Available online at www.sciencedirect.com
SCIENCE DIRECT®
Research in Veterinary Science 76 (2004) 19–29
RVS
www.elsevier.com/locate/rvsc

A field study on the effect of the dietary use of a clinoptilolite-rich tuff, alone or in combination with certain antimicrobials, on the health status and performance of weaned, growing and finishing pigs

D.S. Papaioannou [a], C.S. Kyriakis [a], C. Alexopoulos [b], E.D. Tzika [a], Z.S. Polizopoulou [c], S.C. Kyriakis [a,*]

[a] *Clinic of Productive Animal Medicine, School of Veterinary Medicine, Aristotle University of Thessaloniki, 540 06 Thessaloniki, Macedonia, Greece*
[b] *Clinic of Obstetrics and AI, School of Veterinary Medicine, Aristotle University of Thessaloniki, 540 06 Thessaloniki, Macedonia, Greece*
[c] *Laboratory of Clinical Diagnosis and Clinical Pathology, School of Veterinary Medicine, Aristotle University of Thessaloniki, 540 06 Thessaloniki, Macedonia, Greece*

Accepted 20 August 2003

Abstract

This study was conducted to assess the effect of dietary use of a clinoptilolite-rich tuff (Cp) on health status and performance of weaned, growing and finishing pigs and its compatibility during simultaneous oral administration of antimicrobials (AM) such as enrofloxacin (E) or salinomycin (S). Weaners (720) were assigned in 2 experimental groups and 4 subgroups based on the inclusion or not of Cp and AM in their feed (subgroups: NC, ES, Cp, Cp + ES) in order to evaluate their health status, under PWDS prevailing herd conditions. A second part of the trial aimed to the evaluation of piglet performance under conditions with minimized PWDS herd risks. For this purpose, a second set of 264 weaners were assigned in 2 groups and 4 subgroups, in a respective manner. All piglets remained on-trial until slaughtering age; Cp was incorporated in their feed at a rate of 2% from the day of weaning until slaughtering. The health status evaluation consisted in monitoring piglets for adverse effects related to Cp consumption, average daily diarrhoea scoring during weaning and mortality rate calculations throughout. Performance evaluation included individual weighing at the end of weaning, growing and fattening periods and feed consumption assessments. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ration (FCR) on a pen basis were further calculated. Cp ingestion was well tolerated by the piglets. Simultaneous administration of Cp and AM in feed, resulted in less severe forms of PWDS, which had a shorter clinical course ($P < 0.05$). Mortality decreased ($P < 0.05$) during the weaning period due to AM administration. Concerning mean pig body weight at the end of each production phase, both Cp and AM had favorable effects ($P < 0.05$). ADG estimated for the whole observation period was improved ($P < 0.05$) by Cp-use along with AM. FCR improvements ($P < 0.05$) were noticed during the different stages of growth due to AM or Cp administration, while Cp/AM interaction was noticed only at weaning ($P < 0.05$).

Keywords: Zeolite; Pig; Health; Performance

1. Introduction

Natural zeolites are minerals formed at low temperature. They possess a three-dimensional network of SiO_4 and AlO_4 tetrahedra, with each oxygen ion shared by adjacent tetrahedra. Zeolites are further characterized by the presence of void spaces in the form of channels

* Corresponding author. Present address: P.O. Box 21559, Panorama, 552 10 Thessaloniki, Macedonia, Greece. Tel.: +30-2310-994507; fax: +30-2310-994550.
E-mail address: kyriakis@vet.auth.gr (S.C. Kyriakis).

0034-5288/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2003.08.006

and cavities within their structures. Electrical neutrality is achieved by the presence of alkali or alkaline earth cations in the porous space (Mumpton and Fishman, 1977). Their physical and chemical properties to act as ion exchangers, catalysts and adsorbents have led to a wide range of industrial and agricultural applications (Mumpton, 1999). Clinoptilolite is probably the most common zeolite in volcanic sediments (Sheppard, 1984; Bernal and Lopez-Real, 1993).

Dietary use of zeolites has been shown to improve weight gain rates in growing lambs (Pond et al., 1984), fattening pigs (Pond et al., 1988; Coffey and Pilkington, 1989; Yannakopoulos et al., 2000) and broilers (Fethiere et al., 1994), to enhance the reproductive performance of sows (Cheshmedzhiev et al., 1985; Papaioannou et al., 2002) and to lead to a better feed efficiency and egg productivity in laying hens (Elliot and Edwards, 1991; Olver, 1997). However, some researchers have not observed any response relating to the performance of growing and fattening pigs (Pearson et al., 1985), while a reduction in the daily weight gain of growing pigs fed zeolite has also been reported (Poulsen and Oksbjerg, 1995). These contradictory results could be attributed to factors such as the species, the chemical and structural properties of the zeolite used, its purity and physicochemical properties, as well as the supplemental level used in the diets (Mumpton and Fishman, 1977; Pond and Yen, 1982; Pond et al., 1988). Furthermore, the dietary conditions under which consistent positive responses to zeolite administration are expected, should also be considered (Poulsen and Oksbjerg, 1995), along with the health status of the treated animals (Pearson et al., 1985).

Under field conditions, in-feed strategic medication programmes remain a common policy for the control of persistent infectious diseases (Deen et al., 2001), along with the use of antimicrobial growth promoters for performance enhancement. However, when administered simultaneously via feed, the potential interaction of antimicrobials and zeolites as regards the efficacy of the formers, should always be considered. Due to zeolite's non-specific adsorption property and ion-exchange capacity, a potential interaction of zeolitic particles with antimicrobial feed additives cannot be precluded, even if the cross sectional diameter of an antimicrobial molecule is incompatible with the entry channels of the zeolitic structure – in this case being unable to pass through and be adsorbed on surface of the zeolite particle. Interaction with the external surface of the zeolitic particles is also possible (Lam et al., 1998, 2001). Indisputably, if large quantities of antimicrobial molecules are rendered unavailable to the animals, this could have an undesired effect on both health status preservation and performance enhancement.

The objective of the present study was to assess the effect of a long-term feeding scheme using a clinoptilolite-rich tuff (henceforth referred to as Cp) at the inclusion rate of 2%, on the health status and performance of weaned, growing and finishing pigs of a conventional farrow-to-finish pig farm with a disease history that justified the application of strategic medication programmes.

2. Materials and methods

2.1. The trial farm: disease history

The investigation was carried out on a farrow-to-finish pig unit with a capacity of 450 sows and an annual production of around 8500 fatteners. The programme for disease monitoring in the trial farm was supervised by the Clinic of Productive Animal Medicine of the Aristotle University of Thessaloniki and included serological examinations (performed twice yearly), slaughterhouse checks (performed on a routine basis) and appropriate laboratory tests (i.e., microbiological and histopathological examinations) whenever outbreaks of severe clinical conditions occurred.

The trial farm was infected with porcine reproductive and respiratory syndrome virus since 1993 with sporadic outbreaks of the acute phase of the disease (last one reported in 1996) in both breeding animals and growing/finishing pigs. Enzootic pneumonia was also present, demonstrated by slaughterhouse checks (mycoplasma-like lesions expanding over a 2–15% of the lung surface of the slaughter pigs). Furthermore, the farm had a relatively high incidence of post-weaning diarrhoea syndrome (PWDS), caused mainly by enterotoxigenic *Escherichia coli* strains, but was free of transmissible gastroenteritis and porcine epidemic diarrhoea.

2.2. Experimental materials

The natural zeolite used in the present study was Cp mined from the Pentalofos Paleogene zeolite-rich tuffs of Evros County (Thrace), northeastern Greece. The Cp rock was won and prepared by the company Silver and Baryte Ores Mining Co. (Athens, Greece). Granullometry of Cp was adjusted by crushing and screening the raw material to a size of <1 mm (99% of the particles <0.7 mm). Recently, it has been registered in accordance with Directive 70/524/EEC as additive, No 3, in feedingstuffs intended for pigs, rabbits and poultry (at the maximum inclusion rate of 2%), under the conditions laid down in Annex II to this Regulation (Commission Regulation No. 1245/1999). One batch of this product was used and, according to the supplier, the material's NH_4^+ cation exchange capacity (determined by the

Table 1
Mineralogical composition of the Cp used in the study and chemical composition of the clinoptilolite

Mineralogical composition (*wt%*)[a]							
Clinoptilolite		Feldspar		Micas and clays		Quartz	
77.3 ± 13.3		12.8 ± 6.6		7.7 ± 5.9		2.2 ± 3.9	
Oxides (wt%)[a]							
SiO_2	67.13 ± 1.24	Fe_2O_3		0.08 ± 0.08		CaO	4.34 ± 0.40
TiO_2	0.03 ± 0.04	MnO		0.03 ± 0.04		Na_2O	0.26 ± 0.17
Al_2O_3	12.30 ± 0.30	MgO		1.05 ± 0.2		K_2O	0.94 ± 0.71
Trace elements (ppm)[b]							
Ag	2.6	Cs	4.7	Ni	13	Sr	1400
Ba	260	Cu	7.3	Nb	18	Ta	19
Bi	5.9	Ga	16	Pb	62	V	22
Co	<20	Ge	2.9	Rb	110	W	25
Cr	10	La	32	Sn	3.6	Y	21
Ce	52					Zn	41

[a] Data obtained by Professors Yannakopoulos A. and Kassoli-Fournaraki A. (EU funded research under Brite-Euram Project No. BRE2-CT94-0954: ''Development of industrial and environmental uses of European natural zeolites'') and listed in the material's registration file by Silver and Baryte Ores Mining Co. Mineralogical composition was determined by means of X-ray powder diffraction analysis using a Philips diffractometer, Ni-filtered Cu Kα radiation. Major oxides were determined from polished thin sections using a Jeol JSM-840 scanning electron microscope equipped with a LINK-An 10000 microanalyzer.

[b] Data obtained by Silver and Baryte Ores Mining Co. Trace element content of a representative sample of the material used in the studies, determined by means of atomic absorption spectroscopy.

ammonium acetate method) was 1.50 mEq/g, while its mineralogical and chemical composition is shown in Table 1.

The in-feed antimicrobials (AM) registered in Greece and in the EU and used in the study were enrofloxacin (Baytril premix®, Bayer) and the performance enhancer salinomycin (Salocin premix®, Hoechst).

2.3. Animals and treatments

In the first part of the trial (health status evaluation study), 720 clinically healthy piglets participated (first set of piglets) and were allocated according to their sex in two experimental groups and four subgroups, depending on the inclusion or not of Cp and AM in their feed, as follows:

1. *Cp-group* (360 piglets; 180 females and 180 males): basic on-farm mixed feed;
   - Negative control (NC) subgroup (240 piglets; 120 females and 120 males): feed without AM;
   - ES subgroup (120 piglets; 60 females and 60 males): feed supplemented with AM.
2. *Cp+ group* (360 piglets; 180 females and 180 males): basic on-farm mixed feed containing Cp at the inclusion rate of 2%;
   - Cp subgroup (240 piglets; 120 females and 120 males): feed without AM;
   - Cp + ES subgroup (120 piglets; 60 females and 60 males): feed supplemented with AM.

In the second part of the trial (performance evaluation study), 264 clinically healthy piglets of good physical condition (second set of piglets), having a minimum body weight of 5 kg, were ear-tagged and distributed, according to sex, in two experimental groups and four subgroups, in the same way as it was previously described: 88 piglets (44 females and 44 males) in each one of the NC and Cp subgroups and 44 piglets (22 males and 22 females) in each one of the ES and Cp + ES subgroups.

All piglets were of the same genetic background [(Large White × Landrace) × Belgian Landrace] and had received creep feed free of any antimicrobial or performance enhancer during lactation.

2.4. Management and hygiene of the experimental animals

During the first part of the trial and on a weekly basis, 120 weaned piglets were moved from the farrowing house to the flat-deck unit, where they were randomly allocated to 6 pens (2.5 m × 3 m), each one comprising of 10 female and 10 male piglets. In this way, the first set of piglets was obtained in 6 consecutive weeks. A Restricted randomization method was used, for the assignment of the 6 pens to the treatments/experimental subgroups, each week. Thus, on a weekly basis, a complete randomized design was used with a complete set of subgroups; four subgroups and two pens/replicates per Cp and NC subgroups and one pen/replicate per Cp + ES and ES subgroups, respectively (considering the whole 6-week period of allocation, there were 12 pens/replicates per Cp and NC subgroups and 6 pens/replicates per Cp + ES and ES subgroups). In order to elicit overt disease in piglets, no care was taken to minimize the stress factors associated with PWDS (i.e., no cleaning, no disinfection, ad libitum consumption of a starter feed which in the case of NC and Cp subgroups was free of any AM).

Accordingly, the second set of piglets (second part of the trial) were obtained during the following 4 consecutive weeks. On a weekly basis, 66 weaned piglets were moved from the farrowing house to the flat-deck unit, where they were allocated to 6 pens, each one comprising of 11 female or male piglets The assignment of the pens to the treatments/experimental subgroups was carried out similarly as in the first part of the trial (considering the whole 4-week period of allocation, there were 8 pens/replicates per Cp and NC subgroups and 4 pens/replicates per Cp + ES and ES subgroups). Prior to the allocation, the designated pens were thoroughly cleaned and disinfected. The body weight of the piglets at weaning averaged 6.16 kg and was not significantly different ($P > 0.05$) among the experimental groups, as well as among sex and pen distributions.

The average age for all piglets was 25(±3) days at weaning. On 71(±3) days of age the participating animals were moved from the flat-deck unit to the fattening accommodations, where they remained during the growing period [71(±3)–112(±3) days of age] and the fattening one [113(±3)–161(±3) days of age], using the same pen design, as it was initially set. Prior to the allocation, the designated pens (3 m × 4 m) were thoroughly cleaned and disinfected.

Following the standard management policy of the farm, all piglets were intramuscularly injected with 200 mg iron dextran on the 2nd day of age. Accordingly, and based on the previous disease history of the farm, all pigs were fully vaccinated against *Mycoplasma hyopneumoniae* (on the 7th day of age and 15 days later), Aujeszky's disease and swine influenza [on 90(±3) days of age and a booster shot on 120(±3) days of age]. Additionally, in order to minimize the stress factors associated with PWDS, all the piglets participating in the second part of the study were individually treated at weaning with amperozide (Hogpax®, Bayer; 1 mg kg^{-1} body weight, intramuscularly, SID), a psychotropic drug with potent anxiolytic properties (Kyriakis, 1990).

Considering the 10-week interval for the introduction of all participating animals in the flat-deck unit and the monitoring period for each pig (from weaning age to slaughter age, i.e., 136 days), the trial lasted over a total period of 8 months (July 1998 to January 1999).

Management of the animals and data recording during the study were carried out under the Good Clinical Practice for the Conduct of Clinical Trials for Veterinary Medicinal Products (GCPV) guidelines (EMEA/CVMP/595/98-CONSULTATION, 1998).

2.5. Feeding of the animals

Feed and water were offered ad libitum throughout the study period. The feeds were typical maize–soybean weaning (starter and follow-on), growing and fattening rations, depending on the age of the pigs. The formulation of the basal diets used is given in Table 2. A proximate analysis and an energy determination of adequate basal feed samples was conducted in order to confirm feed composition throughout the study. The basic specifications are also shown in Table 2. The diets were also fortified with vitamins and minerals to meet, or exceed NRC (1998) standards.

The pigs of Cp+ group consumed the basal diets in which Cp replaced an equal quantity of barley. The antimicrobials were incorporated in the feeds of ES and Cp + ES subgroups also replacing an equal quantity of barley, as follows:

- weaners' (starter) feed: 50 mg kg^{-1} enrofloxacin;
- weaners' (follow-on) and growers' feed: 60 mg kg^{-1} salinomycin;
- fattener feed: 30 mg kg^{-1} salinomycin.

In order to assess the ability of the farm's feed mill to mix the necessary ingredients efficiently, samples of each type of final feed (with and without Cp) were collected and forwarded to the laboratory of Veterin S.A. (Aspropyrgos, Athens, Greece) for the determination of Cp inclusion rate. Furthermore, enrofloxacin and salinomycin feed assays were performed in the Laboratory of Aspland and James Ltd (Cambridgeshire, UK). The results were within the analytical limits of the laboratory method used, verifying the efficient feed mixing procedure.

2.6. Health status evaluation

The experimental animals (first set of piglets) were daily observed for signs of disease throughout the monitoring period and any disease problems were recorded. Special attention was given to possible adverse effects noticed in the Cp+ group due to the consumption of Cp. The following parameters were considered: (a) the average daily diarrhoea score during the weaning period (ADDS) and (b) mortality rate throughout the monitoring period.

For each piglet showing diarrhoea, the severity was assessed visually and characterized according to the following scale: 0, normal faeces; 1, pasty; 2, liquid; 3, with mucus; and 4, with blood (Kyriakis et al., 1999). The assessor was an experienced person, who was unaware of the treatment each pen was assigned to. Daily diarrhoea score on a pen basis (DDS) was calculated by multiplying each diarrhoeic piglet by the previous characterization scale of diarrhoea [thus the maximum theoretical daily diarrhoea score was 80 (20 piglets × scale 4)]. ADDS for each pen was calculated by dividing the sum of DDS by the days of the weaning period (observation period).

For all pigs that died throughout the experimental period, the date of death was recorded and a post-mortem examination was performed.

Table 2
Composition (percentage of feed ingredients) and specifications of the basal diets used in the study

Type of feed	Weaning		Growing	Fattening
	Starter	Follow-on		
Age of pigs (days)	25(±3)–42(±3)	43(±3)–70(±3)	71(±3)–112(±3)	113(±3)–161(±3)
Maize	53.2	54.2	43.5	38.5
Barley	9	10	20.5	22
Soybean meal	16	16	19	19
Fish meal	7.5	7.5	5	2.5
Wheat bran	–	–	6	12
Fat (animal)	–	–	2.5	2.5
Milk, dried[a]	10	8	–	–
Dicalcium phosphate	2	2	1.2	1.2
Limestone ground	1	1	1	1
Iodized salt	0.3	0.4	0.4	0.4
L-Lysine–HCl	0.4	0.3	0.3	0.3
Choline chloride	0.2	0.2	0.2	0.2
Vitamin premix[b]	0.2	0.2	0.2	0.2
Mineral premix[c]	0.2	0.2	0.2	0.2
Total	100	100	100	100
Digestible energy (MJ kg^{-1})	13.48	13.38	13.31	13.11
Crude protein (%)	20.8	20.6	19.2	18.1
Calcium (%)	0.91	0.85	0.82	0.76
Phosphorus (%)	0.75	0.72	0.67	0.62
Lysine (%)	1.48	1.23	1.03	0.85

[a] Supplemented with fat (crude content 20%).
[b] Provided the following per kg of diet: vitamin A 15,000 IU; vitamin D_3 2000 IU; vitamin E 30 mg; vitamin K_3 3 mg; vitamin B_1 2.5 mg; vitamin B_2 4 mg; vitamin B_6 3.5 mg; vitamin B_{12} 30 μg; biotin 30 μg; folic acid 0.8 mg; niacin 24 mg; pantothenic acid 16 mg; vitamin C 20 mg.
[c] Provided the following per kg of diet: Fe 100 mg; Zn 100 mg; Mn 40 mg; Cu 20 mg; Co 0.2 mg; I 0.6 mg; Se 0.2 mg; Mg 25 mg.

2.7. Evaluation of performance parameters

After the initial weighing at weaning, the experimental animals (second set of piglets) were further weighed on the 70(±3), 112(±3) and 161(±3) days of age (end of weaning, growing and fattening periods, respectively). Feed consumption per pen was recorded over the same periods. Feed wastage was considered minimal, thus feed disappearance was considered to be a reliable estimate of feed consumption. The average daily gain (ADG) and average daily feed intake (ADFI) were calculated by dividing total pen weight gain and total pen feed consumption by the number of alive-animal days. ADG of dead pigs in the pen, as well as the theoretical feed consumption of each pig until the day of death (from the start of each stage of growth and considering that feed intake was even among pen-mates) were excluded from the calculations. The feed conversion ratio (FCR) per pen was determined as ADFI ADG^{-1}.

2.8. Statistical analysis

Each pen of piglets constituted the experimental unit. Values for all parameters under study were recorded for each experimental unit and statistical analysis was performed by the use of the general linear model procedure of (SAS/STAT®, 2000). In the analysis of variances the variables tested were analyzed as a 2×2 arrangement with Cp, AM and their interaction as factors of the model (Petrie and Watson, 1999). Furthermore, Duncan's multiple range test was used to compare the estimated means of the treatment subgroups. The significance was attained if the P-value was <0.05.

3. Results

3.1. Health status evaluation

Cp was well tolerated by the animals since the consumption of the supplemented feed did not provoke any apparent adverse or side effect on them throughout the two parts of the study.

Pigs which participated in the first part of the study begun scouring 5–8 days after weaning with scouring peaking 4–7 days afterwards and diarrhoea scores returning to normal at 40–45 days of age. It is worth noting that the exacerbation of clinical signs in NC-pigs coincided with the transition from starter to follow-on feed [43(±3) days of age] but scouring piglets were observed transiently in three pens only. After the 50(±3) days of age no scouring piglet was recorded. The actual pattern of the mean DDS of the different treatment

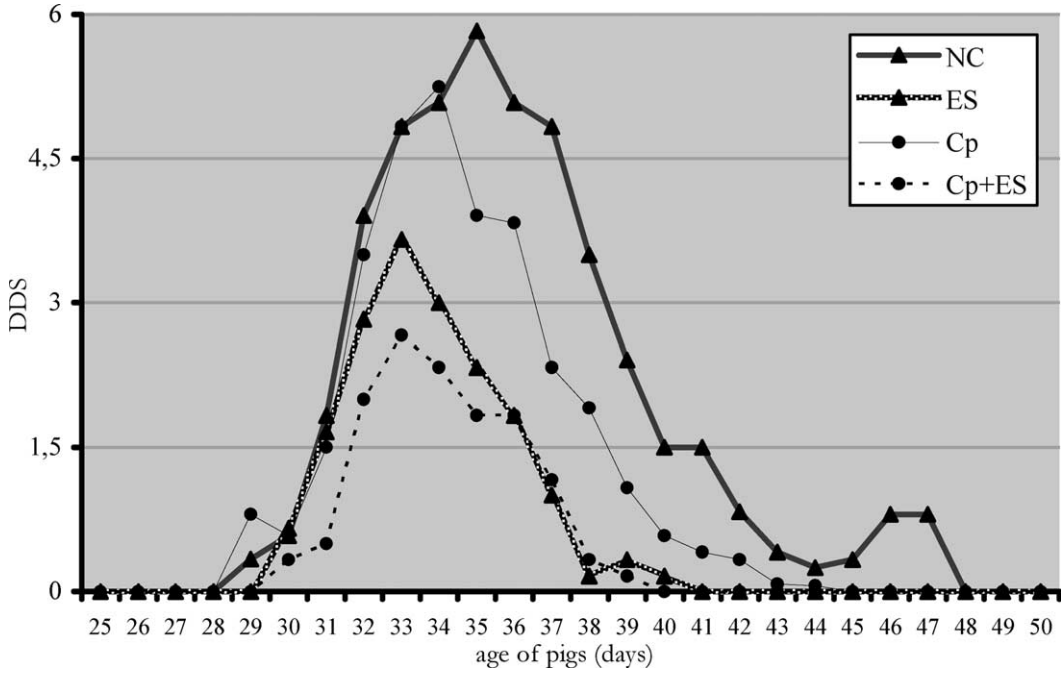

Fig. 1. Pattern of mean daily diarrhoea score (DDS) of the experimental subgroups from weaning to 50(±3) days of age (estimated according to the method described in Section 2).

subgroups until 50(±3) days of age is depicted in Fig. 1. It is clearly demonstrated that the severity and the duration of the clinical signs were reduced in Cp + Es and ES subgroups compared to the NC one. The reducing effect of Cp and AM (mainly enrofloxacin) on ADDS during the weaning period (Table 3) was significant ($P < 0.05$). Furthermore, no statistical Cp/AM interaction was noticed ($P > 0.05$). Adversely, Cp + ES subgroup, as well as ES subgroup, demonstrated a significantly lower ADDS compared to Cp or NC subgroups ($P < 0.05$). The difference among Cp and NC subgroups was also significant ($P < 0.05$).

The prevalent clinical signs observed in sick animals were yellow-greyish or watery diarrhoeic faeces, dehydration in severe cases and perineal staining. Mucus was rarely present, while blood was recorded in 8 piglets (probably cases of complicated origin). In general, piglets of Cp + ES and ES subgroups did not present serious and typical clinical signs of PWDS.

Table 4 presents the mortality rate throughout the first part of the study. During the weaning period, piglets mortality was significantly reduced in the subgroups where AM were used ($P < 0.05$). Similarly, it was slightly reduced by the dietary use of Cp. In the majority of the cases, the gross lesions revealed by a post-mortem examination, were typical of PWDS. The carcasses of the dead piglets were dehydrated, the stomach was often filled with feed and the gastric mucosa was congested. The small intestine was full with watery contents and the colon and rectum contained fluid faeces. Five piglets (2 from Cp subgroup, 1 from ES subgroup and 2 from NC subgroup) died due to respiratory infection, while in 7 cases the cause of sudden death was not identified due to the absence of typical lesions or a delayed necropsy. Although the dietary use of Cp and AM (salinomycin) did not affect significantly the mortality rate during the growing and the fattening period ($P > 0.05$), the overall pig mortality among the experimental subgroups throughout the

Table 3
Effect of Cp and AM[A] on average daily diarrhoea scores (ADDS) of weaners estimated according to the method described in Section 2.

Experimental subgroups					*P*-value		
NC	ES	Cp	Cp + ES				
$n = 12$	$n = 6$	$n = 12$	$n = 6$		Cp effect	AM Effect	Cp–AM interaction
Mean	Mean	Mean	Mean	SD			
0.932[a]	0.383[c]	0.657[b]	0.286[c]	0.296	0.003	>0.001	0.142

[a,b,c] Means in the same row with different superscripts differ significantly ($P < 0.05$).
[A] AM, antimicrobials (mainly enrofloxacin).

Table 4
Ratio of dead/total number of animals of each experimental subgroup and the effect of Cp and AM[A] on mean mortality rates (%), at different stages of growth

Age of pigs (days)	Ratio of dead/total number of animals				Mean mortality rates						
	Experimental subgroups				Experimental subgroups						
	NC	ES	Cp	Cp + ES	NC	ES	Cp	Cp + ES		*P*-value	
					$n = 8$	$n = 4$	$n = 8$	$n = 4$			
					Mean	Mean	Mean	Mean	SD	Cp effect	AM effect	Cp–AM interaction
25(±3)–70(±3)	29/240	5/120	20/40	4/120	12.08[a]	4.16[b,c]	8.33[a,b]	3.33[c]	5.38	0.136[B]	<0.001[B]	0.660[B]
71(±3)–161(±3)	7/211	2/115	6/220	2/116	3.32	1.75	2.73	1.75	2.80	0.769	0.214	0.769
25(±3)–161(±3)	36/240	7/120	26/240	6/120	15.00[a]	5.83[b,c]	10.83[a,b]	5.00[c]	6.36	0.183	<0.001	0.371

[a,b,c] Mean mortality rates in the same row with different superscripts differ significantly ($P < 0.05$).
[A] AM, antimicrobials (enrofloxacin and salinomycin).
[B] Effects on mean mortality rates were obtained after square root values.

observation period remained as in the case of the weaning period. Respiratory infection and volvulus of heavy fatteners' intestine were the prevalent causes of death during the growing and the fattening periods.

3.2. Performance evaluation

During the second part of the study no serious or typical clinical signs of PWDS observed and with few exceptions, scouring waned after 2 injections with long acting amoxicillin (15 mg kg^{-1}, intramuscularly, every other day) without affecting piglets' overall performance. Similarly, the mortality rates during the weaning period were minimized (0% in ES subgroup to 4.54% in NC subgroup), while the respective rates throughout the observation period (from weaning to slaughter) ranged from 2.27% in Cp + ES subgroup to 9.09% in NC subgroup. Necropsies revealed the same causes of death as in the first part of the study (first set of piglets). Postmortem findings indicative of gastrointestinal disease were found in 2 weaned piglets. In addition, 2 fatteners (one in each of the NC and Cp subgroups) were euthanised due to severe arthritic signs.

The results relating to mean pig body weight at the end of the different stages of growth are presented in Table 5. Both Cp and AM (enrofloxacin and salinomycin) had a favorable effect on pig body weight at the end of the weaning, growing and fattening period ($P < 0.05$). In every case, the respective means in ES and Cp + ES subgroups were found to be significantly higher ($P < 0.05$) than the ones obtained in the subgroups in which the animals were not fed with AM-supplemented diets. Furthermore, the differences among Cp and NC subgroups were also significant ($P < 0.05$), with the lower means being recorded in NC subgroup.

Table 6 summarizes the results relating to ADG, ADFI and FCR. ADG was significantly improved by the dietary use of both Cp and AM ($P < 0.05$) during the weaning period. AM treated pigs kept on gaining more weight ($P < 0.05$) during the growing and fattening periods, while a tendency for a higher ADG was recorded in pigs which were on the Cp diet ($P < 0.1$; $P = 0.098$) only during the growing period. However, when estimated for the whole observation period, ADG was found to be improved by the dietary use of both Cp and AM ($P < 0.05$). No statistical Cp/AM interaction was noticed.

Cp administration had no significant effect on ADFI ($P > 0.05$). Conversely, the use of AM significantly reduced ADFI during the weaning period ($P < 0.05$). AM had also a decreasing effect ($P < 0.05$) on FCR during the different stages of growth, as well as during the whole observation period. Apart from the fattening period, Cp effect on FCR improvement was also significant ($P < 0.05$). Statistical Cp/AM interaction was noticed only during the weaning period ($P = 0.001$).

Table 5
Effect of Cp and AM[A] on pig body weight (mean value per pen) at the end of the different stages of growth

Age of pigs (days)	Experimental subgroups					*P*-value		
	NC	ES	Cp	Cp + ES				
	$n = 8$	$n = 4$	$n = 8$	$n = 4$		Cp effect	AM effect	Cp–AM interaction
	Mean	Mean	Mean	Mean	SD			
25(±3)	6.14	6.15	6.13	6.20	0.12	0.222	0.109	0.157
70(±3)	21.69[c]	24.69[a]	23.98[b]	25.21[a]	1.50	<0.001	<0.001	0.131
112(±3)	48.17[c]	53.72[a]	51.60[b]	54.94[a]	2.90	0.001	<0.001	0.082
161(±3)	88.83[c]	97.53[a]	93.18[b]	99.49[a]	4.61	0.005	<0.001	0.248

[a,b,c] Means in the same row with different superscripts differ significantly ($P < 0.05$).
[A] AM, antimicrobials (enrofloxacin and salinomycin).

Table 6
Summary of growth performance data; effect of Cp and AM[A] on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR)

Age of pigs (days)	Experimental subgroups					*P*-value		
	NC	ES	Cp	Cp + ES				
	$n = 8$	$n = 4$	$n = 8$	$n = 4$		Cp effect	AM effect	Cp–AM interaction
	Mean	Mean	Mean	Mean	SD			
ADG (kg)								
25(±3)–70(±3)	0.338[c]	0.403[a,b]	0.388[b]	0.412[a]	0.032	<0.001	<0.001	0.144
71(±3)–112(±3)	0.630[c]	0.691[a,b]	0.657[b,c]	0.708[a]	0.040	0.098	<0.001	0.718
113(±3)–161(±3)	0.828[b]	0.897[a]	0.847[b]	0.909[a]	0.045	0.292	<0.001	0.804
25(±3)–161(±3)	0.603[c]	0.667[a]	0.635[b]	0.680[a]	0.033	0.045	0.000	0.211
ADFI (kg)								
25(±3)–70(±3)	0.681[a]	0.643[b]	0.681[a]	0.659[a,b]	0.024	0.355	0.020	0.340
71(±3)–112(±3)	1.794	1.760	1.773	1.688	0.108	0.334	0.221	0.600
113(±3)–161(±3)	2.69[a]	2.565[b]	2.640[a,b]	2.607[a,b]	0.096	0.889	0.154	0.241
25(±3)–161(±3)	1.741	1.672	1.716	1.671	0.056	0.563	0.119	0.585
FCR								
25(±3)–70(±3)	2.02[a]	1.59[c]	1.75[b]	1.60[c]	0.192	0.001	<0.001	0.001
71(±3)–112(±3)	2.84[a]	2.55[b,c]	2.69[a,b]	2.38[c]	0.220	0.028	<0.001	0.919
113(±3)–161(±3)	3.25[a]	2.86[b]	3.12[a]	2.87[b]	0.194	0.240	<0.001	0.158
25(±3)–161(±3)	2.88	2.51	2.70	2.45	0.188	0.042	<0.001	0.118

[a,b,c] Means in the same row with different superscripts differ significantly ($P < 0.05$).
[A] AM, antimicrobials (enrofloxacin and salinomycin).

4. Discussion

The favorable effect of the dietary use of zeolitic volcanic tuff on the performance of pigs has been demonstrated by several authors (Pond et al., 1988; Coffey and Pilkington, 1989; Yannakopoulos et al., 2000). According to Pearson et al. (1985), the beneficial effect of in-feed zeolites merely lies on a disease-control effect. In the case of sepiolite, a clay mineral, it has been assumed that the dietary use of this additive improves pig intestinal ''health status'' by favoring the enzymatic activity and by binding noxious substances along the gastrointestinal tract (Parisini et al., 1999).

The evaluation of results demonstrated an obvious improvement in minimizing PWDS clinical signs in naturally infected pigs, when fed with a Cp-enriched diet. The reverse effect of Cp on the severity of diarrhoea generally confirms the results reported in other studies, where the in-feed use of zeolites (Cp, phillipsite) acted as a preventing and/or eliminating factor against the clinical presentation of scours in pigs (Vrzgula and Bartko, 1984; Gunther, 1990; Benatti et al., 1994). The suppression of PWDS effects in the pigs of our study which were on the Cp diet was represented by a reduction of the potency and the duration of the clinical signs, as well as a 28.7% reduction of the mortality rate during the weaning period.

Apart from Cp's retarding effect on the intestinal transit (Mumpton and Fishman, 1977) and its property to act as a water adsorber, thus leading to the appearance of more compact and better shaped faeces (Benatti et al., 1994), another possible explanation for the

amelioration of PWDS could stand to the elimination of various predisposing and/or causative factors, which are associated in the culmination of this problem in an interactive way, especially in the immediate post-weaning period. These factors may include an intestinal hypersensitivity to feed antigens or the weaning-induced malabsorption syndrome due to a reduction in the digestive enzyme activity, which can both predispose to infectious enteritis (Wilson et al., 1989; Kyriakis, 1989). Whether Cp is able to adsorb substances that may result in intestinal hypersensitivity phenomena, such as in the case of phillipsite (Marastoni et al., 1996), or prevent the malabsorption syndrome by maintaining or restoring the digestive enzyme activity in newly weaned piglets, cannot be confirmed by our study, although there is evidence that pancreatic enzymes can be adsorbed over the surfaces of clays, forming complexes whose activity is complementary to that observed with the native enzymes, over a wider range of digestive pH (Cabezas et al., 1991).

The pattern of mean DDS (Fig. 1) and the mortality rate in Cp subgroup suggest that the incorporation of Cp in the feed contributed to a control of some extend, rather than the prevention of PWDS. Recent evidence from in vitro studies suggests that zeolites (i.e. Cp, mordenite), as well as other inorganic adsorbents, can efficiently adsorb and sequestrate the heat-labile (LT) *E. coli* enterotoxin (Ramu et al., 1997). Hence, the prevention of enterotoxin binding to cell-membrane receptors and subsequently of the activation of adenyl-cyclase, should be considered as a possible – or even an additional – explanation for Cp minimizing effect on PWDS.

In the present study, the effect of Cp on health status of pigs was tested in comparison with a medicated feed which has been proved to be efficacious in controlling PWDS. The choice of enrofloxacin at the dose rate of 50 mg/kg of feed was based on its spectrum of antimicrobial activity, along with previous clinical observations (Lein et al., 1996; Kyriakis et al., 1997).

Both ADDS and mortality rate during the weaning period were significantly improved in the experimental subgroups where the pigs were treated with enrofloxacin. Administration of this antimicrobial was proved to be a useful tool in controlling PWDS, demonstrating a more pronounced efficacy than this of Cp. From a clinical point of view, the trial results revealed that Cp is compatible with enrofloxacin, since the effect of the antimicrobial on pigs health status was not inhibited by the simultaneous use of Cp. Additionally, more optimal results were obtained in Cp + ES subgroup, where enrofloxacin was used in combination with Cp, possibly demonstrating an additive net effect or an "enhanced-by-Cp" enrofloxacin efficacy. Although the mechanism of action in the aforementioned hypothesis cannot be clarified, possible "hydrogen bond" implicated interactions among the –COOH and $-CH_3$ groups of the antimicrobial molecule and the oxygen atoms and/or the H^+ions of the external surface of the protonated – in the gastric juice – Cp cannot be precluded. In this case, Cp could act as a support of sustained-release vehicle of enrofloxacin molecules, thus prolonging and improving their activity.

From a clinical point of view, the absence of any evidence of incompatibility among Cp and salinomycin was also confirmed. Salinomycin, a member of the polyether ionophore antibiotic group, was used at the registered dose range of a performance promoter. Its mode of action implicates modification of the ion movement across biological membranes (Barragry, 1994), and thus a possible indirect interactive effect on its efficacy due to Cp cation exchange capacity could stand as a major concern as regards their concurrent use in pigs. In addition, similarly to enrofloxacin, an alteration of salinomycin activity, caused by surface-interactions with Cp particles cannot be precluded.

Both AM used in the study (enrofloxacin, salinomycin) had a positive effect on growth performance data throughout the monitoring period in the second part of the study. During the growing and finishing periods, the effects of salinomycin use confirmed previous studies, in which the growth promoting effect was attributed to the increase of the digestibility and the absorption of nutrients (Lindeman et al., 1985). It has to be mentioned that the applied preventive measures had a favorable effect on PWDS clinical presentation. However, the effect of the sporadic occurrence of PWDS – even if minimal – on the performance traits evaluated during the weaning period, makes any attempt to speculate the enrofloxacin and salinomycin effects on a separate basis, rather dicey. This is further emphasized by the fact that the pigs which were on diets free of AM demonstrated a higher ADFI during the same period. This result might have emerged as a consequence of the ameliorative effects of subclinical disease on piglets' growth at the beginning of the weaning period and of a compensatory (higher) piglets' feed consumption during the post-recovery period.

In the case of Cp, the improvements recorded relating to ADG and FCR during the weaning and the growing periods are, in general, confirmatory to the findings of other researchers. According to them, the growth promoting properties of Cp and other zeolites or clays are attributed to a number of mechanisms, which involve the binding and/or remove of noxious compounds derived through microbial activity (i.e. ammonia and p-cresol), the retardation of digesta passage rate through the intestines and the favoring of feed components hydrolysis through an enzyme activity enhancement (Mumpton and Fishman, 1977; Shurson et al., 1984; Pond et al., 1988; Cabezas et al., 1991; Olver, 1997; Parisini et al., 1999).

Cp effect on pigs' performance declined with advancing age. This is in agreement with the observations

of Coffey and Pilkington (1989). A possible explanation could lie on the gradual improvement of enzyme activity with advancing age (Pond and Houpt, 1978) and the subsequent lowering of Cp effect after the maturation of the proper enzyme systems. Besides, Cp effect is also dependent on the dietary composition, especially as regards the crude protein content (Poulsen and Oksbjerg, 1995). It seems that high-protein diets, such as the one used in the study during the weaning period, make Cp effects more pronounced than lower-protein growers' and fatteners' rations.

Compared to AM effect, Cp effect on the performance traits was lower throughout the monitoring period. However, it is worth noting that no statistical Cp/AM interaction was observed, apart from FCR values during the weaning period. The reason for this cannot be readily clarified. Reasons such as these described previously, might influence the extent to which the main effects of Cp and AM during the weaning period could have been demonstrated, causing their limitation and thus leading to a significant interaction in-between them. However, the in-feed combined use of Cp and AM (enrofloxacin, salinomycin) resulted in a more profound overall effect on the performance parameters taken into consideration in the present study, than did Cp or AM alone, as observed throughout the monitoring period.

The conclusion that could be drawn from the present study is that the dietary use of Cp does not provoke any clinical adverse effect to pigs from weaning to slaughter or any evidence of incompatibility with in-feed AM (enrofloxacin, salinomycin), when used simultaneously with them in strategic medication programmes for health status preservation or performance enhancement. Furthermore, Cp was found to represent an efficacious, complemental supportive mean in antimicrobial medication programmes for the control of PWDS, showing obvious growth promoting potential in young growing pigs, and acting synergistically to the respective AM (enrofloxacin, salinomycin) effect.

References

Barragry, T.B., 1994. Veterinary drug therapy. Lea and Febiger, Malvern, Pensylvania.

Benatti, G., Bergero, D., Ladeto, G., Sarra, C., 1994. Effect of a zeolite containing phillipsite on some digestibility rates in pigs. Zootecnica e Nutrizione Animale 20, 153–158.

Bernal, M.P., Lopez-Real, J.M., 1993. Natural zeolites and sepiolite as ammonium and ammonia adsorbent materials. Bioresource Technology 43, 27–33.

Cabezas, M.J., Salvador, D., Sinisterra, J.V., 1991. Stabilization-activation of pancreatic enzymes adsorbed on to a sepiolite clay. Journal of Chemical Technology and Biotechnology 52, 265–274.

Cheshmedzhiev, B.V., Angelov, A., Nestorov, N., Kr''stev, A., Bakalivanov, S., Cheshmedzhieva, R., 1985. Effect of zeolite in the feeding of pregnant and nursing sows and sucking piglets. Zhivotnov "dni Nauki 22, 40–46 (abstract).

Coffey, M.T., Pilkington, D.W., 1989. Effect of feeding Zeolite-A on the performance and carcass quality of swine. Journal of Animal Science 67 (Supplement 2), 36 (abstract).

Deen, J., Dee, S., Morrison, R.B., Radostits, M., 2001. Health and production management in swine herds. In: Radostits, O.M. (Ed.), Herd Health: Food Animal Production Medicine, third ed. W.B. Saunders company, Philadelphia, Pensylvania, pp. 635–764.

Elliot, M.A., Edwards Jr., H.M, 1991. Comparison of the effects of synthetic and natural zeolite on laying hen and broiler chicken performance. Poultry Science 70, 2115–2130.

EMEA/CVMP/595/98-CONSULTATION, 1998. Guideline on Good Clinical Practices: London, UK.

Fethiere, R., Miles, R.D., Harms, R.H., 1994. The utilization of sodium in sodium zeolite A by broilers. Poultry Science 73, 118–121.

Gunther, K.D., 1990. Zeolite minerals in pig and poultry feeding. Schweinewelt 15, 15–19.

Kyriakis, S.C., 1989. New aspects of the prevention and/or treatment of the major stress induced diseases of the early weaned piglet. Pig News and Information 10, 177–181.

Kyriakis, S.C., 1990. Post weaning diarrhoea syndrome (PWDS) of piglets: prevention with amperozide and supporting therapy (STh) without the addition in the weaner feed of any antibacterial. In: Proceedings of the 11th International Pig Veterinary Society Congress, Lausanne, Switzerland, p. 160.

Kyriakis, S.C., Tsiloyiannis, V.K., Lekkas, S., Petridou, E., Vlemmas, J., Sarris, K., 1997. The efficacy of enrofloxacin in-feed medication, by applying different programmes for the control of post weaning diarrhoea syndrome of piglets. Journal of Veterinary Medicine, series B 44, 513–521.

Kyriakis, S.C., Tsiloyiannis, V.K., Vlemmas, J., Sarris, K., Tsinas, A.C., Lexopoulos, C., Jansegers, L., 1999. The effect of probiotic LSP 122 on the control of post-weaning diarrhoea syndrome of piglets. Research in Veterinary Science 67, 223–228.

Lam, A., Sierra, L.R., Rojas, G., Rivera, A., Rodriguez-fuentes, G., Montero, L.A., 1998. Theoretical study of the physical adsorption of aspirin on natural clinoptilolite. Microporous and Mesoporous Materials 23, 247–252.

Lam, A., Rivera, A., Rodriguez-fuentes, G., 2001. Theoretical study of metronidazole adsorption on clinoptilolite. Microporous and Mesoporous Materials 49, 157–162.

Lein, A., Verdonck, M., De Kruif, A., 1996. The efficacy of enrofloxacin in-feed medication (Baytril® 2.5% medicated premix) on the excretion of haemolytic *E. coli* and postweaning diarrhoea. In: Proceedings of the 14th International Pig Veterinary Society Congress, Bologna, Italy, p. 267.

Lindeman, M.D., Kornegay, E.T., Stahly, T.S., Cromwell, G.L., Easter, R.A., Kerr, B.J., Lucas, D.M., 1985. The efficacy of salinomycin as growth promotant for swine from 9 to 97 kg. Journal of Animal Science 61, 782–788.

Marastoni, G., Belladelli, G., Curti, M., Gozzi, E., Panciroli, G., Spreafico, C., 1996. Use of zeolite (phillipsite) in swine haemorrhagic bowel syndrome (H.B.S.). In: Proceedings of the 14th International Pig Veterinary Society Congress, Bologna, Italy, p. 454.

Mumpton, F.A., Fishman, P.H., 1977. The application of natural zeolites in animal science and aquaculture. Journal of Animal Science 45, 1188–1203.

Mumpton, F.A., 1999. La roca magica: Uses of natural zeolites in agriculture and industry. Proceedings of the National Academy of Sciences of the USA 96, 3463–3470.

NRC, 1998. Nutrient Requirements of Swine. 10th rev. ed. Washington, DC: National Academy of Sciences, National Academy Press.

Olver, M.D., 1997. Effect of feeding clinoptilolite (zeolite) on the performance of three strains of laying hens. British Poultry Sciemce 38, 220–222.

Papaioannou, D.S., Kyriakis, S.C., Papasteriadis, A., Roumbies, N., Yannakopoulos, A., Alexopoulos, C., 2002. A field study on the effect of in-feed inclusion of a natural zeolite (clinoptilolite) on health status and performance of sows/gilts and their litters. Research in Veterinary Science 72, 51–59.

Parisini, P., Martelli, G., Sardi, L., Escribano, F., 1999. Protein and energy retention in pigs fed diets containing sepiolite. Animal Feed Science and Technology 79, 155–162.

Pearson, G., Smith, W.C., Fox, J.M., 1985. Influence of dietary zeolite on pig performance over the liveweight range 25-87 kg. New Zealand Journal of Experimental Agriculture 13, 151–154.

Petrie, A., Watson, P., 1999. Statistics for veterinary and animal science. Blackwell Science Ltd, Oxford, UK.

Pond, W.G., Houpt, K.A., 1978. The Biology of the Pig. Cornell University Press, Ithaca, New York, USA. p. 282.

Pond, W.G., Yen, J.T., 1982. Response of growing swine to dietary clinoptilolite from two geographical sources. Nutrition Reports International 25, 837–848.

Pond, W.G., Laurent, S.M., Orloff, H.D., 1984. Effect of dietary clinoptilolite or zeolite Na-A on body weight gain and feed utilization of growing lambs fed urea or intact protein as a nitrogen supplement. Zeolites 4, 127–132.

Pond, W.G., Yen, J.T., Varel, V.H., 1988. Response of growing swine to dietary copper and clinoptilolite supplementation. Nutrition Reports International 37, 797–803.

Poulsen, H.D., Oksbjerg, N., 1995. Effects of dietary inclusion of a zeolite (clinoptilolite) on performance and protein metabolism of young growing pigs. Animal Feed Science and Technology 53, 297–303.

Ramu, J., Clark, K., Woode, G.N., Sarr, A.B., Phillips, T.D., 1997. Adsorbtion of cholera and heat-labile *Escherichia coli* enterotoxins by various adsorbents: an in vitro study. Journal of Food Protection 60, 358–362.

SAS/STAT® 2000. User's Guide, Version 8, Volumes 1, 2 and 3. Cary, NC: SAS® Institute Inc.

Sheppard, R.A., 1984. Characterization of zeolitic materials in agricultural research. In: Pond, W.G., Mumpton, F.A. (Eds.), Use of natural zeolites in agriculture and aquaculture. Westview Press, Boulder, CO, pp. 81–90.

Shurson, G.C., Ku, P.K., Miller, E.R., Yokohama, M.T., 1984. Effects of zeolite A or clinoptilolite in diets of growing swine. Journal of Animal Science 59, 1536–1545.

Vrzgula, L., Bartko, P., 1984. Effects of clinoptilolite on weight gain and some physiological parameters of swine. In: Pond, W.G., Mumpton, F.A. (Eds.), Zeo-Agriculture. Use of Natural Zeolites in Agriculture and Aquaculture. Westview Press Inc., Boulder, Colorado, pp. 161–166.

Wilson, A.D., Stokes, C.R., Bourne, F.J., 1989. Effect of age on absorption and immune responses to weaning or introduction of novel dietary antigens in pigs. Research in Veterinary Science 46, 180–186.

Yannakopoulos, A., Tserveni-gousi, A., Kassoli-fournaraki, A., Tsirambides, A., Michailidis, K., Filippidis, A., Lutat, U., 2000. Effects of dietary clinoptilolite-rich tuff on the performance of growing-finishing pigs. In: Coela, C., Mumpton, F.A. (Eds.), Natural Zeolites for the Third Millenium. De Frede Editore, Napoli, Italy, pp. 471–481.