Effect of in-feed inclusion of a natural zeolite (clinoptilolite) on certain vitamin, macro and trace element concentrations in the blood, liver and kidney tissues of sows

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SUMMARY

The study was conducted to evaluate, under field conditions, the effect of the long-term dietary use of a natural zeolite (clinoptilolite, CLI) and antibacterials (chlortetracycline, CTC) on the concentrations of certain vitamins (vitamin A and vitamin E) and minerals (K, Na, P, Ca, Mg, Cu and Zn) in blood and body tissues of the sow. Twenty-four sows were assigned to two main experimental groups and four subgroups, depending on the presence or absence of CLI and CTC in their feed, respectively. CLI was provided to the sows from weaning, during the service, gestation and lactation periods and up to the date of the next service, while CTC was administered for a 2-week period post-service, as well as for a 2-week period following the allocation of the sows in the farrowing house, around 5 days prior to the expected parturition. Blood samples were collected on the starting day of the trial, on the 30th and the 90th day of each pregnancy, on the day of each parturition and on the day of each weaning. Furthermore, 20 sows were similarly distributed in the same experimental groups and subgroups and at the end of the trial they were slaughtered and liver and kidney samples were collected for biochemical analysis. Neither CLI nor CTC supplementation of the diets had any significant effect on vitamins' and minerals' uptake and their distribution in the body, since there was no alteration in their blood serum and liver/kidney concentrations. Furthermore, no CLI × CTC interaction was noticed. © 2002 Harcourt Publishers Ltd

NATURAL zeolites are crystalline, hydrated allumi-nosilicates of alkali and alkaline earth cations, having three-dimensional structures with interconnecting channels and large voids capable of trapping molecules of proper dimensions (Mumpton and Fishman 1977). The basis of interest in the biological effects of zeolites concerns one or more of their physical and chemical properties, such as ion exchange capacity, adsorption and related molecular sieve properties. When used as dietary supplements, naturally occurring zeolites such as clinoptilolite (CLI), have been reported to improve feed efficiency, thus leading to a beneficial growth response in growing–finishing pigs (Pond et al 1988, Coffey and Pilkington 1989, Yannakopoulos 2000), as well as to enhance the reproductive performance of sows (Mumpton and Fishman 1977, Cheshmedzhiev et al 1985).

However, one of the major concerns, which the use of natural zeolites in animal nutrition arises, is their potential adsorbent and binding effect on essential nutrients such as vitamins and minerals (Pasteiner 1998). In such case, if large quantities of these elements are rendered unavailable to the animals via feed in a long-term basis, the caused nutritional imbalances

might have a non-desired effect on both performance and health status preservation. In order to estimate whether the requirements for essential elements are met, the absorbable or utilisable/available amounts are considered, rather than the total amounts in feed ingredients. Dietary additions of naturally occurring or synthetic zeolites have been shown to alter the blood and tissue mineral concentrations in different species such as pigs (Pond et al 1988), broilers (Scheideler 1993) or dogs (Cefali et al 1995). Pond et al (1984) considered that the lack of response of tissue mineral composition to either CLI or zeolite Na-A suggested that cation exchange capacities of these two zeolites were not associated with adverse effects on normal tissue mineral homeostasis, providing evidence of their safety for dietary use in growing lambs. In addition, some years later, it was demonstrated that although the incorporation of 2 per cent CLI in diets fed continuously to growing–finishing pigs was not associated with adverse effects on tissue mineral element concentrations, however, some observed changes in kidney mineral element status, suggested that the inclusion levels of some minerals in the diet need special attention when CLI is also added (Pond et al 1989). Furthermore, it was reported that feeding 0·5 per cent sodium zeolite A in growing pigs resulted in increased liver and bone Zn content and in decreased serum Ca and inorganic P concentrations (Ward et al 1991).

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It is clear that since the behaviour of different species of zeolites in biological systems varies, along with the complexed physiological processes involved in digestion and absorption of ingested diet constituents, there is a necessity for further investigation in order to establish the safety of the dietary use of zeolites.

The objective of the present study was to delve into the potential effect of the CLI on the vitamins (vitamin E and vitamin A) and mineral elements (K, Na, inorg. P, Ca, Mg, Cu and Zn), in terms of any shift in their blood serum and tissue concentrations. For this purpose, CLI was incorporated in sow's diets at the inclusion rate of 2 per cent, in the presence or absence of an antibacterial agent routinely used in strategic medication programmes for disease-control and subsequent reproductive losses prevention (chlortetracycline, CTC).

MATERIALS AND METHODS

Experimental material

The CLI used in the present study was a naturally occurring inert calcium/potassium/sodium hydrated aluminosilicate having a minimum purity of 85 per cent. It was extracted from a deposit in northeastern Greece (Evros County) and crushed and screened to a size of < 1 mm. The CLI extrusive rock was won and prepared by the company Silver & Baryte Ores Mining Co. (Athens, Greece). Recently, it has been authorized in accordance with Directive 70/524/EEC as additive in feeding stuffs intended for pigs, rabbits and poultry (binder, anti-caking agent and coagulant), under the conditions laid down in Annex II to this regulation (Commission Regulation No 1245/1999 of 16 June 1999). One batch of this product was used and, according to the manufacturing company, the material's cation exchange capacity was $130–150$ mEq 100 g^{-1}, while its chemical composition (on dry sample) was: SiO_2 68·26 per cent, Al_2O_3 13·30 per cent, Fe_2O_3 0·08 per cent, CaO 4·34 per cent, MgO 1·05 per cent, K_2O 0·94 per cent, Na_2O 0·26 per cent, L.O.I. 11·6 per cent.

Animals and treatments

The investigation was carried out on a Greek farrow-to-finish pig unit with a capacity of 450 crossbred sows (Large White × Landrace) under production. As part of a broader trial which aimed to the evaluation of the efficacy of natural CLI and its compatibility with antibacterials, 24 healthy sows in parity four were selected for blood sampling and distributed in two main experimental groups and four subgroups, depending on the inclusion or not of CLI and CTC in their feed, as follows:

z − group (12 sows): basic on-farm mixed feed:
z − A − subgroup (eight sows): feed without CTC;
z − A + subgroup (four sows): feed supplemented with 800 ppm CTC [AUROFAC® (Aureomycin), Roche].

z + group (12 sows): basic on-farm mixed feed containing CLI at the inclusion rate of 2 per cent:
z + A − subgroup (eight sows): feed without CTC;
z + A + subgroup (four sows): feed supplemented with 800 ppm CTC.

Furthermore, 20 healthy sows in parity six were selected for tissue biochemical analysis. They were similarly divided in the same experimental groups and subgroups (seven in z − A −, three in z − A +, seven in z + A − and three in z + A +).

Sows received the trial feed since weaning, during service, gestation, lactation and up to the date of the next weaning (second weaning on trial). Following the hygiene programme routinely applied in the trial farm, CTC was administered to the sows of z − A + and z + A + subgroups for a 2-week period post-service, as well as for a 2-week period following their allocation in the farrowing house, around 5 days prior to the expected parturition. The above mentioned medication was incumbent upon the disease history of the trial farm (see feeding and hygiene programs applied) and prescribed because of the antibacterial's disease-control effect against specific, such as *Leptospira spp.* (Taylor 1999), and non-specific microorganisms (Wilson et al 2000), when fed on a strategic medication basis.

The trial was conducted under the license of Veterinary County Administration of Karditsa (Protocol No: 3412) and under Good Clinical Practice for the Conduct of Clinical Trials for Veterinary Medicine Products (GCPV) guidelines (European Agency for the Evaluation of Medicinal Products 1998).

Feeding and hygiene programmes applied

Two types of feed were used for feeding the sows: a pregnancy feed (PF) and a lactation feed (LF). Both of them were supplemented with commercial vitamin and mineral premixes at the recommended inclusion rates (Vetervit Super Sow 503 and Vetermin 504, Veterin S.A., Aspropyrgos, Attiki, Greece). The specifications of the feed are shown in Table 1. The feeding scheme was as follows: from the day of service until the 21st day of pregnancy, 1·9 kg of PF was administered per day to each sow. From the 22nd until the 56th day of pregnancy the quantity of feed offered daily was 2·4 kg, while from the 57th until the 84th day of pregnancy the respective quantity was 2·8 kg. From this day up to the 110th day of pregnancy, 3·4 kg of the same feed was offered. The composition of the feed at this moment was changed to LF and the quantity offered was gradually decreased, until the day of parturition, on which no feed was given. The feed allowance during the first five days of lactation was gradually increased to the maximum of 2 kg plus 0·5 kg per suckling piglet daily; this schedule was followed until weaning (25 ± 3 days). From day of weaning up to day of service, sows were given 3·5 kg of PF. The feed was offered once daily.

During the trial, samples of each type of final feed were collected and sent to the laboratory of Veterin S.A. (Aspropyrgos, Attiki, Greece) for the

TABLE 1: Specifications* of each type of the basal feed used in the study

	Pregnancy feed	Lactation feed
Digestible energy (Mcal kg^{-1})	3·10	3·16
Crude protein (percentage)	16·2	16·7
Lysine (percentage)	0·72	0·81
Vitamin A (IU kg^{-1})	28920	28630
Vitamin E[†] (mg kg^{-1})	65·7	60·7
Sodium (percentage)	0·19	0·22
Phosphorous (percentage)	0·73	0·78
Calcium (percentage)	0·90	0·92
Magnesium (percentage)	0·24	0·22
Copper (mg kg^{-1})	27·2	28·3
Zinc (mg kg^{-1})	159	144

*represent the mean values found in the analyses of feed samples throughout the trial; [†]as DL-α tocopherol.

determination of CLI inclusion rate. The results were within the analytical limits of the laboratory method used, verifying the efficient feed mixing procedure.

The trial farm's female population was regularly vaccinated against erysipelas, leptospirosis, Aujeszky's disease, swine influenza, atrophic rhinitis, porcine parvovirus disease, colibacillosis (ETEC *Escherichia coli*) and enterotoxaemia (*Cl perfringens* types A and C), since 1995. The vaccination scheme was incumbent upon the past disease history of the farm, the latter being infected with PRRS virus since 1993 and having previous history of porcine enteropathy outbreaks [including the acute (haemorrhagic in gilts and heavy finishing slaughter pigs) and the chronic forms (porcine intestinal adenomatosis in weaning, growing and fattening pigs)], Aujeszky's disease, swine influenza and mycotoxicosis. Occasionally, the presence of *Leptospira bratislava* was also detected by serological examinations in blood samples routinely (twice yearly) obtained from the pigs, as part of the disease-monitoring policy in this farm.

Samples collection protocol—methods of analysis

Blood samples were collected on the starting day of the trial (day of weaning), on the 30th day and 90th day of each pregnancy, at each parturition and at each weaning (second weaning on trial). Samples collection was performed between 09·00 hours and 11·00 hours. Blood samples were drawn via cranial (anterior) vena cava puncture in plain glass tubes with a 15-gauge, 150-mm long needle. After clotting, serum was separated by low speed centrifugation, transferred in plastic vials and forwarded for biochemical analysis.

Vitamin E was evaluated by a fluorometric method (Hansen and Warwick 1969), using a Hitachi F2000 fluorometer. Vitamin A was determined by the colorimetric method described by Roels and Trout (1972), using a Hitachi 2000 spectrophotometer. K and Na were analysed using flame atomic emission spectrophotometry and a Sherwood flame photometer 410. Inorganic P was evaluated by the heteropoly blue method (Boltz 1958). Finally, Ca, Mg, Cu and Zn were determined by means of flame atomic absorption

spectrophotometry, using a Perkin Elmer A Analyst 100 instrument (Perkin-Elmer Co 1996).

At the end of the trial (date of second weaning on trial), the sows selected for tissue biochemical analysis were slaughtered, the liver and the kidneys were detached and samples of around 50 g wet weight were removed using stainless steel scalpels, put into plastic containers and frozen until the day of analysis, which was carried out within 1 month of the day of sampling. The estimated parameters and the methods used were the same, as those described in the case of the serum samples. Vitamins were determined in fresh tissue samples. They were homogenized and after saponification and extraction, they were handled similarly as were serum samples (Taylor et al 1976, Roels and Mahadevan 1967). For the determination of the rest parameters the samples, after drying in an oven at 100°C, they were wet digested using an acid mixture containing concentrated sulfuric, nitric and perchloric acids.

Statistical evaluation

Statistical analysis was performed by the use of the general linear model procedure of SAS (Version 8.1 for Windows, 2000/Site code: 0084912001/SAS Institute Inc., Cary, NC 27513, USA). In the analysis of variance the variables tested were analysed as a 2×2 arrangement with CLI, CTC and their interaction as factors of the model. Furthermore, Duncan's multiple range test was used to compare the estimated means of treatments. The significance was attained if the P-value was $<0·05$.

RESULTS

Changes in the concentrations of serum vitamin A and vitamin E throughout the trial are presented in Table 2. No treatment differences (P > 0·05) were noted among the experimental groups and subgroups. Z-group showed a slightly lower vitamin E concentration on the 30th day of each pregnancy, but this effect was rather incidental since it was not followed by a similar one at the ensuing blood samplings.

Data concerning serum K and Na concentrations are summarised in Table 3, while those referring to P, Ca and Mg concentrations are shown in Table 4. From the results, it is clear that serum macro-element concentrations were not influenced by the administration of CLI and/or CTC (P > 0·05). However, the results revealed a tendency for a lower P concentration in Z-group than in N-group on the 30th day of each pregnancy (P = 0·09). It is worth noting that P concentration were rather low in all subgroups, independently of the diet consumed by the animals, especially in the case of the fourth blood sampling which was performed on the day of each parturition.

Serum Cu and Zn concentrations are presented in Table 5. Although there were no differences among the experimental groups and subgroups (P > 0·05), the mean serum Cu concentration in Z-group tended to be lower compared with the N-group, on the 30th and

TABLE 2:   Effect of presence (+) or absence (−) of clinoptilolite (CLI) and chlortetracycline (CTC) on serum vitamin E and vitamin A concentrations

CLI	−		+					
CTC	−	+	−	+				
Experimental subgroups	Z − A −	Z − A +	Z + A −	Z + A +			Probability	
	Mean* (n = 8)	Mean* (n = 4)	Mean* (n = 8)	Mean* (n = 4)	SD	CLI effect	CTC effect	CLI × CTC interaction
Vitamin E (mg l^{-1})								
Day of weaning	1·56	1·70	1·51	1·72	0·37	0·940	0·310	0·826
30th day of pregnancy	1·96	2·12	1·71	1·82	0·43	0·162	0·476	0·896
90th day of pregnancy	1·73	1·75	1·60	1·70	0·36	0·582	0·740	0·796
Parturition	1·21	1·27	1·16	1·22	0·43	0·806	0·759	1·000
Day of second weaning on trial[†]	1·58	1·65	1·43	1·65	0·33	0·619	0·365	0·619
Vitamin A (μg 100 ml^{-1})								
Day of weaning	22·62	22·50	20·87	21·25	4·89	0·511	0·956	0·912
30th day of pregnancy	21·12	22·00	21·12	20·75	3·55	0·707	0·880	0·707
90th day of pregnancy	20·50	21·50	20·12	21·75	3·05	0·964	0·355	0·824
Parturition	19·50	19·50	19·37	20·50	2·58	0·716	0·640	0·640
Day of second weaning on trial[†]	22·12	21·75	21·75	21·50	2·73	0·807	0·807	0·961

*mean values in the same row do not differ significantly (P > 0·05); [†]25 (3) days after parturition.

TABLE 3:   Effect of presence (+) or absence (−) of clinoptilolite (CLI) and chlortetracycline (CTC) on serum potassium and sodium concentrations

CLI	−		+					
CTC	−	+	−	+				
Experimental subgroups	Z − A −	Z − A +	Z + A −	Z + A +			Probability	
	Mean* (n = 8)	Mean* (n = 4)	Mean* (n = 8)	Mean* (n = 4)	SD	CLI effect	CTC effect	CLI × CTC interaction
Potassium (mEq l^{-1})								
Day of weaning	5·02	4·90	4·97	5·07	0·37	0·719	0·942	0·518
30th day of pregnancy	5·46	5·30	4·96	5·50	0·50	0·472	0·370	0·102
90th day of pregnancy	5·01	5·32	5·27	5·12	0·47	0·884	0·706	0·289
Parturition	5·31	5·15	5·15	5·25	0·52	0·898	0·898	0·590
Day of second weaning on trial[†]	4·82	4·75	5·18	4·97	0·52	0·215	0·538	0·768
Sodium (mEq l^{-1})								
Day of weaning	140·00	139·00	137·75	141·25	4·60	1·000	0·549	0·285
30th day of pregnancy	137·62	144·25	142·87	142·25	9·05	0·690	0·463	0·377
90th day of pregnancy	139·75	135·75	140·75	144·25	6·50	0·103	0·929	0·192
Parturition	136·87	136·25	135·37	137·25	6·76	0·937	0·843	0·693
Day of second weaning on trial[†]	134·12	135·00	134·37	135·50	5·65	0·887	0·706	0·962

*mean values in the same row do not differ significantly (P > 0·05); [†]25 (3) days after parturition.

90th day of each pregnancy (P = 0·08 and P = 0·1, respectively).

Table 6 provides data on the liver and kidney content of the vitamins, macro and trace elements. There was no significant effect due to CLI and/or CTC administration on the investigated parameters (P > 0·05).

DISCUSSION

The preconditions for an adequate provision of essential nutrients to the organism are a sufficient supply via feed and an undisturbed absorption process. So far, according to the authors' knowledge, no research evidence is available concerning the dietary use of zeolites in sows and their potential risk in the utilisation of the dietary nutrients due to their non-specific adsorption property and their cation exchange capacity. It is worth noting that the contradictory results obtained in studies dealing with the overall effects of zeolite supplementation in productive animals diets, could be attributed to factors such as the species and the geographical source of the used zeolite, 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, apart from the disease-control effect, it has been posed that the mode of action of in-feed antibacterials could also have a potential nutritional effect, e.g. increased utilisation of nutrients by the animal. From this point of

TABLE 4: Effect of presence (+) or absence (−) clinoptilolite (CLI) and chlortetracycline (CTC) on serum inorganicphosphorous, calcium and magnesium concentrations

	CLI −		CLI +					
	CTC −	CTC +	CTC −	CTC +			Probability	
Experimental subgroups	Z − A −	Z − A +	Z + A −	Z + A +				
	Mean* (n = 8)	Mean* (n = 4)	Mean* (n = 8)	Mean* (n = 4)	SD	CLI effect	CTC effect	CLI × CTC interaction
Phosphorous (mg 100 ml $^{-1}$)								
Day of weaning	5·06	5·02	4·91	5·15	0·42	0·949	0·611	0·486
30th day of pregnancy	5·12	5·10	4·77	4·47	0·64	0·093	0·563	0·624
90th day of pregnancy	4·80	4·95	4·62	4·42	0·50	0·125	0·910	0·433
Parturition	4·38	4·47	4·26	4·50	0·40	0·785	0·380	0·683
Day of second weaning on trial[†]	4·68	4·77	4·41	4·70	0·42	0·346	0·313	0·587
Calcium (mg 100 ml $^{-1}$)								
Day of weaning	9·40	9·47	9·42	9·10	1·35	0·780	0·837	0·756
30th day of pregnancy	8·58	8·64	8·75	8·65	1·46	0·894	0·971	0·903
90th day of pregnancy	8·69	8·48	8·81	8·69	1·26	0·779	0·779	0·935
Parturition	8·98	8·79	9·07	9·07	1·38	0·726	0·931	0·941
Day of second weaning on trial[†]	8·98	8·75	9·13	9·01	1·28	0·740	0·768	0·928
Magnesium (mg 100 ml $^{-1}$)								
Day of weaning	2·59	2·63	2·61	2·57	0·21	0·831	0·990	0·680
30th day of pregnancy	2·56	2·41	2·50	2·55	0·26	0·630	0·667	0·975
90th day of pregnancy	2·55	2·54	2·41	2·39	0·23	0·166	0·897	0·956
Parturition	2·67	2·71	2·49	2·66	0·26	0·339	0·377	0·619
Day of second weaning on trial[†]	2·57	2·60	2·51	2·56	0·19	0·566	0·653	0·922

*mean values in the same row do not differ significantly (P > 0·05); [†]25 (3) days after parturition.

TABLE 5: Effect of presence (+) or absence (−) clinoptilolite (CLI) and chlortetracycline (CTC) on serum copper and zinc concentrations

	CLI −		CLI +					
	CTC −	CTC +	CTC −	CTC +			Probability	
Experimental subgroups	Z − A −	Z − A +	Z + A −	Z + A +				
	Mean* (n = 8)	Mean* (n = 4)	Mean* (n = 8)	Mean* (n = 4)	SD	CLI effect	CTC effect	CLI × CTC interaction
Copper (μg 100 ml $^{-1}$)								
Day of weaning	180·5	183·0	174·0	181·0	20·9	0·661	0·624	0·816
30th day of pregnancy	190·0	195·0	182·5	177·0	15·4	0·067	0·970	0·435
90th day of pregnancy	194·0	190·0	181·5	178·0	21·4	0·211	0·697	0·979
Parturition	198·5	203·0	197·5	200·0	19·5	0·826	0·701	0·913
Day of second weaning on trial†	190·0	195·0	183·5	185·0	19·3	0·357	0·714	0·843
Zinc (μg 100 ml $^{-1}$)								
Day of weaning	131·5	133·5	136·2	138·2	22·7	0·655	0·850	1·000
30th day of pregnancy	116·6	115·7	112·0	113·0	20·0	0·694	0·995	0·920
90th day of pregnancy	107·2	107·0	110·1	107·0	13·7	0·823	0·793	0·823
Parturition	116·9	120·2	112·4	109·7	17·7	0·364	0·963	0·714
Day of second weaning on trial†	122·2	131·7	115·5	117·2	17·8	0·188	0·479	0·625

*mean values in the same row do not differ significantly (P > 0·05); [†]25 (3) days after parturition.

view, the investigation of any potential interactive effect of CLI and CTC on serum and tissue chemistry of the sows strategically medicated with the latter, could be of additional interest.

Vitamins and minerals are essential in pig nutrition in order for optimal growth, reproduction and lactation to occur. In the modern pig production industry the fortification of the diets with vitamin and mineral pre-mixes is often the rule. In our study the supplied feed was enriched in these nutrients, having inclusion levels above the minimal recommended requirements (National Research Council 1998).

Although the reserves of the sow in vitamin A make it difficult to predict deficiency conditions, literature references indicate that a low dietary intake has been associated with undesirable effects on reproductive activity (Tassell 1967, Chew 1993). Furthermore, the importance of vitamin E in the health status and the reproductive performance of sows has also been well documented (Mavromatis et al 1999, Close and Cole 2000). In our study, the assessment of vitamin E and vitamin A levels in serum, liver and kidney tissue samples revealed an adequate status in all the experimental subgroups. Diet had no significant effect on

TABLE 6: Effect of presence (+) or absence (−) of clinoptilolite (CLI) and chlortetracycline (CTC) on the concentrations of certain biochemical parameters in tissue samples[†]

Liver

CLI	−	−	+	+				
CTC	−	+	−	+		Probability		
Experimental subgroups	Z−A− Mean[†] (n=7)	Z−A+ Mean[†] (n=3)	Z+A− Mean[†] (n=7)	Z+A+ Mean[†] (n=3)	SD	CLI effect	CTC effect	CLI × CTC interaction
Vit E (mg kg⁻¹)	6·64	7·70	7·35	7·56	1·67	0·739	0·471	0·628
Vit A (mg kg⁻¹)	171·8	183·3	164·1	165·6	21·8	0·267	0·564	0·658
K (g kg⁻¹)	6·17	6·30	6·12	6·16	0·49	0·741	0·754	0·865
Na (g kg⁻¹)	2·01	2·03	1·90	2·06	0·23	0·742	0·454	0·550
P (g kg⁻¹)	7·01	7·20	6·80	6·86	0·36	0·144	0·489	0·743
Ca (mg kg⁻¹)	207·5	220·5	216·6	218·2	45·8	0·890	0·767	0·817
Mg (mg kg⁻¹)	665·8	673·6	665·1	661·6	104·9	0·911	0·970	0·921
Cu (mg kg⁻¹)	48·6	51·3	48·5	49·6	6·5	0·803	0·583	0·819
Zn (mg kg⁻¹)	269·1	272·0	271·4	273·0	474	0·949	0·932	0·980

Kidneys

CLI	−	−	+	+				
CTC	−	+	−	+		Probability		
Experimental subgroups	Z−A− Mean[†] (n=7)	Z−A+ Mean[†] (n=3)	Z+A− Mean[†] (n=7)	Z+A+ Mean[†] (n=3)	SD	CLI effect	CTC effect	CLI × CTC interaction
Vit E (mg kg⁻¹)	8·54	8·70	7·98	8·13	0·83	0·194	0·718	0·991
Vit A (mg kg⁻¹)	10·18	10·93	9·01	10·80	2·13	0·544	0·247	0·629
K (g kg⁻¹)	6·28	6·26	6·22	6·26	0·49	0·914	0·971	0·914
Na (g kg⁻¹)	4·01	4·03	3·90	3·93	0·29	0·490	0·865	0·963
P (g kg⁻¹)	6·82	6·86	6·54	6·76	0·44	0·397	0·563	0·681
Ca (mg kg⁻¹)	396·2	406·2	399·6	391·1	73·8	0·884	0·985	0·816
Mg (mg kg⁻¹)	811·3	793·1	795·8	783·9	97·5	0·814	0·774	0·952
Cu (mg kg⁻¹)	30·9	35·7	31·1	35·3	5·37	0·949	0·104	0·911
Zn (mg kg⁻¹)	161·9	163·9	158·3	161·9	25·8	0·840	0·839	0·951

*Tissue samples were collected at the end of the trial (day of second weaning on trial); [†]For each tissue, means in the same row do not differ significantly (P > 0·05)

vitamin concentrations and although a longer observation period over more than one reproductive cycle would more reliably indicate CLI effects on sows' vitamin status (in particular vitamin A status), our results provided evidence of CLI safety relating to the availability of them.

Although there is a lack of literature data relating to the effects of zeolites on sow's serum and tissue chemistry, valuable conclusions have been drawn by comparative studies in growing–finishing pigs and other species. Experiments conducted in growing lambs, hens and growing pigs have demonstrated that plasma and liver Na and K concentrations are not altered by the dietary use of either CLI or zeolite Na-A (Pond and Yen 1983, Pond et al 1984, Roland et al 1993). However, a decreased liver K concentration of weaners and growers in response to CLI dietary inclusion has been reported, attributed to the high K-binding affinity of CLI at the pH of the small intestinal tract (Pond et al 1988). Adversely, a 20 per cent increase of serum K in mice that were on a CLI-enriched diet was demonstrated (Martin-Kleiner et al 2001). As revealed in the current study, no alteration in serum and tissue K and Na concentrations was observed in response to the inclusion of CLI in sow's diet during pregnancy and lactation.

As far as P, Ca and Mg concentrations are concerned, the results of our study indicated no significant differences among the experimental groups and subgroups, except for a tendency for a lower serum P concentration in the group which was on the CLI diet, on the 30th day of pregnancy. Although the results generally support previous observations in lambs, growing pigs and broilers (Pond et al 1984, Vrzgula and Bartko 1984, Dwyer et al 1997), the estimated tendency cannot be readily explained. In general, CLI is considered to be rather stable in the acid environment of the stomach, without undergoing major degradation during its transit through the digestive tract (Shurson et al 1984). Thus, the structural Al and Si ions are not removed from its crystalline matrix to interfere with P availability by forming insoluble phosphates (Valdivia et al 1982), as well as with Ca (Spencer et al 1982) and Mg retention (Valdivia et al 1982). Presumably, the estimation of Al and Si concentration in the tissues of the sows fed the CLI diet, in our study, would have provided more information about the behaviour of the additive during the digestion process and its effect on the utilisation of the dietary mineral elements.

In the current study, serum Cu concentration tended to be lower on the 30th and the 90th day of pregnancy in the group fed the CLI supplemented diet. Pond et al (1989) reported a reduced kidney Cu concentration in growing pigs fed a diet supplemented with 2 per cent CLI. However, the dietary use of CLI in the sows of our study did not result in a similar response. This could be attributed to the different dietary level of Cu used in our study. However, the potential effect of CLI on sow's serum Cu concentration during pregnancy, as revealed in our study, should be further investigated by taking also into consideration the alterations in the metabolic patterns of Cu following the growth and development of

the fetus and placenta at mid-gestation (Rhéaume and Chavez 1989, Richards 1999).

Several studies conducted using lambs, growing-finishing pigs and broilers demonstrated contradictory results relating to the effect of zeolites on serum and tissue Zn content. (Pond 1989, Ward et al 1991, Watkins and Southern 1993). Nevertheless, the results obtained by an earlier study in growing–finishing pigs (Pond et al 1989) support the lack of an alteration response to CLI dietary supplementation on sow's liver and kidney Zn concentrations as demonstrated in our study. Additionally, under the trial's study design, CTC strategic medication had no effect on the blood and tissue mineral content, neither resulted in any interactive effect with CLI.

The conclusion that could be drawn from the results of the current study is that the dietary supplementation of CLI during pregnancy and lactation, alone or in combination with CTC in the case of a necessary metaphylaxis programme application, is not associated with any adverse effect on vitamins and mineral elements uptake and body distribution in sows, which are on a diet adequately fortified with the above nutrients.

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