The long-term administration of a clinoptilolite-supplemented feed to layers and its effect on performance, haematological parameters and metabolic profile

E. Straková[1], P. Suchý[1], I. Herzig[1], V. Šerman[2], N. Mas[2]

[1]Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Science, Brno, Czech Republic
[2]Faculty of Veterinary Medicine, University of Zagreb, Croatia

ABSTRACT: 120 selected layers (Bovans Goldline hybrid) aged 19 weeks with an average weight of 1 735 g were divided into two balanced groups: control group (Group C) and experimental (Group E). Layers were reared in three-tier cages with automatic watering, manual feeding, and at controlled light and temperature regimens. One cage accommodated two layers, the floor surface area was 0.1125 m^2 per layer. The experiment started after a 20-day adaptation period with layers aged 22 weeks (Week 22) and ended when layers were 68 weeks old (Week 68). In a period of initiation (i.e. Weeks 19–38), layers were fed the complete feeding mixture N1. Then they received the feeding mixture N2 until the end of the experiment. Feeding mixtures in both groups (C and E) had the same composition; the only difference between mixtures was that the feeding mixture in the experimental group was enriched with 1% clinoptilolite (i.e. the commercially available additive ZeoFeed). Layers received feeding mixtures and drinking water *ad libitum*. In the course of the experimental period, control layers laid 16 289 eggs while experimental layers laid 16 474 eggs. It follows from the results that the laying intensity in experimental layers was 1.7% higher as compared to control layers, i.e. the number of laid eggs in experimental layers increased by 5.6 eggs per layer. The mean weight of all laid eggs was 66.3 ± 6.25 g in the control group and 65.6 ± 5.44 g in the experimental group ($P \leq 0.01$). Such performance was achieved at the consumption of feeding mixture being 141.7 g per laid egg in the control group and 137.6 g per laid egg in the experimental group. The consumption of feeding mixture in the experimental group was 4.1 g lower than that in the control group. The mean values of parameters monitored in blood plasma such as uric acid, cholesterol, glucose, lactose, calcium, phosphorus, ALP, and LDH in both groups of layers ranged within reference intervals, with no significant differences being detected between both groups.However, statistically significant differences between both groups were found in total plasma protein ($P \leq 0.01$), triacylglycerol levels ($P \leq 0.05$), and magnesium ($P \leq 0.01$), which were elevated in the control group, and in AST ($P \leq 0.05$) whose level in the control group was significantly lower than that in the experimental group. The results of haematological tests performed with layers' blood revealed statistically significant changes in parameters such as the erythrocyte count ($P \leq 0.01$), haemoglobin level ($P \leq 0.01$), and MCHC ($P \leq 0.05$), which were elevated in the experimental group, and in the leukocyte count ($P \leq 0.05$), which was lower in the experimental group, as compared with the control. However, the values found varied within physiological ranges.

Keywords: ZeoFeed; layers; performance; blood tests

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Feeds are not only the source of nutrients but also they contain a number of contaminants which may enter the food chain via animal products. As a result, a considerable effort is being made to develop procedures to diminish the penetration of such substances into the animal body and subsequent contamination in animal products. One of the approaches to address this issue is to use sorbents such as natural zeolites derived from clinoptilolite.

Clinoptilolite is a natural form of zeolite which has a 3D lattice characterized by plentiful internal spaces in the form of channels and cavities with an internal negative charge, which gives it its specific sorption and ion-exchanging properties. Cavities may entrap molecules of different solid, liquid or gaseous substances, for example methane, carbon dioxide, ammonia, steam, etc.

Specific physicochemical properties of clinoptilolite are the prerequisite of its use in animals. Until now, more than 40 kinds of zeolites have been described, with clinoptilolites and mordenites being the most commonly used zeolites in animal nutrition. Clinoptilolites have the capability of selectively exchanging their own ions with the ions from the environment (Boranic, 2000; Melenová et al., 2003). These substances were also shown to have adsorption properties enabling them to bind various mycotoxins, thus reducing their adverse impact (Harvey et al., 1993; Parlat et al., 1999; Skalická and Makóová, 1999; Skalická et al., 2000; Ortatatli and Oguz, 2001; Rizzi et al., 2003). When clinoptilolite is applied to a feed as a feed additive, the utilization of nutrients increases (Olver, 1997); clinoptilolite also improves digestion and is involved in the elimination of heavy metals (Tepe et al., 2004).

Clinoptilolites were shown to enhance significantly the metabolic utilization of nitrogen in poultry and pigs. This means that it is possible to decrease the level of nitrogen-containing substances in a feeding dose without affecting the performance of animals. The elimination of nitrogen via excrements decreases. Sows that were administered feeding mixtures containing clinoptilolite-based formulations for a long period of time showed beneficial effects such as improved fertility, the effects of mycotoxins (zearalenone) were reduced, mortality in piglets decreased and weight gain in piglets also improved (Papaioannou et al., 2002; 2004).

In laying hens, Olver (1997) demonstrated the improved utilization of nutrients from a feed, increased laying intensity, and improved stability of the egg shell. Gezen et al. (2004a,b) reported the increased weight of eggs during the administration of clinoptilolite. One of the great advantages associated with the use of clinoptilolite is that it is able to bind ammonia, thus decreasing ammonia concentrations in animal houses and reducing odour from excrements in animal houses (Amon et al., 1997; Meisinger et al., 2001; Melenová et al., 2003).

The main aim of this experiment performed in defined experimental conditions was to test the long-term administration of feeding mixtures supplemented with 1% of clinoptilolite (ZeoFeed) in laying hens and to determine its effect on selected production, haematological and biochemical parameters of the hepatic and renal function.

MATERIAL AND METHODS

The experiment was performed in accredited experimental enclosures of the Institute of Nutrition, Zoo-technology, and Zoo-hygiene, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno. 120 selected layers (Bovans Goldline hybrid) aged 19 weeks and weighing ca. 1 735 g were divided into two balanced groups: control group (Group C) and experimental group (Group E).

Layers were reared in three-tier cages with automatic watering, manual feeding, at controlled light and temperature regimens. One cage accommodated two layers, the floor surface area was 0.1125 m^2 per layer. The experiment started after a 20-day adaptation period with layers aged 22 weeks (Week 22) and ended when layers were 68 weeks old (Week 68). Eggs were collected manually.

After initiation, i.e. in the period between Week 19 and Week 38, layers received complete feeding mixture N1, followed by the feeding mixture N2 until the end of the experiment. Feeding mixtures contained the same components, except for the feed additive ZeoFeed which was added to the experimental mixture at a level of 1%. Layers received feeding mixtures and drinking water *ad libitum*. Table 1 shows the levels of basic components together with the nutrient composition of feeding mixtures in the control group.

Active substance specification

ZeoFeed used as an additive in this experiment contains min. 80% of clinoptilolite (active substance)

Table 1. Composition of basal diets

Component composition (%)	N1	N2
Wheat	36.16	33.00
Maize	30.00	35.00
Soybean meal	22.00	19.50
Soya oil	1.10	0.95
Monocalcium phosphate	0.92	0.97
NaCl	0.35	0.35
Limestone	9.00	9.83
DL-methionine	0.20	0.17
Biolys 65[1]	0.06	0.07
L-threonine	0.01	–
DB-D 0106[2]	0.20	0.16
ZeoFeed	0	0
Nutrient contents (g/kg)	N1	N2
Dry matter	892.30	893.20
Crude protein	171.50	160.70
Fat	30.17	30.00
Fibre	26.20	25.60
Ash	126.40	133.40
ME MJ/kg	11.45	11.43

[1]contains 50.7% of L-lysine sulphate

[2]supplied per kg of diet N1 and N2: vitamin A 15 000/12 000 IU; vitamin D_3 3 000/2 400 IU; vitamin E 80/64 IU; Fe 50/40 mg; Mn 100/80 mg; Zn 120/96 mg; Cu 10/8 mg; I 2/1.6 mg and Se 0.30/0.24 mg

at max. water content of 6%. It also contains 62% of SiO_2, 14% of Al_2O_3, 2.3% of Fe_2O_3, and 5.5% of CaO. Grain size varies in a range of 0.2–0.5 mm.

Determination of selected metabolic parameters

At the end of the experiment, layers aged 68 weeks (Week 68) were subjected to the puncture of the *v. basilica* to collect blood samples for haematological and biochemical examination. Blood plasma was analysed for the following parameters: total protein, uric acid, cholesterol, triacylglycerides, glucose, lactic acid, aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), calcium, phosphorus, and magnesium, with the biochemical analyzer Cobas EMira using commercially available laboratory kits (Biovendor – Laboratory Medicine, Inc.).

Determination of selected haematological parameters

The following individual haematological parameters were monitored: erythrocyte count (Er), hematocrit (Hk), content of haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume of erythrocyte (MCV), leukocyte count (Le). Haematological examination was performed by conventional methods (Doubek, 2003).

Determination of egg production and feed conversion

The consumption of feeding mixture, daily production of eggs and egg weight were continually monitored and used to calculate the number of eggs per layer, laying intensity, production of egg mass per layer, consumption of feeding mixture per laid egg and per production of 1 kg of egg mass.

Health

During the experiment, the layers' state of health was monitored every day. No clinical symptoms of any disease were observed. Four layers died in both groups (yolk peritonitis, heart failure) during the experiment.

Statistical evaluation

Data with homogeneous distribution was processed using ANOVA followed by a multiple comparison using the Tukey-HSD test in order to identify the pairs of groups with statistically significant differences. Data with non-homogeneous distribution was processed using the Kruskal-Wallis ANOVA followed by a multiple comparison of non-parametric rank-order test (Tukey-type multiple comparisons).

RESULTS AND DISCUSSION

In the course of the experimental period, control layers (Group C) laid 16 289 eggs (100%), while experimental layers (Group E) laid 16 474 eggs (101.14 %) (Table 2). It follows from the results that

Table 2. Performance parameters of layers

Parameter	Group C	Group E	*P*
Total number of laid eggs (pcs)	16 289	16 474	–
Mean egg weight (g)	66.3 ± 6.25	65.6 ± 5.44	$P \leq 0.01$
Number of eggs per layer (pcs)	283.0	288.6	
Laying intensity (%)	89.9	91.6	
Production of egg matter per layer (g)	18 773	18 945	
Consumption of FM per layer (g/day)	127.9	126.2	
Consumption of FM per egg (g)	141.7	137.6	
Consumption of FM per kg of egg mass (g)	2 136.5	2 095.6	

the laying intensity in experimental layers was 1.7% higher than that in control layers, which means an increase in the number of laid eggs by 5.6 eggs per layer in the monitored period.

The mean weight of all laid eggs was 66.3 ± 6.25 g in control layers and 65.6 ± 5.44 g in experimental layers. Differences between the mean weights were highly conclusive ($P \leq 0.01$). The consumption of

Table 3. Results of biochemical tests with blood plasma from layers (n = 30)

	Group	*X*	± SD	V (%)	*P*
Total protein (g/l)	C	56.30	6.564	11.7	
	E	50.95	5.03	9.87	$P \leq 0.01$
Glucose (mmol/l)	C	11.98	6.564	54.8	NS
	E	12.15	1.140	9.38	
Triacylglyce ride (mmol/l)	C	20.06	8.089	40.3	
	E	15.95	6.520	40.9	$P \leq 0.05$
Cholesterol (mmol/l)	C	3.91	1.182	30.2	NS
	E	3.78	0.870	23.0	
Lactose (mmol/l)	C	8.38	1.802	21.5	NS
	E	8.05	1.670	20.8	
Uric acid (µmol/l)	C	337.99	154.443	45.7	NS
	E	290.69	89.770	30.9	
Calcium (mmol/l)	C	7.67	1.494	19.5	NS
	E	7.12	1.290	18.1	
Phosphorus (mmol/l)	C	2.04	0.379	18.6	NS
	E	1.98	0.830	41.9	
Magnesium (mmol/l)	C	2.55	0.741	29.1	
	E	1.23	0.360	29.3	$P \leq 0.01$
AST (µkat/l)	C	2.05	0.312	15.2	
	E	2.42	0.940	38.8	$P \leq 0.05$
ALP (µkat/l)	C	6.77	4.707	69.5	NS
	E	5.15	2.920	56.7	
LDH (µkat/l)	C	15.62	4.419	28.3	NS
	E	13.77	6.050	43.9	

AST – aspartate-aminotransferase; ALP – alkaline phosphatase; LDH – lactate dehydrogenase; NS – statistically non-significant; $P \leq 0.05$; $P \leq 0.01$

feeding mixture at the above-mentioned performance was 141.7 g per laid egg in the control group and 137.6 g per laid egg in the experimental group. The consumption of feeding mixture in the experimental group was 4.1 g lower as compared to the control group.

At the end of the experiment (Week 68), the values of selected parameters of protein, energy and mineral metabolism were determined (Table 3).

The values for monitored parameters such as uric acid, cholesterol, glucose, lactose, calcium, phosphorus, ALP and LDH ranged within reference ranges, with no statistically significant differences being found between both groups (Meluzzi et al., 1992). However, statistically significant differences between both groups were found for total protein ($P \leq 0.01$), triacylglycerols ($P \leq 0.05$), and magnesium ($P \leq 0.01$), which were higher in the control group, and for AST ($P \leq 0.05$), which was lower in the control group, as compared to the experimental group. Statistically very significantly higher values of total protein in the control group correlate with non-significantly higher levels of uric acid, which represents a degradation product of protein metabolism in poultry. The values of total protein found in the experimental group vary within reference ranges (Meluzzi et al., 1992). Clinoptilolites were found to affect the metabolic utilization of nitrogen in poultry and pigs by decreasing the elimination of nitrogen via excrements. Pigs that were administered feeding mixtures containing clinoptilolite-based formulations showed improved feed conversion and health (Papaioannou et al., 2004), and layers receiving such feed showed increased laying intensity and improved quality of eggs (Olver, 1997) but their weight did not increase, which is in good agreement with our experiment. Martin-Kleiner et al. (2001) failed to reveal any changes in the blood serum of mice that received zeolite, except for the elevated levels of potassium. Triacylglycerol levels (TAG) were statistically significantly higher in the control group ranging at the upper limit of the reported reference range while in the experimental group they fall into the reference range (Meluzzi et al., 1992). TAG belongs to a group of basic nutrients. In both groups, the levels of AST were within the reference range although they were statistically significantly higher in the experimental group ($P \leq 0.05$). The elevated activity of AST particularly indicates changes in the liver function being directly proportional to the degree of organ damage. Decreased AST activity is not of practical significance for diagnostics. Decreased levels of magnesium in blood may be associated

Table 4. Results of haematological testing in layers (n = 30)

	Group	X	± SD	V (%)	P
Er (T/l)	C	1.83	0.261	14.3	
	E	2.02	0.270	13.4	$P \leq 0.01$
Hb (g/l)	C	83.79	8.632	10.3	
	E	91.64	10.626	11.6	$P \leq 0.01$
Hk (l/l)	C	0.29	0.019	6.6	
	E	0.30	0.026	8.7	NS
MCHC (%)	C	28.87	1.879	6.5	
	E	30.52	3.468	11.4	$P \leq 0.05$
MCH (pg)	C	46.51	6.931	14.9	
	E	45.90	6.894	15.0	NS
MCV (fl)	C	161.22	22.826	14.2	
	E	150.87	18.796	12.5	NS
Le (G/l)	C	17.80	3.775	21.2	
	E	15.37	3.839	25.0	$P \leq 0.05$

Er – total erythrocyte count; Hb – haemoglobin content; Hk – hematocrit value; MCHC – mean corpuscular haemoglobin concentration; MCH – mean corpuscular haemoglobin; MCV – mean corpuscular volume of erythrocyte; Le – total leukocyte count

NS – non-significant; $P \leq 0.05$; $P \leq 0.01$

with a number of disorders. The lowered level of magnesium in dairy cows fed zeolite was reported by Enemark et al. (2003), who concluded that it might be caused partly by the disturbed resorption function of the intestine or by a magnesium-deficient diet. Feeding mixtures N1 and N2 used in this experiment were not supplemented with magnesium. Papaioannou et al. (2002) observed no changes in serum concentrations of minerals (Ca, P, K, Na, Mg, Cu, and Zn) in sows at the long-term administration of zeolite.

The results of haematological tests of blood from layers revealed statistically significant changes in total erythrocyte count and haemoglobin levels, which were higher in the experimental group, and in total leukocyte count, for which lower values were found in the experimental group (Table 4).

The effect of the long-term administration of a diet containing 1.25 and 2.5% of clinoptilolite on selected haematological parameters in cattle was investigated by Katsoulos et al. (2005), who did not reveal any adverse effect on the monitored haematological parameters (PCV, Hb, WCB). Similarly, Martin-Kleiner et al. (2001) found that the erythrocyte count, haemoglobin content and the levels of blood platelets did not change in mice fed zeolite. The effect of feed supplemented with clinoptilolite administered to layers was also investigated by Vogt (1991). Layers were reared in cages and fed an isoenergetic diet containing 0, 1, 2, and 3% of clinoptilolite. The performance parameters of layers were not significantly affected by supplementing the diet with clinoptilolite. The content of water in excrements decreased, which might be associated with an increase in ash content in excrements. Furthermore, the firmness of the egg shell improved slightly, the height of the white decreased, and the yolk colour improved. No clinical symptoms of any disease were recorded in the course of the experiment. Four laying hens died in each group (yolk peritonitis, heart failure).

When using clinoptilolite – zeolite, one may count with the protection of animals against harmful effects of mycotoxins as well as with the protection throughout the detoxification process. The binding of free water prevents the growth of fungi and spores while the antisintering properties of clinoptilolite make the feeding mixture loose and extend its usability since clinoptilolite prevents the formation of lumps from individual components. The binding of ammonia improves environmental conditions in animal houses for both the animals and the staff, and also improves the digestibility of nutrients in feeds. Considerable reduction in circulating amines improves sensorial properties of meat and eggs. It may also improve food safety in respect to the presence of pollutants.

The results obtained in this experiment confirmed that the long-term administration of clinoptilolite did not have any adverse impacts on layers' state of health but increased egg production and decreased the consumption of feeding mixture maintaining the good state of production health in layers.

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Corresponding Author

Prof. Ing. Eva Straková, Ph.D., Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, 612 42 Brno, Czech Republic
Tel. +420 541 562 672, fax +420 541 562 675, e-mail: strakovae@vfu.cz