The effect of natural zeolite (clinoptilolite) on total bacteria contamination of ostrich eggshells

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The objective of the present study is to determine whether the supplementation of clinoptilolite in ostrich diet or its addition in their nest, has any influence on total bacteria contamination of ostrich eggshells. Two experiments were carried out. In the first experiment 6 families of African Black ostrich breeders each consisted of 3 birds, 1 male and 2 females (trio), were equally divided into 2 groups: Group A (control) was fed a balanced concentrate ration without clinoptilolite supplementation and group B was fed the same concentrate ration supplemented with 1.5% clinoptilolite. Equal numbers of eggs were collected from the 2 groups (June to August 2004). In the second experiment, 8 trios of African Black ostrich breeders were used. Each trio was provided with a nest of approximately 1.5 m diameter and 20 cm depth, placed on the ground. Trios were equally divided into 4 groups: Group A, where 60kg of clinoptilolite was added in the nest, group B, where 30kg of clinoptilolite and 30 kg of sand was added in the nest, group C, where 60kg of sand was added in the nest and group D whose nest was filled with neither clinoptilolite nor sand. Equal numbers of eggs were collected from all experimental groups from June until the end of August 2005. In both experiments, each egg was collected by hand with a sterile plastic glove of one use and was immediately put in a sterile plastic bag and kept refrigerated until next day. For the determination of bacteria contamination on the eggshells, eggs were washed in their sterile plastic bags with phosphate buffered saline (P.B.S.). The results from the first experiment revealed that there were no significant differences (P>0.05) between the total bacteria counts in the 2 groups. In the second experiment eggs from group C were the most heavily contaminated, with bacteria counts of 8.843log (number of bacteria/ml) followed by eggs from group B, D and A with bacteria counts of 6.8117log (number of bacteria/ml), 6.0167log (number of bacteria/ml) and 5.4510log (number of bacteria/ml), respectively. The numerical differences among the 4 groups, although not statistical (P=0.07), they present practical interest as they imply that the addition of clinoptilolite in ostrich nests tended to reduce total bacteria counts on the eggshells. Moreover, it was shown that sand enhances bacteria growth on the ostrich eggshells.

Keywords: natural zeolite; bacteria contamination; ostrich eggs

Introduction

Commercial ostrich farming is an important component of livestock industry in several countries (Cooper, 2000). Ostrich egg quality has significant effect on embryonic development and the health status of the newly hatched chick (Deeming, 1997; Superchi et al., 2002). The high incidence of microbial contamination in ostrich eggs (18-36%) is deemed to be a significant problem, as it results in reduced hatchability (Deeming, 1995; 1996; Deeming and Ar, 1999). Bacterial contamination of the internal egg content could be the result of the penetration of the shell by bacteria deposited on the surface of the egg, after it has been laid (Quarles et al., 1970; Schoeni et al., 1995). Microbiological findings in ostrich eggs have shown that the organisms isolated from the interior egg were mainly enterobacteria (Cabassi et al., 2004). Deeming (1995) reported that a variety of soil and faecal bacteria, as well as fungal species, were isolated from the shell membranes, yolk, albumen and embryo of ostrich eggs. Even though microbes can deposit inside eggs in the oviduct, eggshell contamination usually takes place at the passage of the egg through hen's cloaca (Smith, 1993) or when the egg is laid into an environment contaminated by faecal matter (Bruce and Drysdale, 1991).

Studies in poultry have shown that the housing system may affect the bacteria contamination of the eggshell (De Reu et al., 2003; Protais et al., 2003; De Reu et al., 2005). According to the ostrich farming systems practiced worldwide, breeders are farmed in outdoor paddocks and the farmer or the male bird digs a nest on the ground so that females lay their eggs inside (Cooper, 2000). Improving nest hygiene, as well as breeder bird management is considered to be the simplest way to reduce microbial spoilage. For this purpose Deeming (1995) proposed the addition of coarse sand as a substrate in the nests.

Clinoptilolite is a natural zeolite. Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, having infinite, three-dimensional structures. They are further characterized by the ability to lose and gain water reversibly, to absorb molecules of appropriate diameter (adsorption property or acting as molecule sieves), and to exchange their constituent cations without major change of their structure (ion-exchange property) (Mumpton and Fishman, 1977). Because of their physical and chemical properties, zeolites have been used in animal nutrition since 1960s, mainly to improve animals' performance or as toxin binder for preventing mycotoxicosis and ameliorating the poultry house environment by absorbing ammonia (Mumpton, 1999; Papaioannou et al., 2005). Moreover, natural zeolites have been accepted as promising materials for the immobilization of microorganisms due to their high porosity and large surface area (Bartko et al., 1995; Shindo et al., 2001; Chang et al., 2002; Tsitsishvili et al., 2002; Hrenovic et al., 2003; 2005;) In vitro studies by Ramu et al. (1997) have shown that clinoptilolite can adsorb cholera toxin (CT) and heat-labile (LT) Escherichia coli enterotoxin. Clark et al. (1998) have also proved that clinoptilolite has an excellent capability of adsorbing bovine rotavirus and coronavirus in vitro.

Taking into consideration all the above information, this study was carried out in order to determine whether the supplementation of clinoptilolite in ostrich diet or its addition in their nest, has any influence on the total bacteria contamination of ostrich eggshells. A complementary objective of the study was to test the effects of the river sand, alone or in combination with clinoptilolite, on total bacteria counts of ostrich eggshells.

Materials and methods

Two experiments were carried out for the purpose of this study. In the first experiment 6 families of African Black ostrich breeders each consisted of 3 birds, 1 male aged 6-7 years and 2 females aged 4-5 years (trio) were equally divided into 2 groups: Group A (control) was fed a balanced concentrate ration without clinoptilolite supplementation and group B was fed the same concentrate ration supplemented with 1.5% clinoptilolite (*Table 1*). All rations were calculated on isonitrogenous and isocaloric basis. A total of 30 eggs (15/group) were collected throughout the collection period (June – August 2004).

In the second experiment, 8 trios (8 males aged 7-8 years and 16 females aged 5-6 years) of African Black ostrich breeders were used. Each trio was provided with a nest of approximately 1.5 m diameter and 20 cm depth, placed on the ground. Trios were equally divided into 4 groups: Group A, where 60kg of clinoptilolite was added in the nest, group B, where 30kg of clinoptilolite and 30 kg of river sand was added in the nest, group C, where 60kg of river sand was added in the nest and group D, whose nest was filled with neither clinoptilolite nor river sand. The material from the nest of all groups, except from group D, was replenished approximately every 20 days. A total of 40 eggs (10/group) were collected from June until the end of August 2005.The trios in both experiments were accommodated in outdoor enclosures of 500 m^2 each, separated by wooden fences. A shelter was located at the one side of each enclosure, under which there was a nest, a wooden sheep trough and a plastic barrel, that was filled twice daily with fresh water.

Table1 Ingredient analysis of the rations used in experiment 1 (g/kg).

Ingredients	Group A (Control)	Group B (1.5% Clinoptilolite)
Deh. Lucerne	500	500
Wheat Bran	192.9	122.7
Maize	127	181.8
Soyameal-48	89.9	100.7
Soya oil	23.1	10
Limestone	17.1	19.8
Clinoptilolite	-	15
Vitamin and Mineral Premix	50	50
Total	1000	1000

The zeolitic material used in the experiments was derived from the zeolite deposits of Metaxades (Thrace, Northeastern Greece). It contained approximately 89% clinoptilolite, 6% plagioclastes and feldspars, 3% micas and clay minerals and 2% quartz as determined by X-ray powder diffraction. The material's cation exchange capacity was 226 meq 100 g^{-1} and its chemical composition was: SiO_2: 67.87, $TiO_2 < 0.01$, Al_2O_3: 12.03, $Fe_2O_3 < 0.01$, MnO < 0.01, MgO: 0.89, CaO: 3.10, Na_2O: 0.51, K_2O: 2.48, H_2O: 13.12, Si/Al: 4.8. Clinoptilolite analysis, took place in the laboratory of the Department of Mineralogy-Petrology-Economic Geology of Aristotle University of Thessaloniki. The zeolitic material used in the ostrich rations had particle size < 1.5mm, whereas half of the amount of zeolite added in ostrich nest had particle size < 1.5mm and the other half had particle size 1.5-4mm. The river sand that was used in the second experiment had particle size 1.5-4mm.

The egg collection started two weeks after the onset of each experiment. In both experiments each egg was collected by hand with a sterile plastic glove of one use and was immediately put in a sterile plastic bag and was kept refrigerated until next day. For the determination of bacteria contamination on the eggshells, eggs were washed in their sterile plastic bags with phosphate buffered saline (P.B.S.). In particular, 25 ml of P.B.S was added in each plastic bag and the egg was rubbed through the bag for 1 min. The procedure was repeated after 5 minutes. Then, serial ten fold dilutions of each original fluid were made, followed by a spread plate method. One ml of each dilution was transferred to petri dishes with Tryptose Agar. After 48 hours of incubation at 37 C^0, colony counts were made for the determination of total bacteria concentration on each eggshell. The results were expressed as log (number of bacteria/ml).

For the analysis of the data the statistical program STATISTIX® version 7 for Windows was used. The normality of the data in both experiments was tested with Shapiro-Wilk normality test. At the first experiment, the effect of clinoptilolite supplementation was evaluated using Wilcoxon rank sum test. At the second experiment, Kruskal-Wallis one-way nonparametric analysis of variance was run to determine the statistical significance of the differences among the experimental groups. A significance level of $P \leq 0.05$ was used in all comparisons.

Results and Discussion

The results of the first experiment revealed that the supplementation of clinoptilolite, at the level of 1.5%, in ostrich diet had no significant effect on total bacteria counts of ostrich eggshells (mean±SE: 7.52±0.86 and 6.78±0.06 log (number of bacteria/ml) for groups A and B respectively, $P>0.05$). It was believed that clinoptilolite could decrease the bacteria counts on ostrich eggshell by adhering intestinal bacteria and restricting the microbial population in cloaca, or by reducing faecal moisture. Smith et al. (2000) reported that high excreta moisture can directly increase the microbial contamination of the shell. Unfortunately, there are no relative references considering the dietary effect of clinoptilolite on bacteria eggshell contamination, neither in the ostrich, nor in other poultry. Although the effectiveness of zeolites and especially clinoptilolite on the adsorption and adherence of bacteria is well documented (Bartko et al., 1995; Shindo et al., 2001; Chang et al., 2002; Tsitsishvili et al., 2002; Hrenovic et al., 2003; 2005), clinoptilolite administration via feed at the rate of 1.5% was proved to be ineffective. A possible explanation might be that the rate of clinoptilolite inclusion in the diet was low. It has been proved in wastewater treatment, that higher concentrations of natural zeolites provide larger surface area for the adsorption of bacteria (Hrenovic et al., 2003).

In the second experiment, eggs from group C were the most heavily contaminated followed by eggs from group B, D and A (mean±SE: 5.45±0.72, 6.81±0.75, 8.84±1.02 and 6.01±0.21 log (number of bacteria/ml) for groups A, B, C and D respectively), but the differences among the 4 groups were not significant ($P=0.07$). However, the results imply that the addition of clinoptilolite in ostrich nests, tend to reduce total bacteria counts on the eggshells, especially in comparison with the addition of river sand. This finding is attributed to the fact that clinoptilolite adsorbed and immobilized the bacteria from the nest environment, resulting in a net reduction of their number. As a consequence, the number of free microorganisms able to infect the eggs laid in nests of group A was less than those in the nests of the rest of the groups. Previous studies have proved that the dimensions of bacteria are comparable to the sizes of zeolite crystallites, as well as to the corresponding inter-crystalline pores (Tsitsishvili et al., 2002; Hrenovic et al., 2003). Furthermore, natural zeolites provide a large surface area where microbes are strongly adsorbed and adhering to one another by exrtacellular substances (Bartko et al., 1995; Tsitsishvili et al., 2002; Hrenovic et al., 2003; 2005). The results of the present study, indicate that the number of bacteria that were immobilized onto clinoptilolite were higher than those immobilized onto river sand. This is consistent with the findings of Chang et al. (2002) who reported that the numbers of nitrifying bacteria were greater in biofilm grown on natural zeolite than sand. Similarly, in studies with wastewater treatment, Hrenovic et al. (2005) found that carriers with natural zeolite adsorbed greater number of bacteria than those with sand, a finding that was attributed to the predominantly smooth surface of sand and the rough surface of clinoptilolite particles, which therefore provides a better microenvironment for the adsorption of bacteria. The intermediate number of bacteria eggshell counts found in group B compared to those of groups A and C appears to be reasonable because the nests of this group were filed with both clinoptilolite and river sand in equal percentages.

Based on these findings, it may be concluded that the supplementation of ostrich diet with 1.5% clinoptilolite had no effect on bacteria contamination on ostrich eggshells, whereas the addition of clinoptilolite in ostrich nests tended to reduce total bacteria counts on the eggshells. The results of the second experiment present practical interest as they imply a positive effect on reducing eggshell bacteria counts, but further investigation is required. Moreover, from the materials tested in the second experiment, it was shown that river sand enhances bacteria growth on the ostrich eggshells.

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