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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

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SUMMARY

The study was conducted to evaluate the effect of the long-term dietary use of a natural zeolite (clinoptilolite, NZ) on health status and reproductive performance of sows/gilts and performance of their litters, along with its compatibility with antibacterials (chlortetracycline, CTC) periodically used in medication programmes. Two hundred and forty sows/gilts and their litters were assigned to two main experimental groups and four subgroups, depending on the inclusion of NZ and CTC in their feed. During the trial, frequent sampling of pregnancy feed for mycotoxicological analysis revealed a high contamination level with zearalenone. No adverse or side effects attributed to NZ were noticed. Furthermore, the combined use of NZ and CTC revealed no clinically apparent interactive effect on the availability of the latter. Reproductive performance was significantly improved by the dietary inclusion of both NZ and CTC. The results also suggested that the beneficial effect of NZ could be additionally considered as an indicator of the amelioration of zearalenone exposure consequences.

NATURAL zeolites (NZ) are crystalline, hydrated alluminosilicates of alkali and alkaline earth cations, having three-dimensional structures. They are characterised by the ability to lose and gain water reversibly and to exchange constituent cations without major change of structure (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. In particular, clinoptilolite, one of more than 30 naturally occurring zeolites, has a high affinity for ammonium ions (Mumpton 1984).

The results of experiments on the effects of in-feed application of NZ on pig performance are equivocal. Some researchers have observed beneficial responses on growth (Pond et al 1988, Coffey and Pilkington 1989, Yannakopoulos et al 2000) while others have observed no response relating to the growth of the animals (Pearson et al 1985). Moreover, it was found that the addition of 5 per cent NZ to the rations of pregnant sows 20–90 days after mating, improved feed efficiency values and increased litter size and litter weight at parturition (Ma et al 1979). Adversely, some years later, it was reported that the feeding of 2·5 per cent or 5 per cent of NZ to pregnant sows decreased the ovulation rate but did not affect the embryo-survival rate significantly (Ma et al 1984). The explanation for these contradictory results is not clear. It has been reported that the effect of the dietary NZ in achieving positive performance results may be related to the species and the geographical source of the involved zeolite, its purity and physicochemical properties, as well as the supplemental level used in the diets (Mumpton and Fishman 1977, Pond et al 1988).

Considerable evidence is also available suggesting an overall efficacy of zeolites in binding aflatoxin B_1 and zearalenone (Piva et al 1995, Ramos et al 1996a). It was also demonstrated that feeding zearalenone to rats receiving dietary synthetic anion exchange zeolite resulted in a positive correlation between zeolite and fecal excretion of zearalenone and a negative correlation with the urinary excretion of zearalenone, establishing the hypothesis that zeolite binds zearalenone in the gastrointestinal tract to prevent intestinal absorption (Smith 1980). Furthermore, other reports have shown that some of the toxic effects of aflatoxin B_1 may be counteracted by the incorporation of the natural zeolite mordenite (Harvey et al 1993) or the synthetic zeolite NaA (Miazzo et al 2000) into the diets of growing broiler chickens.

The objective of the present study was to determine the effect of a long-term feeding scheme using a NZ (clinoptilolite) at the inclusion rate of 2 per cent on the health status and reproductive performance of sows/gilts and on the performance of their litters, as well as its

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compatibility with an antibacterial routinely used in strategic medication programmes for reproductive efficiency (chlortetracycline, CTC).

MATERIALS AND METHODS

Experimental material

The clinoptilolite used in the present study was a naturally occurring inert calcium/potassium/sodium hydrated aluminosilicate of volcanic origin, having a minimum purity of 85 per cent. It was extracted from a deposit in north-eastern Greece (Evros County) and crushed and screened to a size of <1 mm. The clinoptilolite extrusive rock was won and prepared by the company Silver & Baryte Ores Mining Co. (Athens, Greece). Recently, it has been authorised in accordance with Directive 70/524/EEC as additive, No 3, in feeding stuffs 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.

The trial farm: management, feeding and hygiene programmes applied

The trial was carried out on a Greek farrow-to-finish pig unit with a capacity of 450 crossbred sows (Large White × Landrace) under production. At the age of 6 months, replacement gilts were housed in groups of six in the service area, where they were served during their second oestrus (double mating 12 and 24 hours after detection of standing oestrus by a teaser boar). Since conception was confirmed by an ultrasound method (using a real time ultrasound device) on the 30th day after service, they were moved in the gestation building and housed individually. Five days prior to the expected farrowing, they were moved to the raised pens of the farrowing house, where they remained until weaning. The same procedure was also applied to sows. Since conception was confirmed after their service (double mating 12 and 24 hours after detection of standing oestrus by a teaser boar), they were moved in individual stalls in the gestation building and 5 days prior to the expected farrowing they were allocated in the raised pens of the farrowing house. Piglets were weaned on the 22nd to 28th day of their life. Both oestrus and farrowing were not induced by the use of any hormone treatment.

Two types of feed were used for feeding the sows/gilts: a pregnancy feed (PF) and a lactation feed (LF). From the seventh day of age until weaning, piglets were offered creep feed (CF) ad libitum. The basic specifications of the feed (on an air-dry basis) were as follows: (a) PF: digestible energy 3·10 Mcal kg^{-1}, crude protein 16·2 per cent, lysine 0·72 per cent, calcium 0·90 per cent, total phosphorus 0·73 per cent; (b) LF: digestible energy 3·16 Mcal kg^{-1}, crude protein 16·7 per cent, lysine 0·81 per cent, calcium 0·92 per cent, total phosphorus 0·78 per cent; (c) CF: digestible energy 3·42 Mcal kg^{-1}, crude protein 21·6 per cent, lysine 1·48 per cent, calcium 0·87 per cent, total phosphorus 0·74 per cent. The feeding scheme was as follows: from the age of 6 months up to the sixth day prior to service 2 kg of LF was administered daily to each gilt, followed by a quantity of 2·4 kg of the same feed until the day of service. From the day of service until the 21st day of pregnancy, 1·9 kg of PF was administered per day to each sow, and 1·6 kg of PF per day to each gilt. From the 22nd until the 56th day of pregnancy the quantity of feed offered daily was 2·4 kg for the sows and 1·9 kg for the gilts, while from the 57th until the 84th day of pregnancy the respective quantities were 2·8 kg and 2·4 kg. From this day up to the 110th of pregnancy, 3·4 kg and 3 kg of the same feed was offered, respectively. The composition of feed at this moment was changed to LF and the quantity offered was gradually decreased, until the day of farrowing on which no feed was given. The feed allowance during the first 5 days of lactation was gradually increased to the maximum of 2 kg plus 0·5 kg per suckling piglet per sow/gilt daily; this schedule was followed until weaning. From day of weaning up to day of service, sows/gilts were given 3·5 kg of PF. Feed was offered manually, twice daily. Piglets had no access to the sow LF.

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. Details are in Table 1. The above mentioned vaccination scheme was incumbent upon the past

TABLE 1: Sows' and gilts' vaccination programme

Gilts		Sows/Gilts		
Against	Age (days)	Against	Pre-farrowing (days)	Post-farrowing (days)
AD, AR	130	C*	35	
SI	140	AD, SI	28	
AD, AR	150	AR, C, ET	21	
SI	160	ER, PV, L		15
PV, L	170			
PV, L	190			

*Only in gilts; AD: Aujezsky's disease; AR: Atrophic rhinitis; SI: Swine influenza; PV: Parvovirus disease; L: Leptospirosis; C: Colibacillosis; ET: Enterotoxaemia; ER: Erysipelas.

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.

For the control of endo/ectoparasites all sows were treated with a single ivermectin injection, at the dose rate of $300\,\mu g\,kg^{-1}$, 14 days prior to the date of farrowing. Ivermectin was also applied to the boars twice yearly.

Experimental animals and treatments

A total of 240 female breeding animals participated in the trial, divided into two main experimental groups and four subgroups, depending on the inclusion of NZ and antibacterial in their feed, as follows:

(Z−) group (120 sows/gilts): basic on-farm mixed feed;
(Z − A−) subgroup (80 sows/gilts): feed without CTC;
(Z − A+) subgroup (40 sows/gilts): feed supplemented with 800 ppm CTC [AUROFAC® (Aureomycin), Roche].
(Z+) group (120 sows/gilts): basic on-farm mixed feed containing NZ at the inclusion rate of 2 per cent;
(Z + A−) subgroup (80 sows/gilts): feed without CTC;
(Z + A+) subgroup (40 sows/gilts): feed supplemented with 800 ppm CTC.

The sows of each experimental subgroup received the trial feed starting from weaning, during service, gestation, lactation and up to the date of service of the next reproductive cycle. Since the weekly farrowing/weaning/service rate of the farm was around 20 sows, the participating animals were segmentally introduced in the trial within a 12-week period. The different experimental groups were run in parallel, whereas the sows were distributed among them almost equally each week. Actually, following the normal replacement rate of the farm's breeding stock, around 25 per cent of these sows were excluded from production due to high parity number, trauma or continuous signs of illness and were replaced by newly selected gilts that were in good clinical condition. Sows, which had accomplished six consecutive reproductive cycles, were also excluded from the trial. The gilts which were going to participate in the trial were monitored daily from the age of 6 months and were actually assigned in the experimental subgroups on the day of their service after the exhibition of the second oestrus. Their mean age (±SD) at that time was 216 ± 9 days. Furthermore, special care was taken in order to ensure an equal parity distribution among the experimental subgroups (Table 2). The equalisation was achieved progressively during the 12-week period that the introduction of the animals in the trial lasted.

TABLE 2: Parity distribution among the experimental subgroups

	Experimental subgroups			
	Z + A−	Z + A+	Z − A−	Z − A+
Gilts	20	11	20	11
1st–2nd reproductive cycle	20	9	20	9
3rd–4th reproductive cycle	26	13	26	13
5th–6th reproductive cycle	14	7	14	7
Total	80	40	80	40

CTC was administered periodically to the sows/gilts of (Z − A+) and (Z + A+) subgroups for a 2-week period post-service, as well as for a 2-week period after their allocation in the farrowing house. The choice of CTC administration was based on its efficacy in preventing reproductive failure due to leptospirosis (Saoulidis et al 2000), ileitis (McOrist et al 1999) and non-specific bacterial infections (Sbiraki et al 2000, Wilson et al 2000). The piglets were assigned in the corresponding groups and subgroups of sows, receiving creep feed without NZ and/or antibacterials.

The first group of sows/gilts enrolled in the trial in the first week of February 1998, while the last group completed the trial (first service after second weaning on trial) in late September 1998, except for a small number of animals which exhibited prolonged weaning-to-oestrus intervals and/or return(s) to oestrus, resulting in a short prolongation of the monitoring period.

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).

Laboratory examinations

During the pre-trial preparation period, the equipment of feed mill was put under service. Furthermore, every 2 months throughout the trial period, samples of each type of final feed (with and without NZ) were collected and forwarded to the laboratory of Veterin S.A. (Aspropyrgos, Attiki, Greece) for the determination of NZ inclusion rate. Following the same procedure, CTC feed assays were performed by the laboratory of Aspland and James Ltd. (Cambridshire, UK). The results were within the analytical limits of the laboratory method used, verifying the efficient feed mixing procedure.

Due to the previous disease history of the trial farm, mycotoxicological analysis of PF samples was routinely performed every month. In order to evaluate the mean monthly contamination level of PF, a representative feed sample was constituted according to the following sampling procedure: 10 feed samples of 500 g each were collected from the troughs every 3 days within the month period. Thus, by the end of each month, 100 samples were collected having a total weight of 50 kg. A final sample of 500 g was obtained from the above collected

TABLE 3: Concentrations of certain mycotoxins in pregnancy feed samples

Month of sampling (1998)	Zearalenone ($\mu g\ kg^{-1}$)	Aflatoxin B_1 ($\mu g\ kg^{-1}$)	Trichothecenes ($\mu g\ kg^{-1}$)
February	200	< 4·5*	140
March	278	< 4·5*	166
April	263	< 4·5*	152
May	285	< 4·5*	143
June	1550	9	310
July	1042	< 4·5*	258
August	622	< 4·5*	244
September	158	< 4·5*	217

*This value means the analytical detection limit.

amount and was forwarded to BIOMIN Laboratories (BIOMIN Gesunde Tierernahrung International Gmbh, Herzogenburg, Austria) for mycotoxicological analyses, which were performed by using HPLC method according to the procedures described by the Association of Official Analytical Chemists (1990). The samples collected throughout the month were stored according to the specific guidelines of the Laboratory (packed in paper bags and kept in dry place), until submission. The results are shown in Table 3.

Observations

Each individual sow/gilt and its litter constituted the experimental unit. All sows/gilts participating in the trial were monitored daily. Special attention was paid for adverse or side effects noticed in the (Z+) group due to the use of NZ. The health status of sows/gilts was assessed regularly during lactation and the incidence of any clinical sign such as inappetence (i.e. consumption of less than the half of the quantity of the feed offered daily, for 2 continuous days), pyrexia (i.e. rectal temperature higher than 39·3°C for 2 continuous days), mastitis (i.e. changes in the appearance of the mammary glands or of the mammary secretion) or vaginal discharge (i.e. presence of purulent or blood-tinged discharge after the fifth day post partum), was recorded. Individual injectable treatments were applied to both control and treated (NZ and/or CTC) sows to control the above mentioned clinical conditions.

During the trial the following dates were recorded per each sow/gilt: (i) entry in the trial (date of weaning for the sows); (ii) oestrus and service; (iii) pregnancy confirmation; (iv) farrowing; (v) weaning; and (vi) subsequent oestrus and service. The following parameters were taken into consideration: (i) weaning-to-first oestrus interval at the commencement of the trial (WOI1), as well as (ii) after the second weaning on trial (WOI2). Furthermore, the mean zearalenone concentration to which the sows/gilts of each experimental subgroup were exposed during pregnancy, based on the mean monthly contamination level of the pregnancy feed, was also evaluated. The following calculations were also performed per each experimental subgroup: (i) anoestrus rate at the commencement of the trial (anoestrus 1: i.e. number of sows failed to come back into heat within a 30 day interval post weaning/total number of sows entered in the trial), as well as after the second weaning on trial (anoestrus 2: i.e. number of animals failed to come back into heat within a 30 day interval post weaning/total number of animals on trial after the cessation of the lactation); (ii) short weaning-to-first oestrus interval rate (i.e. the proportion of animals with a weaning-to-first oestrus interval shorter than 10 days) at the commencement of the trial (WOI1 < 10), as well as after the second weaning on trial (WOI2 < 10); (iii) return to oestrus rate at the commencement of the trial (return to oestrus 1: i.e. the proportion of sows/gilts that re-served after their first oestrus on trial), as well as after the second weaning on trial (return to oestrus 2: i.e. the number of animals re-served within an interval of 44 days/total number of sows/gilts served after weaning); and (iv) farrowing rate based on first service after weaning.

Females with a severe illness (i.e. where no successful treatment could be applied, e.g. severe leg trauma) or with continuing signs of illness (i.e. where signs of disease were present even after a week's treatment), with weaning-to-first oestrus interval longer than 30 days and animals that failed to farrow (i.e. animals which did not complete parturition for any reason, other than abortion) were excluded from the trial.

For each litter the following data were recorded: number of piglets born alive and dead (mummified, stillbirths), piglet malformations, number of piglets weaned, number of piglets dying during lactation and reasons for piglets' mortality, body weight of piglets (kg) at birth and at weaning.

Statistical analysis

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 zeolite, antibacterial and their interaction as factors of the model. Furthermore, the estimated means of treatments were compared with LSD multiple comparison technique. X^2 (Pearson's) test was used to compare frequencies (expressed as actual number of cases in each category/total number of animals examined).

RESULTS

From the 240 sows/gilts used in this study, one gilt in the (Z – A–) subgroup was culled, shortly before the allocation in the farrowing house due to rectal prolapse. Furthermore, one sow in the same subgroup aborted in the last trimester of pregnancy and three gilts and one sow [three in (Z – A–) and one in (Z – A+) subgroups] showed signs of pseudopregnancy (although they exhibited normal oestrus behaviour, they accepted the boar, they were tested positive for pregnancy by ultrasound device and they exhibited mammary development around 90–100 days after service, however, they never farrowed). Moreover, nine sows/gilts [four in (Z + A–), one in (Z + A+) and three in (Z – A–), one in (Z – A+)

subgroups] failed to farrow. After the farrowing and the second weaning on trial, three sows [one in each of the (Z+A−), (Z−A−) and (Z−A+) subgroups] were removed from the breeding stock of the trial farm. One of them had a rear left leg trauma and the other two exhibited signs of the Thin Sow Syndrome.

Table 4 provides details on the fertility and health status parameters examined. At the beginning of the trial there were no differences (P > 0·05) in the performed comparisons, relating to the anoestrus rate (anoestrus 1), the return to oestrus rate (return to oestrus 1) and the proportion of sows that exhibited a weaning-to-first oestrus interval shorter than 10 days (WOI1 < 10). It is noteworthy that four sows [one in each of the (Z+A−) and (Z−A+), as well as two in (Z−A−) subgroups) returned to oestrus twice within a total interval of 50 days after the first-oestrus service. Furthermore, three sows that exhibited return to oestrus [one in (Z+A−) and two in (Z−A−) subgroups] were included in the total number of the nine sows that failed to farrow and thus, they were subsequently excluded from the trial. The farrowing rate was not diversified significantly among the experimental groups and subgroups, although the values obtained in (Z+) and (A+) groups were slightly higher. Likewise, comparing the results among the experimental subgroups, the same parameters after the second weaning on trial (anoestrus rate 2, return to oestrus 2, WOI2 < 10) did not differ. On the contrary, comparing the two main experimental groups (Z+ and Z−), anoestrus rate 2 and return to oestrus rate 2 were significantly lower (P < 0·05) in the (Z+) group. Additionally, among the CTC treated sows (A+), the proportion of them with a weaning-to-first oestrus interval shorter than 10 days after the second weaning on trial (WOI2 < 10) tended to be higher (P = 0·09) than the respective proportion among the non-CTC treated sows (A−).

As also shown in Table 4, there was no difference (P > 0·05) in the incidence of inappetence, pyrexia, mastitis and vaginal discharge during lactation among the main experimental groups (Z+ and Z−). However, CTC effect was pronounced, since among the CTC treated sows (A+) the incidence of the foresaid conditions was significantly lower (P < 0·05), something that was also partially reflected in the results of the comparison among the experimental subgroups. Thus, significant difference (P < 0·05) was noticed relating to the incidence of inappetence, with the lower levels been recorded in the (Z+A+) and (Z−A+) subgroups, where the diets were supplemented with CTC. The same subgroups also tended to show lower occurrence of mastitis (*P* = 0·1) and vaginal discharge (P = 0·09).

Table 5 presents the mean weaning-to-oestrus (WOI) interval at the commencement of the trial and after the second weaning on trial, as well as the mean pregnancy and lactation intervals. Neither NZ nor CTC had any effect on WOI 1. However, WOI 2 was significantly affected by the dietary inclusion of CTC (P < 0·05), with the lower means been reported in (Z+A+) and (Z−A+) subgroups.

The influence of NZ and CTC on the mean number of piglets born alive and weaned, as well as the mean piglet bodyweight (PBW) at birth and weaning and the mean piglet bodyweight gain (PBWG) during lactation are shown in Table 6. The mean numbers of piglets born alive and weaned were increased by the inclusion of both NZ and CTC (P < 0·01). Furthermore, the higher mean number of piglets weaned was reported in the presence of the antibacterial agent. As far as the mean piglet bodyweights at birth and at weaning are concerned, they were both increased (P < 0·01 and < 0·05, respectively) by the supplemental NZ and it was demonstrated to be increased even more, by the presence of CTC (P < 0·01). Zeolite × antibacterial interactions, related to the above mentioned parameters, were also recorded. Finally, the mean piglet body weight gain during lactation was increased by the use of CTC (P < 0·01), while the effect of the supplemental NZ tended also to be significant (P = 0·08).

Table 7 presents the prevalence of litters having at least one piglet with body malformations, such as splayleg or oedematous swelling and reddening of the vulva. All the cases with swelling and reddening of the vulva were noticed within the first 24 hours after birth. The results revealed that the dietary use of NZ in the (Z+) group tended to reduce (P = 0·09) the prevalence of

TABLE 4: Sows/gilts health status and fertility parameters: cases in category/total animals examined (percentage)

	Zeolite (Z)		Antibacterial (A)		Experimental subgroups (ZA)			
	+	−	+	−	Z+A−	Z+A+	Z−A−	Z−A+
Anoestrus 1*	4/89 (4·4)	7/89 (7·8)	3/58 (5·2)	8/120 (6·7)	3/60 (5)	1/29 (3·4)	5/60 (8·3)	2/29 (6·8)
WOI1 < 10*†	75/85 (88·2)	72/82 (87·8)	48/55 (87·3)	99/112 (88·4)	50/57 (87·7)	25/28 (89·3)	49/55 (89·1)	23/27 (85·2)
Return to oestrus 1	12/116 (10·3)	16/113 (14·1)	7/77 (9·1)	21/152 (13·8)	9/77 (11·6)	3/39 (7·6)	12/75 (16·0)	4/38 (10·5)
Farrowing rate‡	100/116 (86·2)	89/113 (78·7)	67/77 (87·0)	122/152 (80·3)	65/77 (84·4)	35/39 (89·7)	57/75 (76·0)	32/38 (84·2)
Inappetence§	39/111 (35·1)	42/103 (40·8)	17/74 (23·0)^{b}	64/140 (45·7)^{a}	31/73 (42·5)^{ab}	8/38 (21·0)^{c}	33/67 (49·2)^{a}	9/36 (25·0)^{bc}
Pyrexia§	29/111 (26·1)	30/103 (29·1)	14/74 (18·9)^{b}	45/140 (32·1)^{a}	22/73 (34·1)	7/38 (18·4)	23/67 (34·3)	7/36 (19·4)
Mastitis§	16/111 (14·4)	18/103 (17·5)	6/74 (8·1)^{b}	28/140 (20·0)^{a}	13/73 (17·8)	3/38 (7·9)	15/67 (22·4)	3/36 (8·3)
Vaginal discharge§	9/111 (8·1)	7/103 (6·8)	1/74 (1·4)^{b}	15/140 (10·7)^{a}	8/73 (10·9)	1/38 (2·6)	7/67 (10·4)	0/36 (0·0)
Anoestrrus 2	8/110 (7·3)^{b}	16/101 (15·8)^{a}	6/73 (8·2)	18/138 (13·0)	6/72 (8·3)	2/38 (5·3)	12/66 (18·2)	4/35 (11·4)
WOI2 < 10†	76/102 (74·5)	58/85 (68·2)	53/67 (79·1)	81/120 (67·5)	47/66 (71·2)	29/36 (80·6)	34/54 (63·0)	24/31 (77·4)
Return to oestrus 2¶	8/102 (7·8)^{b}	15/85 (17·6)^{a}	5/67 (7·5)	18/120 (15·0)	6/66 (9·1)	2/36 (5·5)	12/54 (22·2)	3/31 (9·7)

*Gilts have been excluded; †Weaning-to-first oestrus interval shorter than 10 days; ‡Farrowing rate based on first service after weaning; §During lactation; ¶Within an interval of 44 days after first oestrus service. ^{a,b,c}Frequencies in the same row (under column Z, A or ZA) with different superscripts differ significantly (P < 0·05).

TABLE 5: Effect of the presence (+) or absence (−) of zeolite and antibacterial on weaning-to-first oestrus interval at the commencement of the trial (1) and after the second weaning on trial (2), as well as mean pregnancy and lactation intervals (days)

Zeolite (Z)	+		−					
Antibacterial (A)	−	+	−	+				
Experimental subgroups	Z+A−	Z+A+	Z−A−	Z−A+		Probability*		
	Mean (n)	Mean (n)	Mean (n)	Mean (n)	SD	Zeolite effect (Z)	Antibacterial effect (A)	Z × A interaction
WOI† 1	8·0 (57)	7·4 (28)	7·8 (55)	7·7 (27)	4·6	NS	NS	NS
Pregnancy interval	115·1 (73)	115·3 (38)	115·3 (67)	115·0 (36)	1·2	NS	NS	NS
Lactation interval	24·1 (73)	24·2 (38)	24·4 (67)	24·4 (36)	1·4	NS	NS	NS
WOI† 2	9·1[ab] (66)	8·4[b] (36)	10·3[a] (54)	8·8[b] (31)	3·2	NS	< 0·05	NS

*NS: no significant effect; †: weaning-to-first oestrus interval.
[a,b]Means in the same row with different superscripts differ significantly (P < 0·05).

TABLE 6: Effect of the presence (+) or absence (−) of zeolite and antibacterial on litter size and litter performance parameters

Zeolite (Z)	+		−					
Antibacterial (A)	−	+	−	+				
Experimental subgroups	Z+A−	Z+A+	Z−A−	Z−A+		Probability*		
	Mean (n = 73)	Mean (n = 38)	Mean (n = 67)	Mean (n = 36)	SD	Zeolite effect (Z)	Antibacterial effect (A)	Z × A interaction
No. of piglets born alive	10·01[b]	10·92[a]	9·13[c]	10·47[ab]	1·41	< 0·01	< 0·01	NS
No. of piglets weaned	9·07[b]	10·29[a]	8·12[c]	9·83[a]	1·44	< 0·01	< 0·01	NS
PBW† at birth (kg)	1·41[b]	1·48[a]	1·30[c]	1·40[b]	0·09	< 0·01	< 0·01	< 0·05
PBW at weaning (kg)	6·20[b]	6·44[a]	5·86[c]	6·38[a]	0·52	< 0·05	< 0·01	< 0·05
PBWG‡ during lactation (kg)	4·78[a]	4·96[a]	4·56[b]	4·97[a]	0·50	< 0·1	< 0·01	NS

*NS: no significant effect; †Piglet bodyweight; ‡Piglet bodyweight gain.
[a,b,c]Means in the same row with different superscripts differ significantly (P < 0·05).

TABLE 7: Prevalence of litters with piglets showing malformations: cases in category/total litters examined (percentage)

	Zeolite (Z)		Antibacterial (A)		Experimental subgroups (ZA)			
	+	−	+	−	Z+A−	Z+A+	Z−A−	Z−A+
Splay-legs	11/111 (9·9)	18/103 (17·5)	10/74 (13·5)	19/140 (13·6)	7/73 (9·6)	4/38 (10·5)	12/67 (17·9)	6/36 (16·7)
Swelling and reddening of the vulva	5/111 (4·5)[b]	15/103 (14·5)[a]	6/74 (8·1)	14/140 (10·0)	4/73 (5·5)	1/38 (2·6)	10/67 (14·9)	5/36 (13·9)

[a,b]Frequencies in the same row (under column Z) with different superscripts differ significantly (P < 0·05).

splay-legs and resulted in a lower prevalence (P < 0·05) of piglets with swelling and reddening of the vulva. No difference (P > 0·05) was found concerning the prevalence of splay-legs among the experimenal subgroups. In the (Z+A−) and (Z+A+) subgroups there was a tendency (P = 0·08) for a lower prevalence of litters with piglets showing swelling and reddening of the vulva, comparing to the (Z−A−) and (Z−A+) subgroups.

DISCUSSION

In the present trial, it was shown that the long-term consumption of feed that was supplemented with NZ (clinoptilolite) at the inclusion rate of 2 per cent had no apparent negative effects on the overall performance of sows/gilts. No adverse or side effects were noticed in the treated animals or their offspring, thus confirming a previous report (Pond and Yen 1983), in which it was concluded that NZ, included at the rate of 5 per cent in the diet of the rat through one reproduction and lactation period was not associated with obvious toxicologic or teratogenic effects.

Considering the evaluation of the sows' health status after farrowing, CTC demonstrated a clear beneficial effect, since there was a significant decrease in the incidence of inappetence, pyrexia, mastitis and vaginal discharge among the treated sows. These findings are in accordance with previously reported results of a field trial dealing with the effects of CTC medicated sow's lactation feed (Sbiraki et al 2000) and are confirmatory to the general concept of the usual therapeutic approach which aims to the reduction of the consequences of metritis-mastitis-agalactia syndrome in sows (Klopfenstein et al 1999). On the other hand, one of the major concerns which the use of natural zeolites in animal nutrition arise, is the potential adsorbent effects of them on essential nutrients, such as vitamins and minerals, as well as on the antibacterials which are routinely used in the on-farm strategic medication programmes. In such case, if large quantities of antibacterial molecules are rendered unavailable to the animals via feed, this could have a non-desirable effect on both the performance enhancement and the health status preservation. From a clinical point of view, the trial results revealed no interactive effect of NZ on the availability of CTC, since the beneficial effect of the latter on the sow's health status during lactation was not inhibited by the simultaneous use of NZ.

The cumulative results of the present trial provide clear evidence of the beneficial effect of both additives on sows' and their litters' performance data. The dietary inclusion of NZ had a certain positive effect, as it increased the litter size at both birth and weaning. Also, it was proved to be beneficial to the piglet performance, providing higher piglet bodyweight at both birth and weaning. Similarly, the beneficial effect of CTC on the same parameters, as well as on the WOI1 interval was also clearly demonstrated. This is in agreement with observations, which have been previously reported and have established the positive response of in-feed antibiotics during the breeding period and lactation, as reflected to the improvement of the farrowing rate and of litter size and litter weight at farrowing and at weaning, the reduction of sow's bodyweight loss during lactation and the shortening of the subsequent WOI (Soma and Speer 1975, Maxwell et al 1994, Sbiraki et al 2000). It is noteworthy that the best results were obtained in (Z+A+) subgroup, where CTC was used in combination with NZ, possibly demonstrating an additive net effect.

Few evidence is available related to the effects of naturally occurring zeolites on sows/gilts and their litters' performance and thereupon the mode of action by which NZ exerts its beneficial effects has not been defined clearly yet. However, in studies with growing pigs the potential growth promoting action of NZ has been attributed to the high affinity of NZ for ammonium ions, resulting to the reduction in the uptake of ammonia produced from deamination of proteins during the digestive processes via the intestinal wall (Shurson et al 1984, Pond et al 1988). Ammonia is recognised as a cell toxicant in higher animals (Visek 1978) and the reduction of the amount which the intestinal epithelial cells are exposed to, could lead to a reduction of epithelial turnover, a sparing of energy and a better nutrient utilization. An improvement in the biological value of protein for pigs fed increasing levels of zeolite-A has been reported (Shurson et al 1984). In addition, it was demonstrated that hens which were on a NZ diet showed slightly better rates of lysine and methionine uptakes than those which were not (Olver 1997).

Indeed, these observations could offer an explanation on the improvement of the performance traits resulted by the dietary use of NZ during pregnancy and lactation. The increased piglet body weight at farrowing could be correlated to the improvement in the nutritional efficiency of sow feed during late gestation, in agreement with previously reported observations by Ma et al (1979). Similarly, the suckling piglet performance, as reflected to its body weight gain during lactation and its subsequent weaning weight, is essentially dependent on the ability of the sow to provide sufficient milk of adequate quality and thus, any factor influencing milk production is of considerable importance. In this direction, recent experiments with Holstein cows indicated a beneficial effect of dietary NZ on milk yield (Lopez et al 1992) and on milk quality (Roussel et al 1992). In our trial, the dietary use of NZ tended to improve the mean piglet body weight gain during lactation, resulting in a higher mean value in both (Z+A+) and (Z+A−) subgroups comparing to the control one (Z−A−). These findings are supportive to the hypothesis that NZ enhances sow's feed efficiency during lactation and the sparing effect of energy and nutrients for a better lactation performance, although its establishment requires further investigation in order to be clearly documented.

Zeolites are also included in the nutritional inert adsorbent compounds which may be used for the prevention of mycotoxicosis. In-vitro studies have shown that several non-nutritive adsorbent compounds can form stable mycotoxin-sorbent complexes, while dietary additions of them have shown to ameliorate the in-vivo toxic effects in farm animals of several mycotoxins (Ramos et al 1996b). In the disease history of the trial farm, frequent appearance of impaired reproductive efficiency problems were reported. These were correlated with mould contamination of the feed due to the farm supply with bad quality cereals and the inadequate raw materials storage conditions. Indeed, during the trial, mycotoxicological assays of pregnancy feed samples revealed a low contamination level with aflatoxin B_1 in one case, a medium contamination level with trichothecenes and a high one with zearalenone. In particular, the mean monthly concentration of zearalenone in June and in July, were far exceeded the advisory limit of 250 ppb (Deutsche Bundesministerium für Ernährung, Landwirtschaft und Forsten 2000). Zearalenone possesses potent oestrogenic properties and has been associated with pigs showing anoestrus, delayed return to oestrus post weaning (Étienne and Dourmad 1994), pseudopregnancy, reduced litter size and litter weight (Chang et al 1979). Furthermore, the farrowing of splay-leg piglets (Miller et al 1973) and the oedematous swelling and reddening of the vulva of newborn or suckling piglets (Dacasto et al 1995, Alexopoulos 2001), could be considered as reliable indicators of zearalenone toxicosis in sows. In our study, although the results of the mycotoxicological analysis showed a substantial variation over time, the mean zearalenone concentration to which the animals were exposed during pregnancy, did not differ significantly among the experimental subgroups (659 ppm, 672 ppm, 666 ppm and 658 ppm in (Z+A−), (Z+A+), (Z−A−) and (Z−A+) subgroups, respectively). Our results revealed a tendency for a lower prevalence of splay-legs and a significant reduction of litters with piglets showing swelling and reddening of the vulva in the (Z+) group. It is worth noticing that the piglets showing vulvovaginitis were exclusively derived from litters that had been farrowed in June and in July. In addition, the same group demonstrated a slightly higher farrowing rate, as well as significantly lower anoestrus and return to oestrus rates after the second weaning on trial. These findings implied that the incorporation of NZ in the sow/gilt diet of the (Z+) group probably reduced the detrimental effects of zearalenone toxicosis, maintaining the health status and the reproductive performance at an optimum level. However, an investigation based on slaughterhouse checks of the genital organs for the verification of the actual reasons behind cases of reproductive disturbances such as these that have been recorded in this trial (i.e. anoestrus, return to oestrus, failure to farrow and pseudopregnancy), has to be performed in the future.

Furthermore, the farrowing of litters with larger size could be correlated to the reduction of continuing zearalenone exposure of gestating animals. This observation confirms the previously reported beneficial effects of the dietary use of NZ throughout gestation on the litter size at birth (Ma et al 1979, Cheshmedzhiev et al 1985). However, in these studies the experimental feed was not subjected to mycotoxicological analyses.

The conclusion that could be drawn from the present study is that the nutritional use of NZ, as evaluated from a clinical point of view, does not seem to provoke any obvious adverse effect on sows/gilts or any negative interactive effect on the availability of in-feed CTC. Furthermore, NZ was found to be beneficial for sows/gilts and their litters' performance, as indicated by the improvement of some of the performance related traits estimated in this trial, additionally implying a protective role against the consequences of zearalenone mycotoxicosis. Finally, it is suggested that in future trials dealing with the overall effects of NZ on pigs, a detailed mycotoxicological evaluation of the used rations should also be considered.

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