

## Effects of a clinoptilolite supplement in pig diets on performance and serum parameters

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**ABSTRACT:** The effects of clinoptilolite in pig's diets were examined on sixty Landrace × Yorkshire crossbred pigs of both sexes divided into two groups: 1. control group, with basal diets; 2. treatment, with basal diets supplemented with 5 g clinoptilolite per 1 kg diet (Cp group). Feed and water were available *ad libitum*. Individual live weights were recorded on days 45, 90 and 135 of the experiment. Feed consumptions were recorded weekly. Blood was drawn from the anterior vena cava of each pig at day 135 for the determination of serum biochemical parameters. During the first 90 days of the experiment pigs from the Cp group had higher body weight gain (BWG) compared with the control group (+2.5% and +7.0%, respectively) but in the finishing phase of growing their growth parameters were significantly lower (−4.8%). Blood serum biochemical parameters from all experimental pigs were generally within the normal range. Higher triglyceride concentration, lower total cholesterol concentration and increased activity of AST were recorded in the serum of the Cp group.

**Keywords:** zeolite; growing-finishing pigs; animal feed; cholesterol

Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth metals which possess three-dimensional crystal structures. They have the ability to lose and gain water reversibly, to exchange some of their constituents, and are well known as "molecular sieves". Each zeolite species has its own unique crystal structure and, hence, its own set of chemical and physical properties (Mumpton, 1999).

Zeolite has cationic binding properties of potential value in protecting animals from the tissue accumulation of toxic minerals (Pond and Yen, 1983; Jain, 1999), and also calcium and phosphorus utilization is influenced (Leach et al., 1990; Watkins and Southern, 1991; Frost et al., 1992; Thilising-Hansen et al., 2002). Zeolites are also effective vehicles and carriers for the slow release of some drugs (Dyer et al., 2000; Hayakawa et al., 2000).

Chemically, clinoptilolite is a sodium aluminosilicate,  $(\text{Na}_4\text{K}_4)(\text{Al}_8\text{Si}_{40}\text{O}_{96}) \times 24 \text{H}_2\text{O}$ , with a high ion-

exchange capacity (Mumpton, 1999). Considerable evidence is available suggesting a beneficial effect of the supplementation of clinoptilolite to the diet on the growth of animals (Vrzgula and Bartko, 1984; Pond et al., 1988), and in protecting against ammonia toxicity by binding ammonia *in vivo* (Nakaue and Koelliker, 1981; Shurson et al., 1984). Clinoptilolite has also the ability to adsorb some mycotoxins, especially aflatoxins, and thereby prevents or reduces their adverse effects on metabolic functions in animals (Harvey et al., 1993; Keçeci et al., 1998).

The present study was undertaken to determine the effects of clinoptilolite from southern Serbia in pigs observing the effects on growth performance and on various blood serum biochemical components and to investigate if the addition of clinoptilolite to pig diets at a level of 5 g/kg would negatively affect growth performance and biochemical homeostasis of animals.

Table 1. Ingredients and chemical composition of basal diets

Live weight range	12–35 kg	35–60 kg	60–100 kg
Maize (g/kg)	625	590	560
Barley (g/kg)	150	200	250
Soybean meal (g/kg)	200	180	160
Sodium chloride (g/kg)	5	5	5
Dicalcium phosphate (g/kg)	5	10	10
Calcium carbonate (g/kg)	5	5	5
Premix <sup>a</sup> (g/kg)	10	10	10
Crude protein (CP) (g/kg DM)	191.1	162.4	143.7
Ether extract (g/kg DM)	74.1	67.2	65.3
Crude fibre (g/kg DM)	48.2	59.0	65.2
Ash (g/kg DM)	68.5	68.3	74.0
Calcium (g/kg DM)	7.7	7.1	5.5
Total phosphorus (g/kg DM)	6.4	5.8	5.9
Sodium (g/kg DM)	2.1	2.2	1.7
Lysine (g/kg DM)	11.9	9.2	6.8
Methionine and cysteine (g/kg DM)	7.6	5.1	5.3
Metabolizable energy (MJ/kg)	13.8	13.5	13.2

<sup>a</sup>Commercial vitamin and trace mineral premixes with adequate amounts of active substances for each phase of growth

## MATERIAL AND METHODS

Thirty crossbred (Landrace × Yorkshire) pigs of both sexes with average initial BW of 14.5 kg and average age of 55 days were assigned randomly to one of the treatments. Pigs were allotted randomly to each treatment with weight, sex, and ancestry equalized among treatments. Each group contained seven gilts and six castrated males. The animals were housed in individual pens on a fully slatted concrete floor in a commercial grower house where an automatically controlled natural ventilation and heating system were in operation. Pigs were fed *ad libitum* offering a three-phase program with transition from starter to grower when the average BW was 35.0 kg, and from grower to finisher when the mean BW was 70.0 kg. Feed composition and analysis of basal diets are shown in Table 1. All pigs had free access to water from a nipple adjacent to the feeder. One group was given a control diet without clinoptilolite (control group) and the other an experimental diet with 5 g clinoptilolite per kg of feed (Cp group). The food was tested for possible residuals of lead, cadmium, mercury, mycotoxins, antibiotics, coccidiostats and pathogenic microorganisms. These tests were found negative. The clinoptilolite ore used in this experiment was

of high purity (86% clinoptilolite and 14% mordenite, montmorillonite and other silica minerals) and mined in Southern Serbia. The chemical composition of clinoptilolite ore is presented in Table 2.

Individual live weights were recorded on days 45, 90 and 135 (prior to slaughter) of the experiment and feed consumptions were recorded on the weekly basis. Average daily body weight gains were determined at the end of each 45 days and for all 135 days of the experiment.

Blood was drawn from the anterior vena cava for each pig at day 135 for the determination of serum

Table 2. Chemical composition of clinoptilolite ore

	Ore (g/kg)
SiO <sub>2</sub>	525.1
Al <sub>2</sub> O <sub>3</sub>	101.3
Fe <sub>2</sub> O <sub>3</sub>	24.4
Na <sub>2</sub> O	9.6
K <sub>2</sub> O	31.1
MgO	11.8
CaO	144.2
MnO	0.7
TiO <sub>2</sub>	1.8
H <sub>2</sub> O (800°C)	150.0

Table 3. Effects of dietary supplementation of 5 g/kg clinoptilolite on body weight change and feed conversion ratio in pigs

Parameter	Experimental group	1–45 days	45–90 days	90–135 days	Overall period
BWG (g)	control	0.581	0.741	0.864	0.728
	Cp	0.596	0.793	0.823	0.737
Difference from control (%)		+2.5	+7.0	–4.8	+1.2
Feed conversion ratio (kg:kg)	control	1.92	2.91	2.94	2.59
	Cp	1.88	2.87	2.98	2.58
Difference from control (%)		–2.1	–1.4	+1.4	–0.4

The data are mean values of 15 animals in each group ( $n = 15$ )

BWG = body weight gain

biochemical parameters. Protein content was determined according to the method of Bradford (1976), spectrophotometer determinations (Jenway 6 505 UV/Vis., UK) were compared with bovine serum albumin as a protein standard. Serum concentrations of creatinine, urea nitrogen, glucose, cholesterol, triglycerides, inorganic phosphorus, calcium,

magnesium, iron and chlorides were determined, along with the activities of  $\gamma$ -glutamyl transferase (GGT), alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT) and  $\alpha$ -amylase (AMY) on a clinical chemistry analyzer (Microlab 200, Merck) according to the manufacturer's recommended procedure. Sodium

Table 4. Effects of dietary supplementation of 5 g/kg clinoptilolite on blood serum biochemical parameters of pigs

Experimental group	Control	Cp
Glucose (mmol/l)	4.01 $\pm$ 0.13	3.91 $\pm$ 0.21
Urea nitrogen (mmol/l)	4.78 $\pm$ 0.37	4.57 $\pm$ 0.38
Creatinine ( $\mu$ mol/l)	152.70 $\pm$ 4.39	145.22 $\pm$ 2.66
Total protein (g/l)	78.49 $\pm$ 1.51	77.95 $\pm$ 1.87
Total cholesterol (mmol/l)	2.30 <sup>a</sup> $\pm$ 0.07	1.90 <sup>b</sup> $\pm$ 0.10
Triglycerides (mmol/l)	0.26 <sup>a</sup> $\pm$ 0.02	0.33 <sup>b</sup> $\pm$ 0.02
AST (U/l)	85.58 <sup>a</sup> $\pm$ 3.83	98.02 <sup>b</sup> $\pm$ 3.71
ALT (U/l)	56.35 $\pm$ 2.15	52.64 $\pm$ 2.91
ALP (U/l)	304.54 $\pm$ 11.76	287.13 $\pm$ 13.41
AMY (U/l)	1767.08 $\pm$ 88.46	1656.27 $\pm$ 120.00
GGT (U/l)	30.95 $\pm$ 3.03	32.00 $\pm$ 2.67
Sodium (mmol/l)	148.38 $\pm$ 1.25	148.41 $\pm$ 1.46
Potassium (mmol/l)	5.03 $\pm$ 0.13	4.83 $\pm$ 0.10
Magnesium (mmol/l)	1.25 $\pm$ 0.09	1.33 $\pm$ 0.10
Calcium (mmol/l)	2.54 $\pm$ 0.04	2.52 $\pm$ 0.03
Inorganic phosphorus (mmol/l)	2.88 $\pm$ 0.08	2.89 $\pm$ 0.09
Chloride (mmol/l)	102.23 $\pm$ 0.80	101.25 $\pm$ 0.60
Iron ( $\mu$ mol/l)	25.28 $\pm$ 1.63	22.79 $\pm$ 1.77

The data are mean values  $\pm$  standard error of 15 animals in each group ( $n = 15$ )

<sup>a,b</sup>the values without the same superscripts differ significantly ( $P < 0.05$ )

AST = aspartate amino transferase; ALT = alanine amino transferase; ALP = alkaline phosphatase; AMY = amylase;

GGT = gamma glutamyl transferase

and potassium were determined by flame atomic absorption spectrophotometry (SpectraAA-10, Varian, Australia). Inorganic phosphorus, calcium, magnesium, iron and chlorides were measured with commercially available kits (Randox Laboratories, Ltd., U.K.).

The values obtained for studied indicators were processed using the general linear model procedure of the Statistica for Windows (data analysis software system), Version 6.0. (StatSoft, 2001) and Microsoft Excel Programme. All results were expressed as means and differences in respect to the control group in % – for growth performance results, and mean  $\pm$  standard error (SE) for serum biochemical parameters. Statistical analysis was performed by the analysis of variance (ANOVA), while posthoc comparisons were made using Duncan's multiple range test. Statistical significance was assigned at the level  $P < 0.05$  (Hinkle et al., 1994).

## RESULTS

Daily body weight gain (BWG) and feed conversion ratio (FCR) of the experimental pigs are shown in Table 3. During the experimental period, all pigs fed diets supplemented with Cp were as healthy as those fed the control diet. All the animals were growing normally. The feeding of 5 g Cp/kg diet did not appear to influence the appetite of the pigs. The trend of a higher BWG was observed for the Cp group compared with the control group for days 1–90 (+2.5% and +7.0%). On days 90–135 BWC were significantly lower in the group fed the diet supplemented with Cp (–4.8%). However, total BWC were similar in both experimental groups (+1.2%). FCR was not influenced by the dietary treatment.

Blood serum biochemical parameters of all experimental pigs were generally within a normal range (Table 4). The supplementation of Cp had no significant influence ( $P > 0.05$ ) on the blood serum concentrations of total protein, glucose, creatinine and urea nitrogen. Serum concentrations of triglycerides were significantly higher ( $P < 0.05$ ) in the blood samples from pigs fed diets supplemented with Cp. The pigs fed Cp had significantly lower total cholesterol than those from the control group.

Cp supplementation increased the activities of AST in the blood serum. Cp supplementation to the diets exerted no effect on the blood serum con-

centrations of sodium, potassium, magnesium, calcium, iron, chlorides and inorganic phosphorus.

## DISCUSSION

Different experiments with pigs fed clinoptilolite in their diets have given variable results concerning growth rate, daily weight gain and feed efficiency concerns. Vrzgula and Bartko (1984) and Pond et al. (1988) found that weight gain improved when clinoptilolite was added to the feed. Contrary to these results, some other authors (Shurson et al., 1984; Ward et al., 1991; Poulsen and Oksbjerg, 1995) found that body weight gain was unaffected by clinoptilolite. In the present study the oral administration of clinoptilolite was found to have positive effects on growth performance during the growing phase of the pigs. However, in the finishing phase clinoptilolite decreased weight gain. The feed conversion ratio was unaffected by dietary supplementation of Cp and these results are in accordance with those of Pond et al. (1988) and Ward et al. (1991). The magnesium silicate sepiolite could prolong the retention time of digesta in the gut of pigs and poultry (Ouhida et al., 2000). Longer retention times, slower passage of ingesta through the gastrointestinal tract and increases in the microbial activity in the small intestine could lead to the better utilization of nutrients and affect weight gain.

The concentration, purity and type of supplemented clinoptilolite to the diet as well as the growth phase of animals could be important factors affecting clinoptilolite performance in animals. Environmental conditions and animal species may also interfere with these effects.

The addition of dietary Cp to basal diets decreased the serum concentration of total cholesterol and increased the concentration of triglycerides. These results are not in agreement with the analysis of Harvey et al. (1993) in chickens. In their experiments the concentration of total serum cholesterol increased (though not significantly) and the concentration of triglycerides was unaffected by the dietary supplementation of zeolitic ores. The mechanism of this effect is not clear. It is assumed however that longer retention times in the gastrointestinal tract and increases in the microbial activity in the small intestine could promote the deconjugation of bile acids (Ouhida et al., 2000). One possible explanation is that clinoptilolite adsorbs bile acid

salts on its surface in the digestive tract, decreasing the level of total cholesterol in the blood serum, thereby influencing the need for their synthesis *de novo*. Thus, the lowering of serum cholesterol could be a result of its increased utilization for the synthesis of bile acid salts. The type, concentration, and purity of used clinoptilolite, and animal species could interfere with this effect.

The serum AST activity was significantly higher in pigs fed clinoptilolite but remained in the normal range for pigs. This could be an indication of tissue damage if it was accompanied by the increased activity of other serum enzymes, especially ALT. However, the activities of all other measured enzymes were within the normal range for pigs and similar to the control group. This is in agreement with the results of other authors (Vrzgula and Bartko, 1984; Harvey et al., 1993). Although zeolites could induce alterations in mineral absorption and electrolyte balance (Watkins and Southern, 1991), clinoptilolite used in this study did not negatively affect the balance of serum electrolytes, which is in agreement with results of Vrzgula and Bartko (1984).

It can be concluded from our experiment that the dietary addition of 5 g/kg of clinoptilolite has beneficial effects on pigs' performance in growth phases but exerts a decreasing effect in the finishing phase. A decrease in the total serum cholesterol concentration and the effect on the serum lipid status are of great interest and the mechanism of clinoptilolite action should be further studied. It is important to note, however, that the dietary addition of clinoptilolite has no adverse effects on other serum biochemical parameters and does not affect the normal physiological homeostasis of animals.

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