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Araştırma Makalesi

EFFECTS OF DIETARY NATURAL ZEOLITE ON THE TESTICULAR WEIGHT, BODY WEIGHT AND SPERMATOLOGICAL CHARACTERISTICS IN RATS

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Ratlarda Doğal Zeolitin Testis Ağırlığı, Vücut Ağırlığı ve Spermatolojik Özellikler Üzerindeki Etkileri

Özet: Zeolitler iyon değişim özelliğine sahip doğal veya sentetik kristalize aliminyum slikat bileşikleridir. Kırk yılı aşkın süredir hayvanlarda yem katkı maddesi olarak kullanılmalarına rağmen zeolitlerin spermatolojik özellikler üzerindeki etkileri bilinmemektedir. Bu çalışmada, erkek ratlarda zeolitin vücut ağırlığı, testis ağırlığı ve spermatolojik özellikler üzerindeki etkilerinin belirlenmesi amaçlandı. Araştırmada 24 adet 8 haftalık Sprague–Dawley erkek rat kullanıldı. Ratlar çalışma başlangıcında tartılarak rastlantısal olarak dört gruba ayrıldı. Yüzde yirmi dört protein içeren standart toz rat yemine %0 (kontrol), %2, %4 ve %6 oranında zeolit karıştırılarak besleme grupları oluşturuldu. Daha sonra hazırlanan bu toz karışım pellet haline getirilerek 8 hafta süre ile ratların beslenmesinde kullanıldı. Çalışma sonunda ratların canlı ağırlıkları kaydedildi ve sodium thiopental anestezisi uygulandı. Daha sonra testisler, epididimisler, vesicula seminalisler ve ventral prostat çıkarılarak çevre dokularından temizlendi ve testis ağırlıkları kaydedildi. Kauda epididimis sıvısı pipet aracılığı ile aspire edilerek spermatozolojik muayeneler yapıldı.

Çalışma süresince elde edilen ortalama canlı ağırlık artışı kontrol (%0), %2, %4 ve %6 zeolit gruplarında sırasıyla 49.37±17.18, 44.84±10.25, 39.45±5.98 ve 47.09±11.49 g olarak tespit edildi (P>0.05). Testis ağırlığı ve spermatozoon motilitesi bakımından gruplar arasında istatistiksel farklılık bulunamadı (P>0.05). Ancak diğer bütün gruplar ile karşılaştırıldığında, %6 zeolit grubunun şekilsiz baş (P<0.001), boyun-orta kısım bağlantı hataları (P<0.01), ve toplam morfolojik bozukluk (P<0.001) oranları önemli derecede yüksek bulundu.

Bu çalışmadan, yüksek oranlardaki zeolitin erkek hayvanlarda spermatozoon morfolojisini olumsuz yönde etkileyebileceği ve çiftlik hayvanlarında zeolitin reprodüksiyon üzerindeki etkilerinin incelenmesinin gerektiği sonuçları çıkarıldı.

Anahtar Kelimeler: Zeolit; Rat; Spermatolojik özellikler; Motilite; Morfoloji

Effects Of Dietary Natural Zeolite On The Testicular Weight, Body Weight And Spermatological Characteristics In Rats

Abstract: Zeolites are natural or synthetic crystalline aluminosilicates with ion exchanging properties. Although zeolites have been used as animal feed additive for more than 40 years, there is no report about the effects of zeolites on spermatological traits. The aim of this study was to designate the effects of zeolite on the testicular weight, live body weight and spermatological characteristics in rats. Twenty-four, 8-week-old, Sprague–Dawley male rats were used in the study. Rats were weighed and randomly divided into control and feeding groups at the beginning of the study. Feeding groups were formed by adding zeolite at rates of 2%, 4% and 6% to standard rat food. Subsequently the prepared rations were pelleted in 1 cm diameter and fed to the rats for 8 weeks. At the end of the study live weights of all rats were recorded and all animals were anesthetized with sodium thiopental. Then, the testes, epididymis, seminal vesicles and ventral prostate were removed, cleared from the surrounding tissues and testicular weights were recorded for each animal. Cauda epididymis fluid was aspirated by a pipette and spermatological examinations were made.

The mean live weight gain during the study was 49.37±17.18, 44.84±10.25, 39.45±5.98 and 47.09±11.49 g in control (0%), 2%, 4% and 6% zeolite groups, respectively (P>0.05). No statistical difference with regard to testis weight and spermatozoon motility could be determined among the groups (P>0.05). However, when compared with all groups the rates of amorphous head (P<0.001), bent at cephalo-caudal junction (P<0.01), and total morphological defects (P<0.001) of the 6% zeolite group were found to be significantly higher.

It was concluded that high rates of zeolite could affect the spermatozoon morphology negatively in male animals and the effects of zeolite on reproduction in farm animals require to be studied.

Key Words: Zeolite; Rat; Spermatological characteristics; Motility; Morphology

Introduction

Natural zeolites, mining of which is easier and cheaper than other mines, are found in large reserves **(33).** It has been known that zeolites are aluminum silicate compounds having infinite three-dimensional crystalline structures containing soil alkali or alkali elements such as K, Na, Ca, Mg. At present it has been reported that use of natural zeolites develops by utilizing features of ion-exchange, water and gas absorption. They exhibit absorbent features because there are gaps in their molecular structures **(24).** Areas where these features of natural zeolites, significant industrial raw materials are especially used in recent years, can be categorized under 4 groups as pollution control, mine-metallurgy, human health and agriculture and animal husbandry.

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In human medicine, zeolites have been applied as antidiarrheal remedies **(27),** for the external treatment of wounds and athlete foot, and for the removal of ammonia ions from kidney dialysates **(17).** Beneficial effects of zeolites in various disease states, including tumors, have been described **(15).**

In veterinary medicine, there is evidence in the literature that the use of natural zeolites has favorable effects on the growth and performance of ruminant animals **(16, 23).** In addition, dietary zeolites improves weight gain of fattening pigs **(34),** feed efficiency and egg production in laying hens **(9).** Furthermore, some studies suggest that natural zeolites may have a beneficial effect against aflatoxicosis and other health disorders **(36)**. Zeolites have been used as animal feed additive substance in stockfarming for more than 40 years. It was determined that fattening performance, slaughter and carcass characteristics of animals fed with feed containing zeolite at different rates were positively affected at various rates between 1.5% and 15% when compared with animals fed with normal feed **(5, 16, 20, 23).**

As far as we know there have been no studies carried out about the effects of zeolite on the testes and spermatological characteristics although its use as an animal feed additive substance has been gradually getting prevalent. In the present study we aimed to determine the effects of zeolite added to rat food at rates of 0%, 2%, 4% and 6% on the testicular weight, live body weight and spermatological characteristics in rats.

Table 1: Analyses of Turkish Manisa Gördes Natural Zeolite

Tablo 1: Türkiye'nin Manisa Gördes Bölgesindeki Zeolitin Analizi

Heavy metal analysis (ppm)				**Chemical analysis (%)**					
As <50	Pb <50	Mo <5	Cu <10	SiO2	71.0	Al_2O_3	11.8	Na_2O	0.4
Cd <5	Se <50	Ni <10	Co <10	CaO	3.4	K_2O	2.4	TiO_2	0.10
Zn 52	Mn 164	Cr 12	Fe 84	Fe_2O_3	1.7	MgO	1.4		

Materials and Methods

Zeolitic Material

Turkish Manisa Gördes zeolite, obtained from Rota Mining Corporation (Istanbul, Turkey), was used as the porous template medium in the present work. Clinoptilolite was the predominant mineral (95%) present in the natural zeolite used. Chemical (%) and heavy metal analyses (ppm) of the zeolite were given in Table 1.

Preparing of Foods

The basal food used in the study is standard powdered rat food containing 22.41% protein. The rat food was obtained from Elazığ Feed Factory. Four different rations were prepared by adding zeolite at a weight of 0% (control), 2%, 4% and 6% food, respectively. Subsequently the prepared rations were pelleted in 1 cm diameter and fed to the rats for 8 weeks. Since there is no zeolite protein and digestible energy, the raw protein and metabolic energies of the rations were estimated according to the rates of the control food. The raw protein (RP) and metabolic energy (ME) value of the rations of control, 2%, 4% and 6% zeolite groups were estimated as respectively 22.41% and 3203 kcal/kg, 22.58% and 3229 kcal/kg, 22.65% and 3211 kcal/kg and 22.45% and 3225 kcal/kg.

Crude ash, crude oil, dry matter and crude protein analyses of the diets were conducted with Weenders Analysing Method **(1)** and the crude cellulose amount was conducted according to Crapton and Maynard **(5)** at the laboratory of the Department of Animal Nutrition and Nutritional Diseases, Veterinary Faculty, Dicle University.

Animals and Treatments

A total of 24 healthy adult male Sprague–Dawley rats (aged 8 weeks, average 306 g weighing) were obtained from the Dicle University, Veterinary Faculty, Experimental Research Centre (DUSAM) laboratory, Diyarbakir, Turkey. They were kept under standard laboratory conditions (12-h light: 12-h dark and 24±3°C). Feed and water were provided as *ad libitum*. Rats were randomly divided into 4 equal groups (n = 6), according to zeolite ratios (0%, 2%, 4% or 6% zeolite) of the diets. This study was approved by the animal ethic committee of Dicle University. Live weights of the rats were recorded on the first day and at the end of the experiment lasting for eight weeks.

At the end of the experiment all animals were anesthetized with sodium thiopental (4mg/kg body weight, i.p.). The testes, epididymis, seminal vesicles and ventral prostate were removed and seperated from the surrounding tissues, and than testicular weights were recorded for each animal. Sperm progressive motility was evaluated by an earlier method described by Sonmez et al. **(29).** For this purpose, fluid was obtained from the caudal epididymis with a pipette and diluted to 2 ml with Tris Buffer Solution. A phase-contrast microscope with a hot plate was used for the examinations. The system was pre-warmed (35°C) and the percentage of motility was evaluated visually at 400X magnification. Motility estimations were performed from three different fields in each sample. The mean was used as the final motility score.

There is no agreed general classification for the determination of morphology of rat spermatozoa **(28).** In our study, morphological examinations were carried out in 9

categories: Acrosome integrity, hookless, head without tail, amorphous head, bent at cephalo-caudal junction, proximal droplet, tail without head, coiled tail and total morphological defects **(2).** Buffered-formol saline was used for morphological examinations **(11).** Morphological examinations were carried out under 1000X magnification of a phase-contrast microscope. Two hundred cells were calculated for each morphologic evaluation **(31).**

Statistical Analyses.

In the statistical comparisons among the groups, the one-way analysis of variance (ANOVA) was used. The significance controls of the differences between the groups were determined by Duncan's test **(6)**. All statistical analyses were carried out using SPSS program v. 10.0 for Windows. Means and standard errors (mean+S.E.M.) were presented in the tables.

Results

Mean final fattening weights of the control, 2%, 4% and 6% zeolite groups were found to be 358.0±17.00, 352.0±10.92, 345.7±6.03 and 353±12.46 g (P>0.05) and the mean live weight gain obtained during the experiment was observed as 49.37±17.18, 44.84±10.25, 39.45±5.98 and 47.09±11.49 g, respectively (P>0.05). At the end of the experiments, right and left testes weights were observed as 3.46±0.09 and 3.40±0.14, 3.61±0.06 and 3.65±0.19, 3.32±0.09 and 3.20±0.21, and 3.62±0.10 and 3.37±0.08 g in control, 2%, 4% and 6% zeolite groups, respectively (P>0.05, Table 2).

Table 2: Testes and Mean Body Weights in All Groups

Tablo 2: Gruplara Göre Testis ve Vücut Ağırlıkları

Groups	Control (0%)	2% Zeolite	4% Zeolite	6% Zeolite
Body weight (g)				
Day 0	308.63±1.23	307.16±1.16	306.25±1.13	305.91±2.43
Day 56	358.00±17.00	352.00±10.92	345.67±6.03	353.00±12.46
Mean live weight gain (g)	49.37±17.18	44.84±10.25	39.45±5.98	47.09± 11.49
Testes weight				
Right	3.46±0.09	3.40±0.14	3.61±0.06	3.65±0.19
Left	3.32±0.09	3.20±0.21	3.62±0.10	3.37±0.08

Epididymal sperm motility, and abnormal sperm rates are presented in Table 3. No differences in sperm motility were observed among groups. Zeolite treatment did not show any significant effect on the acrosome integrity, hookless, head without tail,

proximal droplet, tail without head and coiled tail of the sperm. However, the group fed with 6% of zeolite had significantly higher amorphous head (P<0.001), bent at cephalo-caudal junction (P<0.01), and total morphological defects (P<0.001) compared to the other groups.

Table 3: Effects of Different Levels of Zeolite on Semen Characteristics in Rats

Tablo 3: Farklı Düzeylerde Kullanılan Zeolitin Rat Sperması Üzerindeki Etkisi

Groups	Control (0%)	2% Zeolite	4% Zeolite	6% Zeolite
Motility (%)	60.00±6.32	60.83±3.27	66.67±1.05	60.83±5.83
Acrosome defects (%)	0.67±0.42	0.50±0.34	0.50±0.50	1.00±0.63
Hookless (%)	0.67±0.33	0.67±0.33	0.33±0.21	1.17±0.40
Head without tail (%)	0.17±0.17	0.00±0.00	0.50±0.34	1.00±0.45
Amorphous head (%)	2.67±0.33^{a}	4.00±0.58^{a}	6.83±0.83^{a}	20.83±4.36^{b**}
Bent at cephalo-caudal junction (%)	4.50±0.96^{a}	5.67±0.92^{a}	7.17±0.98^{a}	16.67±3.42^{b*}
Proximal droplet (%)	0.33±0.21	0.33±0.21	0.17±0.17	0.67±0.42
Tail without head (%)	1.83±0.48	2.33±0.88	2.33±0.67	2.33±0.56
Coiled tail (%)	2.33±0.61	2.83±0.79	2.00±0.73	3.83±1.58
Total morphologic defects (%)	13.17±1.74^{a}	16.33±1.52^{a}	19.83±1.76^{a}	47.50±7.10^{b**}

^{ab}: Mean values in the same row with different superscripts differ significantly,

*:P<0.01,

**: p<0.001.

Discussion

In animal husbandry, various feed additive substances are added to animal feed to increase production. Studies about the use of zeolite in animal husbandry as additive substance to animal feed in order to investigate their effects on the fattening performance of cattle, lamb, goat and avian species have been raported in recent years. Previously, researchers indicated the beneficial effect of zeolites to increase biomass production in fisheries **(21),** to promote weight gain of chicken **(9),** swine **(35)** and sheep **(8),** to improve the quality of animal products such as eggs **(13)** or wool **(8)**, to

reduce bacterial contamination of the gut **(32)** and to counteract harmful effects of ingested toxic substances **(35).** Different dietary levels of zeolite were tested in different animal species by many researchers. In studies on pigs, zeolite was used between 0.5% and 5% of the diet (**22, 34, 36**), and between 0.16% and 1% (**7, 9**) or 3–5 g/kg body weight **(12)** on chicken. In the present study the effects of zeolite, added at rates of 2%, 4% and 6% to the diet, on the testicular weight, live body weight and spermatological characteristics were investigated in rats.

The studies on various animal species demonstrate that zeolite generally have positive effects on body weight in animals **(4, 26, 30).** Our results have demonstrated that zeolite at rates between 2% and 6% has no positive effect on the live weight of rats. Similarly Olver **(19)** reported that the use of zeolite in avian feed had no effects on body weight gain. Similar to the body weight results, testes weights of the rats demonstrated no differences among the groups at the end of the study ($P>0.05$).

Although natural zeolites were used as additives in various industries in poultry **(18)** and animal breeding **(25),** no reports described the effects of zeolite on spermatological characteristics in the animal. No differences in epididymal sperm motility were observed among groups. Zeolite treatment did not show any significant effect on the acrosome integrity, hookless, head without tail, proximal droplet, tail without head and coiled tail of the sperm. However, the group fed with 6% of zeolite had significantly higher amorphous head ($P<0.001$), bent at cephalo-caudal junction ($P<0.01$), and total morphological defects ($P<0.001$) compared to the other groups.

Zeolite added to animal diet has been reported to be capable to have three different effects in vivo. The first one is the ion-exchange characteristics of zeolites and their capability in changing the ionic composition and pH of the stomach-bowel fluid **(10)**. The second one is the capability to free the silicate anions in the zeolite structure. Electrolyte balance in the liver, kidneys, testes and blood were reported to change because of this effect **(15).** The third one is their capability in absorbing digestion products of small molecular weight due to the absorbent characteristic of the zeolite. For example zeolite is reported to be able to drag and excrete glucose, amino acids, vitamins and mineral substances or larger molecules from the body via feces **(35)**. In a study it was reported that feces contained more balanced feeding elements and consequently more valuable organic manure was obtained by adding zeolite to the feed of the animals with rumen **(20)**. This information implies us that the absorption of some vitamins (such as vitamin E) and mineral substances, very vital in reproduction, by the zeolite can affect spermatozoon production and spermatozoon morphology negatively. The investigations have raported that violent testis degenerations were formed in vitamin and mineral deficiency by means of suppression of gonadotropin excretion **(3, 14).**

In our present study, observation of the negative effect of zeolite on the spermatozoon morphology only in 6% of the zeolite group shows us the necessity of exceeding a particular threshold value for the appearance of this negative effect. Since our study was carried out using male rats, it is not possible to express an evidential opinion on the effect of zeolite on the reproduction of farm animals. Still, adding zeolite at high rates to increase fattening performance cannot be a more rationalistic approach until establishing the effects of zeolite on the reproduction of farm animals.

Conclusion

Our conclusion provided clues to the possible negative effects of zeolite on the production and morphology of spermatozoon in animals. Consequently, it has been concluded that the effects of zeolite on the reproduction of farm animals is also required to be investigated.

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