0.221
P (mg/dL)	9.55 ± 0.64	8.48 ± 0.33	9.87 ± 1.45	9.70 ± 1.15	0.149
K (mg/dL)	6.00 ± 0.46	5.56 ± 0.36	6.17 ± 0.78	6.25 ± 0.56	0.073
Mg (mg/dL)	2.62 ± 0.41	2.68 ± 0.34	2.83 ± 0.49	2.82 ± 0.19	0.714
Na (mg/dL)	143.93 ± 2.56	144.74 ± 0.99	144.55 ± 2.39	144.48 ± 0.83	0.496
Cl (meq/L)	101.87 ± 1.92	102.46 ± 1.00	103.73 ± 2.91	102.85 ± 1.16	0.170
Glucose (mg/dL)	159.72 ± 4.36	173.90 ± 5.04	197.35 ± 5.73	160.25 ± 5.99	0.220
Urea (mg/dL)	46.08 ± 2.72	42.68 ± 1.19	44.37 ± 2.34	42.65 ± 2.03	0.656
Creatinine (mg/dL)	0.57 ± 0.03	0.54 ± 0.02	0.57 ± 0.03	0.58 ± 0.02	0.771
Uric acid (mg/dL)	1.63 ± 0.14	1.56 ± 0.29	1.73 ± 0.24	1.63 ± 0.09	0.945
Alkaline phosphatase (U/L)	766.82 ± 154	816.44 ± 158	662.90 ± 134	702.37 ± 124	0.882
Fe (mg/dl)	198.33 ± 18	207.20 ± 15	205.33 ± 14	214.33 ± 14	0.906
Fe binding (mg/dL)	325.50 ± 14	317.80 ± 15	323.67 ± 16	314.17 ± 6	0.919
Total Fe (mg/dL)	523.83 ± 15	525 ± 15	529 ± 7	528.50 ± 12	0.988
Tot. protein (g/dL)	6.53 ± 0.08	6.60 ± 0.12	6.82 ± 0.15	6.68 ± 0.10	0.358
Albumin (g/dL)	3.03 ± 0.05 b	3.08 ± 0.04 ab	3.20 ± 0.04 a	3.17 ± 0.03 a	0.044
Globulin (g/dL)	3.50 ± 0.08	3.51 ± 0.11	3.62 ± 0.13	3.53 ± 0.07	0.813
Triglycerides (mg/dL)	42.45 ± 1.58 b	43.57 ± 1.67 b	58.40 ± 6.57 a	54.02 ± 5.32 ab	0.045
Cholesterol (mg/dL)	83.63 ± 3.58	80.32 ± 2.65	84.35 ± 4.91	81.00 ± 2.62	0.485
HDL cholesterol (mg/dL)	55.18 ± 2.63	54.44 ± 2.09	57.32 ± 3.08	54.85 ± 1.95	0.470
LDL (mg/dL)	19.00 ± 0.85	17.72 ± 1.21	15.37 ± 2.14	15.33 ± 0.74	0.096
VLDL (mg/dL)	8.68 ± 0.26 b	8.77 ± 0.37 b	11.00 ± 1.13 a	11.32 ± 0.67 a	0.020

[a, b] Within each row, means superscript with the same letter are not significantly different (P<0.05)

Protein and energy consumption values reflect the feed intake and these values of our trial were similar to the findings of different researches, who use different zeolites (HSCAS, clinoptilolite) with different animal species such as broiler, quail and lamb (Pond, 1989; Başalan *et al*., 2005; Şehu *et al.*, 2005). Nonetheless, our results are not parallel with the findings of some studies with swine, broiler, dairy cow and rat (Ward *et al*., 1991; Schell *et al*., 1993; Santurio *et al.*, 1999; Malagutti *et al*., 2002; Watts *et al.*, 2003; Afriyie-Gyawu *et al.*, 2005; Katsoulos *et al.,* 2005; Alexopoulos *et al.*, 2007; Miles & Henry, 2007). This is plausibly due to toxin free fresh feeds used in the experiments.

Zeolites affect mineral metabolism especially Ca and P, feeding performance and feed efficiency, changing blood pH, increasing bicarbonate levels and lowering Cl levels. When clinoptilolite added to higher Ca containing diets, the better results were obtained (Ballard & Edwards, 1988; Wisser *et al.*, 1990; Roland *et al.*, 1993). However, adding of supplement to lower Ca containing broiler diets were ineffective (Leach *et al.*, 1990). It was also stated that zeolite did not effect serum K and Na levels, but increased in the Ca levels (Roland *et al*., 1993). Similar results with the present study were obtained by the administration of clinoptilolite in sheep (Pond, 1989; Forouzani *et al.,* 2004), in dairy cow (Katsoulos *et al.*, 2005; Katsoulos *et al.*, 2006);

in mice (Martin-Kleiner *et al.*, 2001); and swine (Malagutti *et al.*, 2002; Kyriakis *et al.,* 2002; Prvulović *et al.*, 2007; Alexopoulos *et al.,* 2007; Trckova *et al.*, 2009).

It is suggested that clinoptilolite possibly supplies a significant amount of minerals to the diets of animals, providing that some minerals are present in this material in a form that can be assimilated by the body (Pond & Mumpton, 1984). Relatively small clinoptilolite rates probably did not alter significantly the serum mineral concentrations in this trial. However, our results were not parallel with the findings in mice diets in which blood serum K levels were increased with clinoptilolite (Martin-Kleiner, 2001). Clinoptilolite levels in our trial did not effect serum macro mineral contents and so it can be said that there were no synergetic or antagonistic relationships between clinoptilolite levels and macro mineral contents in rat feed.

Generally, there were no significant effect of many kind and levels of zeolites on serum biochemistry (health) parameters which they are parallel with our results (in pig diets, Malagutti *et al.,* 2002; Alexopoulos *et al.*, 2007; Prvulović *et al.*, 2007, in rat diets, Abdel-Wahhab *et al.*, 1999; Abdel-Wahhab *et al.*, 2002; Afriyie-Gyawu *et al.*, 2005; Abbes *et al.,* 2007, in mice diets, Martin-Kleiner *et al.,* 2001; Abbes *et al.*, 2006b).

However, there were limited reports on the effects of zeolites on blood serum parameters, which were inconsistent with the findings of our results (for serum Ca, P & Cl - Kurtoğlu *et al.*, 1998; for serum triglyceride, BUN - Abdel-Wahhab *et al.*, 2002; for serum HDL, LDL levels - Abbes *et al*., 2006b; for total cholesterol, triglyceride and AST levels - Prvulović *et al.,* 2007; for urea nitrogen, cholesterol and glucose levels - Alexopoulos *et al*., 2007).

In literature findings, additional clinoptilolite slightly increased the feeding costs, it prevent laboratory animals from toxic and teratogenic effects of the diet originated mycotoxins (Abdel-Wahhab *et al.,* 1999). Zeolites can be used in any kind of animal feeds as natural and cheap preservatives as compared to synthetic toxin binders, stored under in unsuitable undeveloped countries. It is suggested that clinoptilolite be preferred in order to prevent domestic animals and their products from carcinogenic side effects. In order to determine safety margins and therapeotic windows of clinoptilolite, different kind and doses of supplement may be used for different animal species in further experiments. Protective and curing properties of zeolites can be considered to be used in animal mixed feeds.

In conclusion, dietary use of clinoptilolite at 2, 4 and 6% did not cause any adverse clinical and biochemical effects on the health status of rats. The reason of unchanged liver enzyme levels are due to lower or lack of toxin in the feeds. Another reason for the stability in the serum parameters could be explained by the lower levels of clinoptilolite in rat diets.

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