# Effects of long-term dietary supplementation with clinoptilolite on the health status and on the performance of poultry

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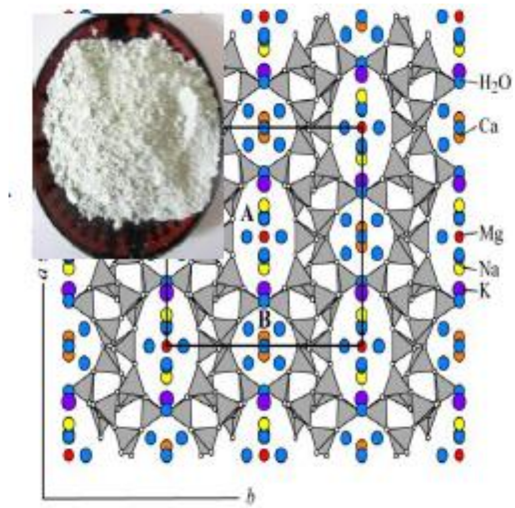
*J.C. Guilmain Inc : Importation and Distribution of Zeolite ore (Clinoptilolite)*

## Preamble




*Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations which possess three-dimensional crystal structures. They have the ability to lose and gain water reversibly, to adsorb molecules of appropriate cross-sectional diameter and to exchange their constituent cations (NH4+, Na+, K+, Ca2+) without major change in their structure. In animal nutrition, natural zeolites are used mainly to improve production. Researchers had demonstrated that their inclusion to animal diets has favorable effects on the prevention and/or treatment of certain farm animal diseases. They have the ability 1) to convert ammonia into ammonium ions that can be easily absorbed 2) to reduce significantly the fecal relative humidity and 3) to be a significant source of minerals acting as cofactors*. *In poultry industry, the non-nutritive clays such as zeolites and particularly clinoptilolite is preferred because of its high binding capacities against aflatoxins and its reducing effect on aflatoxin-absorption from the gastrointestinal tract (Harvey et al., 1993, Parlat et al., 1999, Oguz and Kurtoglu 2000).*

*Besides, 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. As a source of mineral such as aluminium, silicium, calcium, iron, sodium, clinoptilolite is gaining highly consideration from poultry farmers who add it to the dietary of the birds to prevent the damaged and cracked eggshell and to reduce significantly the occurrence of soft eggshell and to strengthen the bones of the birds.*

*Finally, on high poultry production and particularly in broiler houses, clinoptilolite is used as a component of the litter to improve the environmental conditions; hence the health status of the birds and their performance.*

## General Introduction

The inclusion of clinoptilolite in the ration of growing animals (laying hens, chicks ...) optimises not only their sanitary/health status; but also their zootechnical performance. Clinoptilolite is fixing ammonia nitrogen produced from the de-amination of amino acids obtained by the breakdown of proteins during digestion. It also decreases the residue level produced as a result of normal microbial activity in the digestive tract.

These residues are toxic to the poultry. The high levels of ammonia in the intestine are responsible for an enhanced renewal of epithelial cells of the digestive tract providing the birds with more energy and nutrients for this cell renewal without being detrimental to their growth. The binding of excess of ammonia could favour an optimisation of the performance of intestinal microflora.

***Source of minerals (Clinoptilolite):*** A number of inorganic elements are essential for normal growth and reproduction of animals. Those required in *gram quantities* are referred to as *macro minerals*, and this group includes calcium (Ca), phosphorus (P), sodium (Na), chlorine (Cl), potassium (K), magnesium (Mg), and sulfur (S). The macro minerals are important structural components of bone and other tissues and serve as important constituents of body fluids. They play vital roles in the maintenance of acid–base balance, osmotic pressure, membrane electric potential, and nervous transmission. Those elements required in *milligram or microgram amounts* are referred to as the *trace minerals*. This group includes cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), zinc (Z), and perhaps chromium (Cr) and fluorine (F). The trace minerals are present in body tissues in very low concentrations and often serve as components of metalloenzymes and enzyme cofactors or as components of hormones of the endocrine system.

Generally, it is accepted that ammonia levels in the poultry houses not exceed 25ppm. The use of clinoptilolite either as a direct supplementation in the poultry feed or as a component to the litter decreases the ammonia and the odor emissions and thus improves the quality of the air.

Clinioptilolite adsorbs toxins produced by fungal contaminants of raw materials present in the feed and responsible for infectious disease and for the reduction of growth performance of the birds.

## Challenges of the poultry farming

The modern poultry industry aims at high production and better quality at a low cost. This, in addition to an increase in the demand for poultry meat and eggs of quality, necessitates constant, efficient and goal-oriented healthcare to prevent the development of diseases. Therefore, the poultry industry is progressively integrating state of the art technologies 1) to prevent outbreaks that may have a significant negative effect on trade globally, 2) to improve the rearing management. In other words, the challenges are:

- to maintain the birds in good health in a safe environment;
- to strengthen the bones of the birds to avoid dychondroplasia;
- to reduce the heat stress related to high temperatures during the summer period;
- reduce the volatile organic compounds (VOCs) of the chickens' droppings;
- to prevent infectious diseases related to mycotoxins.

**Objectives of the study**

The aim of this study (review) is to elucidate the effects of the long term dietary inclusion of zeolite (clinoptilolite) on health status and on performance of the birds (laying hens, broilers, chicks etc.). This study will also demonstrate the positive impact of the clinoptilolite as a component of the litter in the performance of the birds. While the inclusion of clinoptilolite in the diets of the laying hens is shown to improve significantly the metabolic utilization of nutrients from a feed and to increase laying hens intensity and stability of eggshell (Olver, 1997), its positive effect is associated with its capacity to eliminate heavy metals (Tepe et *al.*, 2004).

## I – Effect of the dietary supplementation of clinoptilolite on the health of the poultry.

*a) Direct inclusion of the clinoptilolite in the daily feed*

*1-a ) Impact on the blood and serum characteristics :* According to Gezen et *al.*, 2009, a long term inclusion of clinoptilolite at 2% to the poultry diet translates into a significant increase of the calcium in the blood of the chickens (Tab.1) . This increase is associated to the selective capacity of the clinoptilolite to retain or release the calcium during the digestive process of the chickens (Roland *et al.*,1985). The bioavailability of the calcium is critical for the bone formation of growing chicks and important for the strengthening of the bones of chickens and for the prevention of dychondroplasia. Specifically, the effect of the addition of clinoptilolite on bone metabolism indicators in broiler chickens was studied by Leach et *al.*, (1990) who reported weight gains in broilers and the enhanced deposition

Tab.1 The effect of clinoptilolite on tibia ash, mineral concentrations, blood calcium and manure dry matter in laying hens.

| Parameters | Groups | | | | | |
|---|---|---|---|---|---|---|
| | N | Low Ca (3.5%) | Optimum Ca (4.2%) | 1% CLP | 2% CLP | P |
| Tibia ash (%) | 8 | 62.0±0.69 | 62.5±0.56 | 60.8±0.92 | 61.6±1.33 | NS |
| Tibia calcium (%) | 8 | 22.8±0.97 | 21.6±0.89 | 21.4±1.16 | 21.7±0.90 | NS |
| Tibia phosphor (%) | 8 | 11.4±0.50 | 10.7±0.03 | 10.4±0.24 | 10.7±0.27 | NS |
| Blood calcium (mmol/L) | 8 | $3.50^{b}$±0.15 | $3.78^{ab}$±0.09 | $3.57^{ab}$±0.04 | $3.94^{a}$±0.08 | * |
| Manure dry matter (%) | | | | | | |
| At the beginning | 4 | 22.4±1.19 | 23.5±1.42 | 23.7±1.53 | 23.9±0.33 | NS |
| At the end | 4 | $21.0^{b}$±0.85 | $21.0^{b}$±0.38 | $23.4^{a}$±0.85 | $23.4^{a}$±0.38 | ** |

[a, b] Mean values within a row with different superscripts are significantly different. * $p<0.05$, ** $p<0.01$.
NS = Not significant, results are expressed as means±standard errors.

of calcium in bones in the case of deficient or boundary level of calcium, thus relieving the symptoms of rickets. These findings were confirmed by Machacek et *al.*, 2010 who demonstrate that an inclusion of clinoptilolite at 4% into the diet of the birds contributes to a better use of the microelements and particularly of the calcium and the phosphorus. The efficacy can be explained by the capacity of the clinoptilolite to slow down the passage of pre-digested food through the intestine which leads to the increased utilization of nutrients and microelements.

*1-b) Impact on the manure and the environment of the chickens:* As shown in the Tab. 1, the inclusion of clinoptilolite at 2% induced an increase of the manure dry matter; confirming the ability of the clinoptilolite to bind free water and to prevent fungi and conidia related disease.

In the same vein, the binding of ammonia by clinoptilolite improves environmental conditions in poultry houses. According to Olver, 1983, 1989, 1997 and Oztuk et *al.*, 1998, the inclusion of clinoptilolite significantly reduced the odor of the chicken's droppings due to the ammoniac and other volatile organic compounds (VOCs). This improved environment will prevent any respiratory problem of the birds.

*1-c ) Impact on the immune system of the chickens:* The effect of the long term inclusion of the clinoptilolite in the immune system of the poultry had also been studied by Strakova et *al.*, 2008 (Tab.2). According to the authors, the addition of the clinoptilolite to the diet of the layers revealed statistically significant changes in total erythrocyte count and haemoglobin levels, and in total leukocyte count.

Tab.2 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; $P \leq 0.05$; $P \leq 0.01$

*1-d) Impact of the inclusion of clinoptilolite on the mycotoxins:* Mycotoxins and particularly aflatoxins are secondary metabolites produced by certain fungi belonging to the genus *Aspergillus* and can occur as natural contaminants of poultry feed (Leeson et *al.*, 1995, Oguz 1997). The chronic exposure of poultry to these toxins is causing "aflatoxicosis", an important disease associated with severe economic losses and health problems in poultry (Edds and Bortell 1983, Kaya et *al.*,1990, Leeson et *al.*,1995, Santurio et *al.*,1999).

Aflatoxicosis in poultry is characterised by listlessness, anorexia with lowered growth rate, poor food utilisation, decreased weight gain, decreased egg production, and increased susceptibility to environmental and microbial stresses, and increased mortality (Bailey et *al.*, 1998, Kubena et *al.*, 1998). Also associated with aflatoxicosis is anaemia (Huff et *al.*, 1988, Kececi et *al.*, 1998), inhibition of immune function (Celik et *al.*, 1996, Gabal and Azzam 1998), hepatotoxicosis (Edrington et *al.*, 1997, Kiran et *al.*, 1998), mutagenesis, teratogenesis, carcinogenesis, and haemorrhage (Edds and Bortell 1983).

In the last 10 years, several studies have been performed to detoxify AF in contaminated food and foodstuffs and to minimise the deleterious effects of aflatoxins in broilers (Kubena et *al.*, 1990, 1993, Harvey et *al.*, 1993, Jindal et *al.*, 1994, Abo-Norag et *al.*, 1995, Bailey et *al.*, 1998) and other poultry species (Sjamsul et *al.*,1990, Kubena et *al.*, 1991, Parlat et *al.*, 1999).

Most recently, Ortatatli and Oguz, 2001 studied the ameliorative effects of dietary clinoptilolite on pathological changes in broiler chickens during aflatoxicosis. According to the authors, the addition of 1.5% or 2.5% of the clinoptilolite to the aflatoxin (AF) containing diet of the broilers decreased both the incidence of affected broilers and the severity of lesions in the organs of chicks (Tab.3). In this study, the clinoptilolite acted as

Tab.3 Effect of clinoptilolite (CLI) on relative organ weights for broiler chicks fed on diet containing 2.5mg/kg total aflatoxin (AF) diet at 1 to 21 days of age*

| Treatment | | | Relative organ weights[1] | | | | |
|---|---|---|---|---|---|---|---|
| AF | CLI (per cent) | | | | | | |
| | 1·5 | 2·5 | Liver | Kidney | Spleen | bursa of Fabricius | Thymus |
| − | − | − | 3·338 (0·19)[bc] | 0·874 (0·02)[d] | 0·099 (0·01)[ab] | 0·273 (0·01) | 0·582 (0·04)[b] |
| + | − | − | 3·876 (0·13)[a] | 1·095 (0·05)[bc] | 0·124 (0·01)[a] | 0·288 (0·01) | 0·544 (0·03)[b] |
| − | + | − | 3·471 (0·12)[ab] | 0·857 (0·03)[d] | 0·079 (0·01)[b] | 0·277 (0·01) | 0·687 (0·02)[a] |
| + | + | − | 3·789 (0·12)[abc] | 1·182 (0·05)[ab] | 0·110 (0·01)[ab] | 0·272 (0·02) | 0·559 (0·03)[b] |
| − | − | + | 3·291 (0·16)[c] | 0·980 (0·03)[cd] | 0·092 (0·01)[ab] | 0·265 (0·02) | 0·565 (0·02)[b] |
| + | − | + | 3·645 (0·17)[abc] | 1·272 (0·09)[a] | 0·121 (0·01)[a] | 0·294 (0·01) | 0·585 (0·03)[b] |

[a–d]Values within columns with no common superscripts are significantly different ($P < 0·05$), according to the Duncan's multiple range tests.
*Values represent the mean (SEM) of six groups of 10 broiler chicks each per treatment.
[1]g of organ 100 $g^{-1}$ of live bodyweight.

a sequestering agent against the aflatoxin in feeds through its adsorption and therefore reducing the aflatoxin bio-availability in the gastrointestinal tract. As an inert and non-toxic matter for poultry (Olver 1997) approved by the European Union Commission since 2000 onwards (Bluthgen and Schwertfeger 2000), the addition of clinoptilolite to the poultry diet is an encouraging approach to prevent the deleterious effect of aflatoxicosis in poultry's health.

### *a) Addition a as component of the litter*

One of the most important challenges in poultry production is the control of litter quality. The latter has been seen as a priority in modern poultry industry to avoid environmental and birds welfare problems and also to reduce productivity losses (Francesch and Brufau, 2004). Over the last few years several strategies have been suggested for the control of litter quality including nutritional manipulation (Francesch and Brufau, 2004) or management practices (McCrory and Hobbs, 2001; Patterson and Adrizal, 2005). The incorporation of the clinoptilolite up to 25% in the litter resulted in the reduction of the litter moisture content (Karamanlis et *al.*, 2008; Tab.4) confirming the previous findings of Eleroglu and Yalcin, 2005 who observed a decrease of 30% of the moisture content of the litter consisting of wood shavings when the clinoptilolite is added at 25%.

Tab.4: Impact of the clinoptilolite on the quality of the parameters of the litter samples

| Parameters | Sampling day | Treatment groups[1] BS (n = 10) | ZS (n = 10) | BSz (n = 10) | ZSz (n = 10) | SEM | p value | Overall effect |
|---|---|---|---|---|---|---|---|---|
| Moisture (% ww) | 14 | 32.25 | 31.67 | 27.09 | 27.28 | 3.45 | 0.643 | NS |
| | 28 | 46.02 | 39.07 | 42.14 | 40.97 | 4.49 | 0.769 | NS |
| | 42 | 51.74 | 49.41 | 46.02 | 44.59 | 3.01 | 0.381 | NS |
| Organics (% TS) | 14 | $94.46^{a}$ | $91.80^{a,b}$ | $85.82^{b}$ | $78.98^{c}$ | 1.81 | 0.002 | *** |
| | 28 | $86.03^{a}$ | $83.00^{b}$ | $78.98^{c}$ | $75.91^{d}$ | 0.91 | <0.001 | *** |
| | 42 | $83.29^{a}$ | $78.39^{b}$ | $80.50^{a,b}$ | $74.75^{c}$ | 1.08 | 0.003 | *** |
| Kjeldahl nitrogen (g/kg ww) | 14 | $17.11^{a}$ | $17.72^{a}$ | $14.49^{a,b}$ | $12.68^{b}$ | 1.40 | 0.075 | NS |
| | 28 | 22.88 | 23.55 | 23.03 | 21.91 | 2.01 | 0.958 | NS |
| | 42 | 24.80 | 23.24 | 23.46 | 22.50 | 1.90 | 0.897 | NS |
| Nitrogen ammonia (g/kg ww) | 14 | $0.90^{a}$ | $0.54^{b}$ | $0.46^{b}$ | $0.62^{b}$ | 0.07 | 0.014 | * |
| | 28 | $3.28^{a}$ | $2.82^{a}$ | $2.75^{a}$ | $2.52^{b}$ | 0.19 | 0.124 | NS |
| | 42 | $6.16^{a}$ | $5.20^{a,b}$ | $5.34^{a,b}$ | $4.56^{b}$ | 0.57 | 0.392 | NS |
| $BOD_5$ (g/kg ww) | 14 | $134.00^{a}$ | $113.20^{a}$ | $83.60^{b}$ | $70.80^{b}$ | 8.32 | 0.105 | NS |
| | 28 | $129.60^{a}$ | $110.60^{a,b}$ | $100.85^{b}$ | $86.70^{b}$ | 7.83 | 0.009 | ** |
| | 42 | $132.00^{a}$ | $115.40^{a,b}$ | $110.56^{a,b}$ | $105.70^{b}$ | 8.55 | 0.198 | NS |

[1] Treatment groups: BS = Basal diet and sawdust, ZS = 2% clinoptilolite diet and sawdust, BSz = Basal diet and sawdust with 2 kg/m$^2$ clinoptilolite. ZSz = 2% clinoptilolite diet and sawdust with 2 kg/m$^2$ clinoptilolite.
[a, b, c, d] Means in the same row with different superscript differ significantly (p<0.05).
* p<0.05, ** p<0.01, *** p<0.001.

In their studies, Karamanlis et *al.*, 2008 demonstrated the significant reduction of the ammonia emissions when the clinoptilolite is added to the broilers' diet and to their litter. They concluded to the improvement of the poultry health with the low levels of ammonia (Tab.5).

Tab.5: Overall ammonia emissions of compartments according to treatment

| | Period (days) | Treatment groups[1] BS (n = 56) | ZS (n = 56) | BSz (n = 56) | ZSz (n = 56) | SEM | p value | Overall effect |
|---|---|---|---|---|---|---|---|---|
| Ammonia emission (ppm) | 29-42 | $23.64^{a,b}$ | $27.00^{b}$ | $20.55^{a}$ | $21.71^{a}$ | 1.20 | 0.002 | ** |

[1] Treatment groups: BS = Basal diet and sawdust, ZS = 2% clinoptilolite diet and sawdust, BSz = Basal diet and sawdust with 2 kg/m$^2$ clinoptilolite. ZSz = 2% clinoptilolite diet and sawdust with 2 kg/m$^2$ clinoptilolite.
[a, b] Means in the same row with different superscript differ significantly (p<0.05). ** p<0.01.

In fact, high ammonia levels in the poultry house are generated as a result of the fermentation of chicken excreta (Valentine, 1964; Carlile, 1984; Whyte, 1993). This may cause cerato-conjunctivitis of the eyes, reduce the rate and the depth of respiration and increase sensitivity due to irritation of mucus in the respiratory tract (Valentine, 1964; Carlile, 1984; Whyte, 1993). This would reduce the rate of development and decrease feed efficiency (Reece *et al*., 1980) and performance (Homiden *et al*., 1997) of the birds. As shown in the Tab.6, the results of Karamanlis *et al.*, 2008 showed that the inclusion of clinoptilolite both as feed and litter additive had a beneficial effect on broilers health and growth.

Tab.6: Growth of broilers according to treatment and age

| Age (days) | Treatment groups (Live weight (g))[1] | | | | SEM | p value | Overall effect |
|---|---|---|---|---|---|---|---|
| | BS (n = 130) | ZS (n = 130) | BSz (n = 130) | ZSz (n = 130) | | | |
| 0 | 39.8 | 39.8 | 39.3 | 39.7 | 0.28 | 0.448 | NS |
| 14 | 399.4[a,b] | 405.3[a] | 390.9[b,c] | 384.4[c] | 3.21 | <0.001 | *** |
| 28 | 1,283.3[a] | 1,334.1[b] | 1,333.5[b] | 1,325.5[b] | 9.62 | <0.001 | *** |
| 42 | 2,317.5[a] | 2,474.1[b] | 2,468.1[b] | 2,482.2[b] | 16.5 | <0.001 | *** |

[1] Treatment groups: BS = Basal diet and sawdust, ZS = 2% clinoptilolite diet and sawdust, BSz = Basal diet and sawdust with 2 kg/$m^2$ clinoptilolite. ZSz = 2% clinoptilolite diet and sawdust with 2 kg/$m^2$ clinoptilolite.

[a, b, c] Means in the same row with different superscript differ significantly ($p<0.05$).

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

## II– Effect of the dietary supplementation of clinoptilolite on the performance of the poultry

For many years nutritionists have recognized that certain non-nutritive materials affect the nutritive efficiency of diets of animals. Among these materials, zeolites and particularly clinoptilolite has been shown to minimize the adverse effects of aflatoxins on feed intake, performance and feed conversion efficiency.

### *a) Direct inclusion of the clinoptilolite in the daily feed of poultry.*

*1-a) Impact on the performance of laying hens:* In their studies, Moghaddam *et al.*, 2008 demonstrated the positive impact of the clinoptilolite in the performance of the laying hens. According to the authors, the inclusion of the clinoptilolite in the daily feed not only induced a significant increase of the egg production (number of eggs per laying hen) and an increase of the egg mass but also improved the quality of the eggshell. More specifically, the clinoptilolite inclusion increased the egg mass/weight in the first 30 to 36 weeks of age and tended to increase it in the further period as shown in Tab.7. The heaviest eggs were obtained with an inclusion of 4.5% clinoptilolite in the laying hen diets. Similarly, in his study by three strains of laying hens, Olver, 1997 found that hens on the diet containing clinoptilolite (zeolite) laid more eggs per hen than those fed no clinoptilolite. Most recently, the increase of the daily egg production was demonstrated by Strakova et *al.*, 2008. During the experimental period, control layers (Group C) laid 16 289 eggs (100%), while experimental layers (Group E) laid 16 474 eggs as confirmed in Tab.8. It follows from the results that 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.

Tab.7: Effect of zeolite supplementation of diets of various Ca and NPP contents on laying hens performance

| Zeolite (%) | Ca (%) | NPP (%) | Egg production (%) | | Feed intake (g day$^{-1}$ per hen) | | Feed efficiency (feed/egg) | | Egg mass (g day$^{-1}$ per hen) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 30-36 weeks | 36-42 weeks | 30-36 weeks | 36-42 weeks | 30-36 weeks | 36-42 weeks | 30-36 weeks | 36-42weeks |
| 0.0 | 3.26 | 0.25 | 86.66 | 80.34 | 107.21 | 102.72 | 2.21 | 2.25 | 48.85 | 45.50 |
| 0.0 | 3.26 | 0.19 | 90.07 | 86.67 | 104.16 | 96.37 | 2.02 | 1.97 | 51.55 | 48.87 |
| 0.0 | 2.45 | 0.25 | 88.72 | 80.00 | 101.15 | 94.32 | 2.01 | 2.05 | 50.33 | 45.99 |
| 0.0 | 2.45 | 0.19 | 90.07 | 83.86 | 105.71 | 103.29 | 2.06 | 2.13 | 51.27 | 48.31 |
| 1.5 | 3.26 | 0.25 | 88.78 | 87.67 | 108.17 | 103.05 | 2.11 | 2.03 | 51.36 | 50.76 |
| 1.5 | 3.26 | 0.19 | 85.90 | 87.50 | 103.72 | 99.05 | 2.10 | 1.94 | 49.34 | 50.96 |
| 1.5 | 2.45 | 0.25 | 89.10 | 87.67 | 103.67 | 98.64 | 2.05 | 1.96 | 50.71 | 50.29 |
| 1.5 | 2.45 | 0.19 | 86.86 | 88.33 | 104.73 | 102.97 | 2.16 | 2.05 | 48.58 | 50.17 |
| 3.0 | 3.26 | 0.25 | 87.41 | 82.83 | 102.35 | 94.46 | 2.06 | 2.02 | 49.78 | 46.81 |
| 3.0 | 3.26 | 0.19 | 89.10 | 86.00 | 104.98 | 97.51 | 2.04 | 1.91 | 51.55 | 50.99 |
| 3.0 | 2.45 | 0.25 | 86.27 | 84.42 | 106.84 | 106.25 | 2.20 | 2.20 | 48.63 | 48.33 |
| 3.0 | 2.45 | 0.19 | 91.67 | 89.67 | 106.07 | 103.30 | 2.00 | 1.99 | 53.05 | 52.02 |
| 4.5 | 3.26 | 0.25 | 83.98 | 85.33 | 105.16 | 100.98 | 2.17 | 2.04 | 48.42 | 49.50 |
| 4.5 | 3.26 | 0.19 | 89.43 | 86.67 | 102.58 | 98.53 | 2.02 | 1.98 | 50.81 | 49.83 |
| 4.5 | 2.45 | 0.25 | 88.14 | 87.67 | 106.39 | 101.85 | 2.06 | 1.98 | 51.58 | 51.41 |
| 4.5 | 2.45 | 0.19 | 86.86 | 86.33 | 108.52 | 101.53 | 2.14 | 2.00 | 50.66 | 50.70 |
| SEM | | | 1.908 | 1.433 | 3.729 | 4.967 | 0.069 | 0.093 | 1.212 | 0.979 |
| | | | Probability | | | | | | | |
| Zeolite | | | NS | *** | NS | NS | NS | NS | NS | *** |
| Calcium | | | NS | NS | NS | NS | NS | NS | NS | NS |
| Phosphorus | | | NS | ** | NS | NS | NS | NS | NS | ** |
| Zeolite×Calcium | | | NS | NS | NS | NS | NS | NS | NS | NS |
| Zeolite×Phosphorus | | | NS | * | NS | NS | NS | NS | * | ** |
| Calcium×Phosphorus | | | NS | NS | NS | NS | NS | NS | NS | NS |
| Zeolite×Calcium×Phosphorus | | | NS | NS | NS | NS | NS | NS | NS | NS |

NS: Not Significant; *p<0.05; **p<0.01; ***p<0.001

Tab.8: 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 | |

In general, the positive effect of the clinoptilolite in the performance on laying hens is related to several parameters. Nevertheless, it has been well documented that the inclusion of clinoptilolite in the laying hens' diet provides a high concentration of calcium to the birds. Moreover, the high content of aluminium included in the clinoptilolite may complex with the phosphorus (P) and may reduce its bioavailability; hence improve the quality of eggshell (Roland, 1990; Elliot and Edwards, 1991).

*1-b) Impact of the inclusion of clinoptilolite on broiler chickens' live weight and dietary intake:*

While some reports indicated that no direct effect of the administration of the clinoptilolite on weight gain and on feed conversion in broiler chickens (Ozturk et al., 1998), some others are confirming the positive effect of the long term inclusion on the growth performance. According to Strakova *et al.*, 2008, the improved performance of the broiler chicken fed with clinoptilolite (0.5, 1.5 and 2.5%) is likely to be associated with the improved utilization of nutrients (Olver 1997) and detoxifying effects of clinoptilolite (Harvey et al. 1993; Skalická and Makoová 1999; Skalická et al. 2000; Parlat et al. 1999; Ortatatli and Oquz 2001; Rizzi at al. 2003). The efficacy can be explained by slower passage of pre-digested food through the intestine which leads to the increased utilization of nutrients from the feeding dose, particularly nitrogen (Mumpton and Fishman 1977; Melenova et al.2003). In the same vein, Safameher, 2008 demonstrated the long term effect of the clinoptilolite on broilers' body weight, dietary intake, food conversion and liver (Tab.9).

Tab.9: Production parameters and liver weight (g/kg) of chicks fed different ratios

A : Basal diet prepared with uncontaminated diet (control).
B : Basal diet containing Aflatoxin (0.5 mg AF $kg^{-1}$).
C : Basal diet containing Aflatoxin (1mg AF $kg^{-1}$).
D : Basal diet containing clinoptilolite (20 g $kg^{-1}$).
E : Basal diet containing Aflatoxin (0.5 mg AF $kg^{-1}$) + clinoptilolite (20 g $kg^{-1}$).
F : Basal diet containing Aflatoxin (1mg AF $kg^{-1}$) + clinoptilolite (20 g $kg^{-1}$).

| | Experimental | | | | |
|---|---|---|---|---|---|
| | Body weight gain (g $day^{-1}$) | Dietary intake (g $day^{-1}$) | Feed conversion ratio | Liver (g 100 $g^{-1}$) | |
| Group | Day 42 | Day 42 | Day 42 | Day 21 | Day 42 |
| Control | 38.1±0.7$^{a}$ | 74.4±2.2$^{a}$ | 1.95±0.15$^{a}$ | 5.7±0.57$^{a}$ | 3.3±0.32$^{a}$ |
| A* | 30.2±2.2$^{b}$ | 69.2±3.2$^{b}$ | 2.22±0.08$^{b}$ | 7.12±0.35$^{b}$ | 5.3±0.3$^{b}$ |
| AA** | 27.3±1.02$^{b}$ | 64.1±3.1$^{c}$ | 2.35±0.09$^{b}$ | 7.45±0.46$^{b}$ | 5.9±0.33$^{b}$ |
| CLI *** | 39.5±1.2$^{a}$ | 78.8±3.7$^{a}$ | 1.99±0.15$^{a}$ | 5.9±0.45$^{a}$ | 3.35±0.4$^{a}$ |
| A+CLI | 35.5±1.3$^{a}$ | 71.8±2.0$^{a}$ | 2.05±0.56$^{a}$ | 6.3±0.3$^{a}$ | 3.5±0.3$^{a}$ |
| AA+CLI | 34.16±1.0$^{ab}$ | 71.2±1.4$^{ab}$ | 2.1±0.05$^{ab}$ | 6.87±0.6$^{ab}$ | 3.84±0.26$^{a}$ |

Feeding started from the first day of housing and parameters calculated on days 21 and 42 days of age. Data presented as mean±S.D. Mean values in same column without common letters (a-c) differ significantly ($p<0.05$). * A = AF (0.5 mg $kg^{-1}$), **AA = AF (1 mg $kg^{-1}$), **CLI = 20 g $kg^{-1}$

As shown in the Tab.9, chickens fed on diet prepared with adding clinoptilolite (groups E and F) were normal with respect to dietary intake, body weight gain and feed conversion ratio (FCR). In contrast, in chickens fed with Aflatoxin (AF)-containing diet (groups B and C), there was a significant reduction ($p<0.05$) in feed intake concomitant with 20.7-27.3% reduction in body weight gain particularly during the second phase of rearing (42 days) and increased FCR as compared to control. According to Oguz and Kortorglu 2000, the inclusion of 15g/kg of clinoptilolite to the 2.5mg AF-containing diet significantly ameliorated the adverse effect of aflatoxin on performance (Tabs.10, 11 & 12),. Further, the protective effect of 15g/kg of clinoptilolite against the toxic effects of aflatoxins was greater than that 25mg/kg of clinoptilolite and the lower concentration of clinoptilolite was inert and non-toxic for broiler chickens.

Tab.10: Effect of clinoptilolite (CLI) on body weight gain for broiler chicks fed on diet containing 2.5mg total aflatoxin (AF) diet at 1 to 21 days of age.

| AF | CLI addition | | Body weight gain (g) | | | | d 1 to d 21: change from control (%) |
|---|---|---|---|---|---|---|---|
| | 15 g/kg | 25 g/kg | 1 to 7 d | 8 to 14 d | 15 to 21 d | 1 to 21 d | |
| – | – | – | 69·58±3·40$^{a}$ | 160·10±6·51$^{a}$ | 191·40±10·65 | 424·71±11·80$^{a}$ | 0 |
| + | – | – | 60·38±2·20$^{b}$ | 138·38±5·00$^{b}$ | 179·78±4·92 | 378·51±11·42$^{b}$ | –10·88 |
| – | + | – | 73·42±2·29$^{a}$ | 165·76±4·87$^{a}$ | 177·22±7·14 | 416·41±9·56$^{ab}$ | –1·96 |
| + | + | – | 71·22±3·45$^{a}$ | 164·50±3·90$^{a}$ | 200·90±13·57 | 436·65±18·45$^{a}$ | +2·81 |
| – | – | + | 71·03±1·21$^{a}$ | 154·22±4·95$^{a}$ | 174·35±9·51 | 399·61±10·73$^{ab}$ | –5·91 |
| + | – | + | 70·71±1·55$^{a}$ | 152·19±5·35$^{ab}$ | 186·10±6·63 | 409·00±10·85$^{ab}$ | –3·70 |

$^{a-b}$ Values within columns with no common superscripts are significantly different ($P<0·05$), according to multiple range test.

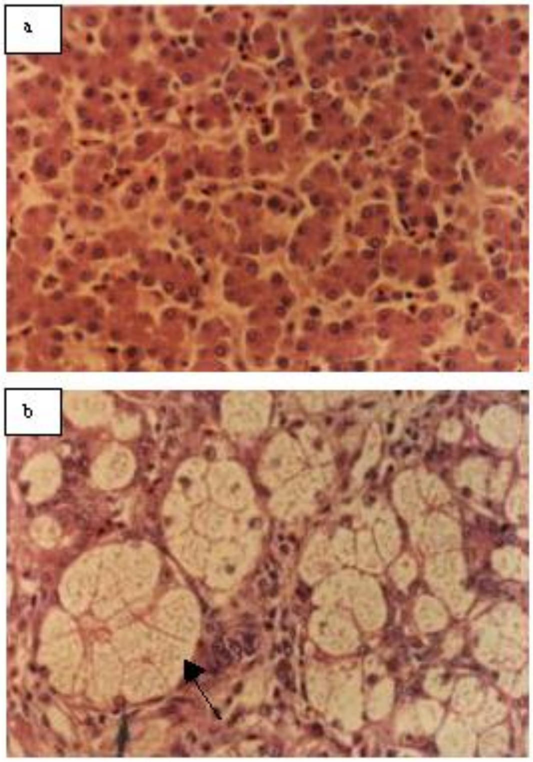
Tab.11: Effect of clinoptilolite (CLI) on food conversion ratio and mortality values for broiler chicks fed on diet containing 2.5mg total aflatoxin (AF) diet at 1 to 21 days of age.

| AF | CLI addition | | Food conversion ratio (g food/g gain) | | | | Change from control (%) | Mortality (dead/total) |
|---|---|---|---|---|---|---|---|---|
| | 15 g/kg | 25 g/kg | 1 to 7 d | 8 to 14 d | 15 to 21 d | 1 to 21 d | | |
| – | – | – | 1·64±0·067 | 1·94±0·081 | 1·86±0·220$^{ab}$ | 1·81±0·102$^{ab}$ | 0 | 4/60 |
| + | – | – | 1·89±0·130 | 2·03±0·074 | 1·82±0·120$^{ab}$ | 1·96±0·110$^{a}$ | +8·28 | 2/60 |
| – | + | – | 1·63±0·060 | 1·97±0·120 | 2·26±0·193$^{a}$ | 2·04±0·086$^{a}$ | +12·70 | 3/60 |
| + | + | – | 1·75±0·054 | 1·89±0·098 | 1·94±0·200$^{a}$ | 1·88±0·080$^{ab}$ | +3·86 | 6/60 |
| – | – | + | 1·88±0·096 | 1·87±0·081 | 1·38±0·068$^{b}$ | 1·67±0·049$^{b}$ | –7·74 | 1/60 |
| + | – | + | 1·83±0·110 | 2·13±0·133 | 1·85±0·112$^{ab}$ | 1·94±0·064$^{a}$ | +7·18 | 6/60 |

$^{a-b}$Values within columns with no common superscripts are significantly different ($P<0·05$), according to multiple range test.
Values represent the mean±SEM of 6 groups of 60 broiler chicks *(6 replicates of 10 broilers in each)* per treatment.

The histopathologic observations of the liver also provide evidence of the adverse effect (positive effect) of the clinoptilolite against the deleterious impact of the aflatoxins (§. Fig.1 & Fig.2).

Fig. 1 Histopathology of liver. a) Liver section (x400) of normal chicken (Gr.A) stained by H and E ; b) Section (x200) shows hepatic tissue of chickens given dietary aflatoxin at a concentration of 1000ppb.




of

*b) Addition of clinoptilolite a as component of the litter: Impact on the*

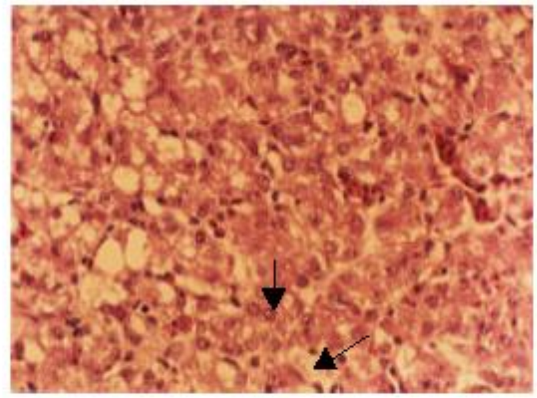


Fig. 2: Liver from 1000 ppb AF -treated plus CLI group. slight granular degeneration (arrowes) and fatty change H and E×400

*performance of poultry.*

The influence of natural zeolite, consisting mainly of clinoptilolite and mordenite, as a component of the litter material in broiler houses on the performance of the broilers had been demonstrated by Eleroglu and Yalcin, 2005. According to the researchers, the live weight gain, feed consumption, feed efficiency, viability were significantly improved when the zeolite was added to the litter made of wood shavings. Further, the authors found that the inclusion of the zeolite at different levels reduced the occurrence of leg and body abnormalities of broilers and the litter moisture content.

Tab.11: Effect of different levels of inclusion of clinoptilolite (CLI) and modernite (MOD)to the litter on the chicks performance and on the moisture content.

| Formulations / Parameters | %0 CLI-MOD | %25 CLI-MOD | %50 CLI-MOD | %75 CLI-MOD |
|---|---|---|---|---|
| Feed consumption and feed efficiency | 3547 | 3381 | 3472 | 3421 |
| Mean live weights | 1935 | 1970 | 1996 | 1978 |
| Feed efficiency (g feed/g weight gain) | 1.83 | 1.71 | 1.74 | 1.73 |
| Percentage of moisture | 36.2 | 25.2 | 23.6 | 21.8 |

Table11 is the summary of the next tables presented below.

Mean live weights (g ± s.e.) of broilers during different stages of the trial reared on different types of litter material

| Weeks | Litter treatments* | | | |
|---|---|---|---|---|
| | Wood shavings 0% zeolite | Wood shavings 25% zeolite | Wood shavings 50% zeolite | Wood shavings 75% zeolite |
| 1 | 146 ± 7.0 | 145 ± 3.6 | 148 ± 2.7 | 146 ± 3.6 |
| 2 | 378 ± 7.0 | 378 ± 3.6 | 385 ± 5.0 | 382 ± 6.2 |
| 3 | 701 ± 9.5 | 707 ± 7.2 | 720 ± 6.9 | 714 ± 9.9 |
| 4 | 1082 ± 9.6[a] | 1110 ± 7.8[b] | 1122 ± 10.4[b] | 1112 ± 10.5[b] |
| 5 | 1489 ± 26.1[a] | 1540 ± 14.2[b] | 1558 ± 11.4[b] | 1544 ± 7.9[b] |
| 6 | 1935 ± 10.0[a] | 1970 ± 11.4[b] | 1996 ± 10.1[b] | 1978 ± 13.1[b] |

* Means within the same row with superscripts, a-b, differ significantly at P > 0.05

Feed consumption and feed efficiency of the broilers reared on litter containing different proportions of wood shavings and zeolite

| Weeks | Litter treatments* | | | |
|---|---|---|---|---|
| | Wood shavings 0% zeolite | Wood shavings 25% zeolite | Wood shavings 50% zeolite | Wood shavings 75% zeolite |
| | Cumulative feed consumption (g) | | | |
| 1 | 141 ± 4.04 | 134 ± 6.08 | 137 ± 5.51 | 132 ± 5.29 |
| 2 | 497 ± 15.9 | 506 ± 17.1 | 508 ± 19.5 | 505 ± 19.16 |
| 3 | 1060 ± 55.2 | 1027 ± 28.7 | 1060 ± 43.7 | 1044 ± 30.3 |
| 4 | 1846 ± 56.1 | 1753 ± 52.2 | 1769 ± 72.6 | 1734 ± 78.0 |
| 5 | 2695 ± 114.5 | 2643 ± 15.7 | 2684 ± 47.9 | 2619 ± 75.7 |
| 6 | 3547 ± 68.4 | 3381 ± 67.5 | 3472 ± 92.0 | 3421 ± 79.7 |
| | Feed efficiency (g feed/g weight gain) | | | |
| 1 | 0.96 ± 0.06 | 0.92 ± 0.04 | 0.93 ± 0.02 | 0.90 ± 0.02 |
| 2 | 1.31 ± 0.03 | 1.34 ± 0.04 | 1.32 ± 0.04 | 1.32 ± 0.03 |
| 3 | 1.51 ± 0.06 | 1.45 ± 0.03 | 1.47 ± 0.04 | 1.46 ± 0.05 |
| 4 | 1.70 ± 0.04[a] | 1.58 ± 0.03[b] | 1.57 ± 0.05[b] | 1.56 ± 0.05[b] |
| 5 | 1.81 ± 0.04[a] | 1.71 ± 0.02[b] | 1.72 ± 0.02[b] | 1.69 ± 0.04[b] |
| 6 | 1.83 ± 0.04[a] | 1.71 ± 0.04[b] | 1.74 ± 0.04[b] | 1.73 ± 0.04[b] |

* Means within the same row with superscripts, a-b, differ significantly at P > 0.05

Percentage of moisture (% ± s.e.) at the end of the trial in litter containing different proportions of wood shavings and zeolite

| Week | Litter treatments* | | | |
|---|---|---|---|---|
| | Wood shavings 0% zeolite | Wood shavings 25% zeolite | Wood shavings 50% zeolite | Wood shavings 75% zeolite |
| 6 | 36.2 ± 3.91[a] | 25.2 ± 5.94[b] | 23.6 ± 3.97[b] | 21.8 ± 3.99[b] |

* Means within the row with superscripts, a-b, differ significantly at P > 0.05

### Conclusion

With the fast growing demand worldwide, one of the most important challenges that the poultry is facing is to produce a sufficient quantity of poultry products that meet the strict requirements for food safety. Feeds for chicks may contain a number of anti-nutritional substances which may enter food *via* animals and their products. These substances may not only have a deleterious impact on the health and the performance of the birds; but may also render their products unfit for human consumption. Suitable procedure had been sought to prevent the contamination of animals with these toxic substances and to control the subsequent penetration of these substances into the food chain. Clinoptilolite is currently used in the poultry industry as a suitable feed additive that prevent such contamination. Due to its physical structure, this type of zeolite (clay) binds the mycotoxins and contributes to their elimination; hence adversing the deleterious effect of the toxins on the health and the performance of the animals. Despite this critical effect, many authors also reported the positive impact of the long term inclusion of the clinoptilolite on birds performance traits: increase of weight gain, improvement of eggshell quality, increase of eggs laid per hen, strengthening of leg bones, resistance to heat stress and improvement of environment (air quality) of poultry rearing houses.

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