Long-term application of clinoptilolite via the feed of layers and its impact on the chemical composition of long bones of pelvic limb (*femur* and *tibiotarsus)* and eggshell

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ABSTRACT: Selected 120 layers, hybrid breed Bovans Goldline, were divided into two balanced groups: control (C) and experimental (E) group. The layers were raised in three-floor cage technology with automatic watering, manual feeding, in the environment with regulated lighting and thermal schedule. The actual experimental period started in the 22nd week and ended in the 68th week of age of the layers. In the period following the filling of the cages the layers received complete feed mixture N1, subsequently, until the end of the experiment, they received feed mixture N2. The feed mixtures for Group C and E were of the same composition, with the only difference that the feed mixture designed for the experimental group contained 1% of clinoptilolite (commercial additive ZeoFeed) as a substitute for the same portion of wheat.. The layers consumed the feed mixtures and drinking water *ad libitum*. In the layers of Group E the intensity of egg laying increased by 1.1%, which corresponds to an increased number of laid eggs 3.08 per layer. The average weight of laid eggs was 66.3 ± 6.25 g in the layers of Group C, 65.6 ± 5.44 g ($P \le 0.05$) in the experimental layers of Group E. In Group E the consumption of feed mixture per one egg was 4.1 g lower when compared to the control. In 100% dry matter the eggshell of layers that received clinoptilolite (Group E), as opposed to Group C, had a statistically significantly higher ($P \le 0.01$) content of crude protein, calcium and magnesium. Almost the same and insignificant difference in values was found in ash and phosphorus. The values of the same indicators were statistically significantly higher in both monitored bones (*femur* and *tibiotarsus)* ($P \le 0.05$; $P \le 0.01$) during the application of clinoptilolite (Group E), with the exception of P in *femur*, where an insignificant rise in the level of P occurred. The rise in the concentration of Mg in Group E was of particular importance: in *femur* by 50.4%, in *tibiotarsus* by 32.4%. If we compare the monitored values in *femur* and *tibiotarsus*, we can see that the levels of ash, Ca and P are higher in *tibiotarsus*, and the levels of crude protein and Mg are lower. The long-term application of clinoptilolite favourably influenced the lodgement of Ca, P, Mg and crude protein in the eggshell as well as in the bones (*femur, tibiotarsus*), increased the egg production and reduced the consumption of feed mixture per one egg, while the good productive health of the layers was maintained.

Keywords: Zeofeed; crude protein, ash; Ca; P; Mg; egg yield; consumption of feed mixtures

The reason for the use of natural zeolite – clinoptilolite in animal applications is its special physical and chemical properties. Clinoptilolite has the ability to selectively exchange its own cations for ions from the environment (Melenova et al., 2003). During the application of clinoptilolite as a feed supplement the utilization of feed nutrients increases (Olver, 1997), it positively affects the digestive mechanism and is involved in the elimination of heavy metals (Tepe et al., 2004). In these substances adsorption properties towards a range of mycotoxins have been proved as well as their influence on the mitigation

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of their adverse effects (Parlat et al., 1999; Skalicka and Makoova, 1999; Skalicka et al., 2000; Ortatatli and Oguz, 2001; Rizzi et al., 2003).

Studies devoted to the application of these substances to layers represent a specific field (Roland et al., 1985, 1993; Rabon et al., 1991; Olver 1997; Wu-Haan et al., 2007). According to Nys (1999) synthetic zeolites with a high capacity of cation exchange slightly improve the eggshell quality since they create a complex with Ca. Evaluated through the specific weight of the eggshell, the majority of experiments attests the positive influence of zeolites on its quality. A positive effect was observed above all during the marginal level of Ca (2.75%) in the diet (Roland et al., 1985) and during the elevated temperature of the environment (Keshavarz and McCormick, 1991).

The economics of egg production is significantly influenced by the quality of eggshell, which is, in turn, in addition to attendance, markedly influenced by nutritional, genetic and environmental factors (Nys, 1999). Damaged and cracked eggshell occurs in 6 up to 8% of laid eggs, most often in the last third of the egg laying, after the 53rd week of age of the layers (Bain, 1991). Effects of different factors which influence the quality have been studied and described. These are for example the use of different resources of Ca (Lichovnikova, 2007), night supply of an adequate level of Ca in the time of the eggshell formation (Nys, 1999), supply of more coarsely ground particles containing Ca (1–4 mm) (Guinotte and Nys, 1991; Koreleski and Swiatkiewicz, 2004; Hernandez-Sanchez et al., 2006), intake of NEFA, which influences the retention of Ca (Nys, 1999), optimum intake of P, Cl, Na and K, but also of magnesium which can have, in its surplus, a negative impact on the shell (Nys, 1999).

The aim of the study was to find out the impact of a long-term application of clinoptilolite on the chemical composition of long bones (*femur, tibiotarsus*) and eggshell of layers.

MATERIAL AND METHODS

The experiment was conducted in an accredited experimental poultry house of the Institute of Nutrition, Zootechnology and Zoohygiene, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.

Selected 120 layers, hybrid breed Bovans Goldline, at 19 weeks of age and of the weight approx. 1 735 g, were divided into two balanced groups: control (C) and experimental (E) group. The layers were raised in three-floor cage technology with automatic watering, manual feeding, in the environment with the regulated lighting and thermal schedule. Two layers were placed into one cage, and the floor area per layer was 0.1125 m^2. In the period following the filling of the cages, from the 19th to the 38th week of layer age, the layers received a complete feed mixture N1, subsequently, until the end of the experiment, they received a feed mixture N2. Feed mixtures had the same proportions of components with the only difference that mixtures prepared for the experimental Group E contained, as a substitute for the same portion of wheat, 1% of clinoptilolite (commercial additive ZeoFeed). The composition of feed mixture N1 (g/kg): dry matter 890.0, crude protein (N×6.25) 171.5, fat 38.5, fibre 26.2, ash 127.7, Ca 37.2, P 5.7 and ME 11.5 MJ/kg. Feed mixture N2 (g/kg): dry matter 891.2, crude protein (N×6.25) 170.6, fat 38.4, fibre 25.9, ash 127.5, Ca 37.3, P 5.71 and ME 11.37 MJ/kg. The addition of 1% of clinoptilolite did not markedly influence the nutrient composition of the feed mixtures applied to Group E. The layers consumed the feed mixtures and drinking water *ad libitum*.

The actual experimental period started after a 20-day adaptation interval in the 22nd week of age of the layers, and the experiment was finished in the 68th week of age of the layers. The collection of eggs was done individually, always from 30 layers at the same time, altogether nine times in five-week intervals. In total 270 eggs were taken from each group and analysed.

Specification of the active substance

The additive used, ZeoFeed (http://www.ekozym.cz/fileadmin/pdf/datasheety/ZeoFeed.pdf), contains at the maximum humidity of 6% at least 80% of the active substance clinoptilolite. Further it contains min. 62% SiO_2, max. 14% Al_2O_3, max. 2.3% Fe_2O_3 and max. 5.5% CaO. Granularity fluctuates in the range from 0.2 to 0.5 mm.

Determination of egg production and conversion of feed

Continuously, the daily production and the weight of eggs were monitored, the number of eggs per layer was counted as well as the consumption of feed mixture per one laid egg.

Chemical composition of bones and eggshell

After the termination of the experiment, in the 68th week of age of the layers, the layers were slaughtered and the *femur* and *tibiotarsus* were taken from the right pelvic limb. In the original matter of bones and eggshell, dry matter, crude protein (N×6.25), ash, calcium, phosphorus and magnesium (AOAC, 2001) were specified and the established values were expressed in 100% dry matter. The applied feed mixtures were analysed in the same way.

Health condition

In the course of the experiment the health condition of the layers was daily monitored and no clinical symptoms of a disease were registered. During the experiment four layers perished in both groups (yolk peritonitis, heart failure).

Statistical evaluation

The acquired data were statistically evaluated. ANOVA was applied to process the data with homogeneous variance and subsequently, multiple comparisons by Tukey-HSD test (Zar, 1999) were used to find the pairs of classes with statistically significant differences. For the data with heterogeneous variance Kruskal-Wallis ANOVA was used and subsequently, multiple comparisons by the non-parametrical serial test (Tukey-type multiple comparisons; Zar, 1999) were applied.

RESULTS AND DISCUSSION

During the experimental period, i.e. from 22nd to 68th week of layer age, the layers of Group C laid 16 289 eggs (100%), the layers of Group E 16 474 eggs (101.14%). The layers of Group E, receiving clinoptilolite, had higher intensity of egg laying by 1.1%, which in the monitored period represents an increased number of laid eggs 3.08 per layer. The average weight of laid eggs was 66.3 ± 6.25 g in the layers of Group C, while it was lower in the layers of Group E: 65.6 ± 5.44 g. The differences between the average weight values were significant ($P \leq 0.05$). The recorded egg production was reached at the consumption of feed mixture 141.7 g per one egg in Group C, in Group E the consumption was 137.6 g, which was the lower consumption per one egg by 4.1 g. In layers that received clinoptilolite Olver (1997) also proved significant improvement in the utilization of feed nutrients, increase in egg laying and stability of eggshell and reduced moisture of droppings. Similarly like in our experiment, he did not register the higher weight of eggs during the application of clinoptilolite which was confirmed also by Gezen et al. (2004).

In 100% dry matter, the eggshell of the layers that received clinoptilolite (Group E) had a statistically significantly higher ($P \leq 0.01$) content of crude protein, calcium and magnesium contrary to Group C (Table 1). The values of ash and phosphorus were almost the same, with insignificant differences. According to Nys (1999) synthetic zeolites slightly improve the eggshell quality, natural clays have only a fractional influence. Roland et al. (1985) recorded a positive influence at a low level of Ca in the diet, whereas Keshavarz and McCormick (1991) at a higher ambient temperature.

The values of the same indicators were statistically significantly higher ($P \leq 0.05$; $P \leq 0.01$) in both

Table 1. Chemical composition of eggshell in 100% dry matter (g/kg)

	Group C (control) n = 270 $\bar{x}$ ± SD	Index	Group E (clinoptilolite) n = 270 $\bar{x}$ ± SD	Index	P-value
Crude protein	53.0 ± 7.89	100	55.4 ± 9.06	104.5	3.29**
Ash	941.6 ± 14.69	100	940.5 ± 18.82	99.88	0.79 NS
Ca	353.2 ± 11.0	100	356.8 ± 11.82	101.0	3.73**
P	1.12 ± 0.12	100	1.13 ± 0.15	100.9	0.29 NS
Mg	1.07 ± 0.08	100	1.10 ± 0.11	102.8	4.07**

Crude protein = N×6.25; NS = not significant; $^{**}P \leq 0.01$

Table 2. Chemical composition of *femur* of the layers in 100% dry matter (g/kg)

	Group C (control) n = 270 $\bar{x}$ ± SD	Index	Group E (clinoptilolite) n = 270 $\bar{x}$ ± SD	Index	P-value
Crude protein	340.6 ± 20.31	100	381.7 ± 33.74	112.1	5.71**
Ash	570.6 ± 36.91	100	604.7 ± 27.94	106.0	4.03**
Ca	218.5 ± 18.12	100	226.8 ± 13.66	103.8	2.08*
P	99.9 ± 5.19	100	102.5 ± 7.52	102.6	1.59 NS
Mg	2.82 ± 0.25	100	4.24 ± 0.41	150.4	16.21**

Crude protein = N×6.25; NS = not significant;); *$P \le 0.05$); **$P \le 0.01$

Table 3. Chemical composition of *tibiotarsus* of the layers in 100% dry matter (g/kg)

	Group C (control) n = 270 $\bar{x}$ ± SD	Index	Group E (clinoptilolite) n = 270 $\bar{x}$ ± SD	Index	P-value
Crude protein	333.2 ± 21.72	100	354.7 ± 21.33	106.4	3.87**
Ash	574.7 ± 41.22	100	611.5 ± 24.74	106.4	4.20**
Ca	208.8 ± 10.77	100	231.8 ± 10.42	111.0	8.42**
P	97.4 ± 7.39	100	105.4 ± 5.16	108.2	4.84**
Mg	2.90 ± 0.20	100	3.84 ± 0.58	132.4	8.35**

Crude protein = N×6.25; **$P \le 0.01$

monitored bones (*femur* and *tibiotarsus)* during the application of clinoptilolite (Group E), with the exception of P in *femur*, where an insignificant rise in the level of P occurred (Table 2 and 3). The rise in the concentration of Mg in Group E was of particular importance; in *femur* by 50.4%, in *tibiotarsus* by 32.4%. Watkins and Southern (1991) also reported higher values of ash during the application of zeolite.

If we compare the observed values in *femur* and *tibiotarsus*, we can see (Table 2 and 3) that the levels of ash, Ca and P are higher in *tibiotarsus*, the levels of crude protein and Mg are lower.

Overall, we can claim that during the application of zeolites – clinoptilolite we can count on a set of favourable effects. These are protection of animals against adverse effects of mycotoxins, retention of free moisture, stimulation of liver towards intoxication processes, retention of free moisture in the feed, which consequently reduces the production of fungi and conidia, anti-caking power and causes the improvement of lousiness and durability of feed mixtures. A significant asset of the use of clinoptilolite is its ability to bind ammonia and thus to reduce its concentration in the stable environment (Amon et al., 1997; Meisinger et al., 2001; Melenova et al., 2003). This is the question of the factors which show up in the sphere of animal welfare and subsequently in the animal production.

The attained results proved that the long-term application of clinoptilolite had a positive effect on the lodgement of Ca, P, Mg and crude protein both in the eggshell and in the bones; the egg production increased and the consumption of feed mixture per one egg was reduced, while maintaining the good productive health of the layers.

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