Dietary effect of artificial zeolite on performance, immunity, faecal microflora concentration and noxious gas emissions in pigs

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Abstract

A total of 48 crossbred (Landrace×Yorkshire×Duroc) pigs (2 months old; 28.38±2.62 kg body weight) were randomly assigned to either a control (basal diet) or 0.5% artificial zeolite (AZ) [basal diet + 0.5% AZ, dry matter (DM) basis] dietary treatment group in a completely randomized block design. Growth performance, immunity, muscle composition, carcass quality, faecal microflora concentration and noxious gas emissions were then investigated. No significant variation was observed in average daily gain (ADG), average daily feed intake (ADFI) or gain:feed [kg gain/kg dry matter intake (DMI)] ratio between treatments. The IgM and IgA levels remained unchanged, whereas the IgG level increased in the AZ dietary group relative to the control (P<0.05). Carcass quality, muscle composition and cholesterol level were also unaffected by AZ supplementation (P>0.05). Although AZ had no significant effect on fecal yeast and Lactobacillus spp. concentrations, a significant reduction was observed for Bacillus spp. in growing and E.coli both in growing and finishing pigs (P<0.05). Additionally, AZ supplementation led to a reduction in faecal ammonia (NH₃), sulphur dioxide (SO₂) and hydrogen sulphide (H₂S) gases relative to the control (P<0.05). Based on these results, AZ had positive effects on faecal E.coli concentration and noxious gas emissions; however, further study with different levels of AZ to better understand its effects on growth performance of grower to finisher pigs is warranted.

Introduction

Pig production and noxious gas emissions are important issues associated with global meat production. High concentrations of agricultural air pollutants are related to human and animal health, ecological damage, loss of nitrogen as fertilizer, and malodorous emissions (Bull and Sutton, 1998; Čupr et al., 2005; Webb et al., 2005). Ammonia (NH₃), hydrogen sulphide (H₂S) and sulphur dioxide (SO₂) are the primary gaseous contaminants emitted from animal waste. Ammonia accelerates the formation of fine particulate in the atmosphere and plays a crucial role in the acidification and eutrophication of ecosystems (Krupa, 2003). In livestock buildings, NH₃ causes adverse effects on production, health and welfare (Banhazi et al., 2008). Ammonia emissions have significantly increased in many developed countries, as well as in some developing countries over the last 50 years (Aneja et al., 2008). About 15% of NH₃ emissions are associated with global pig production (Olivier al., 1998). Hydrogen sulphide is considered the most dangerous gas in animal buildings and manure storage. Indeed, H₂S is responsible for many animal and human deaths in animal facilities (Field, 1980; Oesterhelweg and Puschel, 2008; Taiganides and White, 1969). The United States Environmental Protection Agency (USEPA) has defined SO₂ as one of six criteria pollutants, and it is known to be a major precursor to acid rain. Additionally, trace amounts of SO₂ in the atmosphere exert a significant effect on global climate change (Ward, 2009). Over the last decade, researchers have focused on development of unconventional feed resources. The results of these studies have suggested that supplementation of feed with absorbents leads to improved animal performance (Chen et al., 2005b; Dakovic et al., 2005). It is well known that feeds not only provide nutrients for animals, but also contain a number of contaminants that may enter the food chain via animal products. Various studies have suggested that adsorbents such as clays, bentonites, zeolites, phyllosilicates and synthetic aluminosilicates can suppress mycotoxin bioavailability and its detrimental effects on animals via binding of aflatoxins, zearalenone and ammonium (Abbès et al., 2006; Abdel-Wahhab et al., 1999, 2002).

Zeolites are crystalline hydrated aluminosilicates of alkali and alkaline earth cations composed of three-dimensional frameworks of SiO₄ and AlO₄ tetrahedra linked through shared oxygen atoms to form open crystal lattices with approximately uniform pores of molecular dimensions. Zeolites are porous materials characterized by the ability to lose and gain water reversibly, to adsorb molecules of appropriate cross-sectional diameter (via adsorption or by acting as molecular sieves) and to exchange their constituent cations without major changes in their structure (ion-exchange property) (Filippidis et al., 1996; Mumpton and Fishman, 1977). Chen et al. (2005a) also reported the antibacterial and antymycotic activity of Biotite V (61.90% SiO₂, a commercial mineral additive) and stated that this property was due to its three-dimensional structure and ion exchange capacity. Clinoptilolite specifically adsorbs NH₄⁺ and it is therefore believed having the ability improve feed protein digestion (Leung et al., 2007). Owing to these properties, zeolites are used in a wide range of industrial and agricultural applications, particularly in animal nutrition (Mumpton, 1999). The beneficial effects of dietary zeolites include improved ADG and/or feed conversion in pigs (Mumpton and Fishman, 1977; Petkova et al., 1982), enhanced reproductive performance of sows and increased birth weight of piglets (Papaoannou et al., 2002). Recent investigations have focused on utilization of dietary natural and artificial zeolites to reduce environmental pollutants such aerial NH₃, H₂S and...
SO₂, which are the main components of pig manure that contribute to environmental pollution (Zahn et al., 1997). Indeed, zeolites with molecular sieving properties are capable of adsorbing approximately 30% of gas (Mumpston and Fishman, 1977). However, the performance of zeolites is related to the type used, its purity and physicochemical properties, as well as the amount added to the diet. This study was conducted to evaluate the effects of artificial zeolite (AZ) on growth performance, body immunity, carcass quality, faecal microflora concentration and noxious gas emissions in growing to finishing pigs.

Materials and methods

The experiment was performed at the experimental farm of Sunchon National University, Jeollanam-do, South Korea and the protocol of the current experiment was approved by the Animal Care and Use Committee of Sunchon National University.

Composition of the artificial zeolite

The experimental AZ was obtained from Goryochonggeon Co. Ltd., Korea. The main components of this product were, 45.86% SiO₂, 25.44% Al₂O₃, 10.77% Fe₂O₃, 8.52% Na₂O, 4.48% CaO, 2.28% MgO, 1.32% TiO₂, 0.71% SO₃, 0.30% K₂O, 0.14 P₂O₅, 0.11% MnO, 0.05% ZrO₂, 0.05% Cl, 0.03% CuO and 0.03% ZnO [Semi-quantitative (SQX) analysis, Rigaku, Japan].

Experimental design, animals and diets

A total of 48 (Landrace x Yorkshire x Duroc) crossbred pigs (2 months old; 28.38 ± 2.62 kg body weight) were used in this three months growth trial. Pigs were alloted by the initial body weight (BW) into two dietary treatments in a completely randomized block design. Each dietary treatment consisted of four replications, with six pigs per replication. The experimental dietary treatments were a control (basal diet) and 0.5% AZ [basal diet + 0.5% AZ, dry matter (DM) basis]. Commercial pellet pig grower and finisher diet (Table 1) were used as a basal diet formulated to meet the nutrient requirements recommended by the NRC (1998). The AZ diet was prepared separately by adding AZ at 0.5% (on DM basis) according to the weight/weight ratio. The crude protein, available phosphorus and calcium were measured on a DM basis (AOAC, 2000). Pigs were reared in an environmentally controlled house on a slatted-floor system in 24 adjacent pens and allowed ad libitum access to feed and water through a self-feeder and a nipple waterer throughout the experiment.

Measurements and analysis

Body weights were measured monthly from the beginning to the end of the experiment. Feed intake was determined by measuring feed residue monthly from the start of the experiment. The gain:feed ratio was then obtained by dividing the body weight gain by the feed intake.

For immunological analysis, blood samples were collected from the jugular vein of individual pigs at 2 months after the start of the experiment (growing) and at the end of the experiment (finishing). Following collection, sera were separated from blood samples by centrifugation at 4°C and 1610×g. Immunoglobulins (IgG, IgA, and IgM) were then determined using pig IgG (Cat. No. E 100-104), IgA (Cat. No. E 100-102) and IgM (Cat. No. E 100-100) ELISA Quantification kits (Bethyl Laboratories Inc., Montgomery, TX, USA) according to manufacturer’s instructions. Each experiment was run in duplicate and the results represent the means of three experiment. The absorbance of each well was measured using a micro plate reader (Thermo Lab Systems, Helsinki, Finland) at 450 nm (correction wavelength, 570 nm). The results were expressed as mg/mL of serum.

At the end of the experiment, pigs were slaughtered at a local commercial slaughterhouse in Suncheon, South Korea. After measuring the carcass weight, carcass grades were determined by a trained carcass evaluator according to Korea Institute for Animal Products Quality Evaluation (KAPE, 2010). In South Korea, pork carcasses are graded both in quality and conformation grade. In the present study, the quality grading was done based upon characteristics such as marbling, lean colour, and conditions of belly streaks and was scored as 3 point for grade 1+, 2 point for grade 1, and 1 for grade 2. The carcass conformation was graded as A, B and C by assessing carcass weight, backfat thickness, balance, muscle and fat conditions and finish, etc., and was scored as 3 point for A grade, 2 point for B grade and 1 point for C grade. Within approximately 2 h from slaughter, samples were stored at -20°C until required for analysis. To investigate the meat chemical compositions, longissimus muscles from the loin area were removed and ground using a meat grinder. The moisture, crude protein, crude fat and crude ash contents were then determined using the AOAC methods (AOAC, 2000). Additionally, the cholesterol concentration of the meat was determined according to the method described by King et al. (1998) using a gas chromatograph (DS 6200, Donam Co., Seongnam, Gyeonggido, South Korea).

At the end of the trial fresh faecal samples were collected directly from the rectum of the pigs and preserved at -20°C until analysis.

Table 1. Ingredients and chemical compositions of experimental diet.

| Ingredients, % fed basis | Grower, day 1 to 60 | Finisher, day 60 to 90 |
|-------------------------|---------------------|------------------------|
| Yellow corn             | 45.15               | 45.15                  |
| Wheat                   | 23.00               | 25.00                  |
| Wheat bran              | 4.00                | 4.00                   |
| Soybean meal            | 18.00               | 16.00                  |
| Limestone               | 0.98                | 0.78                   |
| Calcium phosphate       | 1.10                | 1.10                   |
| Salt                    | 0.25                | 0.25                   |
| Vit-min. premix²        | 0.55                | 0.55                   |
| Animal fat              | 2.50                | 2.50                   |
| Molasses                | 4.30                | 4.50                   |
| L-Lysine                | 0.17                | 0.17                   |
| Chemical composition³   | 3265.00             | 3265.00                |
| ME, kcal/kg             | 18.00               | 16.00                  |
| Crude protein, % DM     | 0.70                | 0.50                   |
| Available phosphorus, % DM | 0.55            | 0.45                   |
| Lysine, % DM            | 0.95                | 0.80                   |
| Methionine, % DM        | 0.30                | 0.27                   |

ME: metabolizable energy; DM: dry matter. “Premix provided the following nutrients per kg of diet: vitamin A, 3,000,000 IU; vitamin D₃, 2,100,000 IU; vitamin E, 15,000 IU; vitamin K, 2000 mg; vitamin B₁₂, 1500 mg; vitamin B₆, 4000 mg; vitamin B₃, 1000 mg; vitamin B₃, 15 mg; calcium pantothenate, 6500 mg; niacin, 20,000 mg; biotin, 110 mg; folic acid, 600 mg; Co, 300 mg; Cu, 3500 mg; Mn, 55,000 mg; Zn, 40,000 mg; I, 600 mg; Se, 130 mg.” Calculated values, based on National Research Council (1998) tabular values.

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pre-numbered pigs in sterile polyethylene bags, via manual stimulation of the internal and external anal sphincters to avoid further contamination. To determine the faecal microbrial concentration, 1 g faecal samples were serially diluted in sterile saline (9 mL) and then cultured on agar media in duplicate. De Man, Rogosa, and Sharpe (MRS) media was used to culture lactic acid bacteria (LAB), while nutrient broth (NB) was used for *Bacillus* spp. and yeast and mold (YM) agar for yeast (Difco, Detroit, MI, USA). Colonies were counted after incubation for 24-48 h at 37°C and the microflora concentration was expressed as log_{10} cfu/g faeces.

Pig faeces and urine were collected separately from two pigs in each replicate pen on last 2 days of the trial (three times in a day) and stored immediately at -20°C until use. At the end of the trial, total sampled urine and faeces for each replication were thawed and then homogenized. After which approximately 3 kg of stock slurries [fresh faeces (1.5 kg) and faeces-urine (0.75 kg+0.75 kg)] were incubated in plastic fermentation chambers equipped with a circulating fan for uniform distribution of heavy and light gases. The chambers contained a hole in the top cover to facilitate gas measurements, which were made using an attached flexible rubber tube that was clipped tightly at the end. Samples were collected in triplicate for each treatment and allowed to ferment for five days at room temperature (25°C), after which the gas was sampled using a Gastec (model GV-100) gas sampling pump (Gastec Corp., Japan); Gastec detector tube No. 3M (10-1000 ppm) and 3La (2.5-200 ppm) for NH_{3}; No. 4LK (1-400 ppm) and 4LT (0.1-4 ppm) for H_{2}S and detecting tube 5Lb (0.05-10 ppm) for SO_{2}. Prior to measurement, the slurry samples were shaken manually for approximately 30s to disrupt any crust formation on the surface and homogenize the samples. The measurements were repeated on day 10 and 15 after the initial measurement and the concentration of each gas was determined based on the average of three measurements.

**Results and discussion**

**Growth performance and immunity**

Zeolites (natural and synthetic) have shown both positive and negative effects on animal performance. Mumpton and Fishman (1977) described the stimulating activity of zeolite particles in the stomach and intestinal tract and reported that they ultimately improved animal health. Therefore, we investigated whether supplementation with AZ would exert positive effects on the growth performance of pigs. However, no significant effects on ADG, ADFI or feed efficiency of growing-finishing pigs were observed (Table 2), which is in agreement with the results reported by Chen *et al.* (2005a, 2005b) and Yan *et al.* (2010). In contrast, a significant increase in feed conversion efficiency relative to the control was observed in pigs provided clinoptilolite supplemented diets (Mumpton and Fishman, 1977). These variations among studies are likely due to the use of different adsorbents, animals and environmental conditions. Clinoptilolite is the best suited among all zeolites, as swine feed additive to improve NH_{4} retention and protein digestibility (Leung *et al.*, 2007).

Previous findings has suggested that zeolites may significantly affect the regulation of the immune system. In the present study, IgM and IgG level remained unchanged, whereas the IgG level increased (P<0.05) significantly in both growing and finishing stages in the AZ dietary group relative to the control (Table 3).

| Table 2. Effect of artificial zeolite on growth performance of growing to finishing pigs over 90 days. |
|-------------------------------------------------|-----------------|-----------------|
| Treatment                                      | Pooled SE       | P               |
| ADG, kg                                        |                 |                 |
| Months 0-1                                      | 0.96            | 0.90            |
| Months 1-2                                      | 0.96            | 0.95            |
| Months 2-3                                      | 0.74            | 0.45            |
| Months 0-3                                      | 0.89            | 0.42            |
| ADFI, kg DM                                    |                 |                 |
| Months 0-1                                      | 2.05            | 0.11            |
| Months 1-2                                      | 3.00            | 0.11            |
| Months 2-3                                      | 3.07            | 0.07            |
| Months 3                                        | 3.04            | 0.44            |
| Gain/feed, kg gain/kg DMI                      |                 |                 |
| Months 0-1                                      | 0.50            | 0.11            |
| Months 1-2                                      | 0.32            | 0.07            |
| Months 2-3                                      | 0.32            | 0.03            |
| Months 3                                        | 0.32            | 0.72            |

ADG, average daily gain; ADFI, average daily feed intake; DM, dry matter; DMI, dry matter intake. Data are the mean values of four replicate groups with six pigs per replication (n=24). Significant difference (P<0.05); tendency (P<0.10).

| Table 3. Effect of artificial zeolite on serum immunoglobulin level of growing to finishing pigs. |
|-------------------------------------------------|-----------------|-----------------|
| Pigs                                           | Treatment       | Pooled SE       | P               |
| IgM, mg/mL                                     |                 |                 |
| Growing                                        | 11.94           | 1.43            | 0.550           |
| Finishing                                      | 32.54           | 1.84            | 0.661           |
| IgG, mg/mL                                     |                 |                 |
| Growing                                        | 88.05           | 1.57            | 0.004           |
| Finishing                                      | 143.74          | 2.04            | 0.042           |
| IgA, mg/mL                                     |                 |                 |
| Growing                                        | 6.22            | 0.98            | 0.894           |
| Finishing                                      | 7.83            | 1.13            | 0.730           |

IgM, immunoglobulin M; IgG, immunoglobulin G; IgA, immunoglobulin A. Data are the mean values of four replicate groups with three pigs per replication (n=12). Significant difference, P<0.05; tendency, P<0.10.
Since there is no specific mechanism of AZ that may enhance intestinal antibody absorption in growing and finishing pigs, these differences may have been due to the binding effect of AZ. Ueki et al. (1994) reported that silica, silicates, and aluminosilicates may act as non-specific immunostimulators in a manner similar to that of the superantigens (SAgs), a class of powerful, immunostimulatory bacterial and viral toxins that are able to cause a number of diseases characterized by fever and shock. Unlike conventional antigens, SAgs bind as unprocessed proteins to particular motifs of the variable region of the β chain (Vß) of the T-cell receptor (TcR) outside the antigen-binding groove and to invariant regions of major histocompatibility complex (MHC) class II molecules on the surface of antigen-presenting cells (APCs). As a consequence, SAgs, in nanogram to picogram concentrations, stimulate up to 10 to 30% of the host T-cell repertoire, whereas in conventional antigenic peptide-TcR binding, only 1 in 10^5 to 10^6 T cells (0.01-0.0001%) is activated (Muller-Alouf et al., 2001).

**Muscle characteristics and composition**

AZ had no significant effect on carcass quality grade and conformation grade of pork (Table 4), which is consistent with the results of a study conducted by Yan et al. (2010). The toxic cation absorption capacity of zeolite prevented adverse effects on metabolic functions in hogs (Pond et al., 1993), resulting in no effect on carcass quality. Supplementation of the diet of swine with clinoptilolite at 4 to 8% had no significant effect on carcass quality. Dietary supplementation with ZnO supported zeolite (Z-ZnO) decreased the viable count of E. coli in pig (Hu et al., 2013). Hrenovic et al. (2012) also demonstrated that ZnO exhibited higher antimicrobial abilities than ZnO.

Pig farming results in severe pollution of the environment through noxious gas emissions, which must be addressed in intensive animal agriculture. Exposure to high levels of noxious gases such as volatile sulphurs, phenols, indoles and volatile amines in livestock and animal agriculture. Exposure to high levels of noxious gases such as volatile sulphurs, phenols, indoles and volatile amines in livestock and animal agriculture.

**Table 4. Effect of artificial zeolite on carcass quality, muscle composition (g per 100 g) and cholesterol content (mg per 100 g) of growing to finishing pigs.**

| Treatments               | Control | Zeolite 0.5% | P  |
|--------------------------|---------|--------------|----|
| Carcass quality          |         |              |    |
| Carcass quality grade a  | 1.95    | 2.00         | 0.06 | 0.614 |
| Conformation grade b     | 2.48    | 2.52         | 0.08 | 0.750 |
| Muscle composition       |         |              |    |
| Moisture                 | 71.32   | 70.23        | 2.49 | 0.770 |
| Crude protein            | 24.82   | 26.06        | 1.98 | 0.680 |
| Cholesterol              | 81.57   | 85.17        | 1.94 | 0.260 |

*Grade 1+<3; grade 1=2; grade 2=1 (according to KAPE, 2010); #grade A=3; grade B=2; grade C=1. Data are the means of four replicate groups with three samples per replication (n=12). Significant difference, P<0.05; tendency, P<0.10.

**Table 5. Effect of artificial zeolite on faecal microflora concentration of growing-finishing pigs (Log10 cfu/g).**

| Pigs                  | Treatment | Control | Zeolite 0.5% | P  |
|-----------------------|-----------|---------|--------------|----|
| Yeast                 | Growing   | 7.10    | 6.68         | 0.73 | 0.710 |
|                       | Finishing | 5.77    | 5.71         | 0.75 | 0.957 |
| Lactobacillus spp.    | Growing   | 8.14    | 9.06         | 0.68 | 0.394 |
|                       | Finishing | 8.49    | 8.97         | 1.22 | 0.798 |
| Bacillus spp.         | Growing   | 6.80    | 4.99         | 0.45 | 0.045 |
|                       | Finishing | 6.25    | 6.30         | 0.80 | 0.965 |
| E. coli               | Growing   | 5.27    | 4.25         | 0.18 | 0.022 |
|                       | Finishing | 5.87    | 4.18         | 0.16 | 0.002 |

Data are the means of four replicate groups with three samples per replication (n=12). Significant difference, P<0.05; tendency, P<0.10.

**Table 6. Effect of artificial zeolite on faecal noxious gas emission (ppm) of growing to finishing pigs.**

| Treatment | Control | Zeolite 0.5% | P  |
|-----------|---------|--------------|----|
| NH₃       | 61.00   | 20.67        | 3.61 | 0.002 |
| SO₂       | 0.46    | 0.33         | 0.02 | 0.009 |
| H₂S       | 4.50    | 2.88         | 0.30 | 0.020 |

Data are the means of four replicate groups with three samples per replication (n=12). Significant difference, P<0.05; tendency, P<0.10.
farms not only adversely affects the health of animals and workers, but can also cause environmental problems such as nitrification and acidification of rain (Ferket et al., 2002; Le et al., 2005; Ushida et al., 2003). In the present study, AZ treatment led to a significant reduction in NH₃, SO₂ and H₂S emissions relative to the control treatment (P<0.05) (Table 6), which is consistent with the results reported by Chen et al. (2005a). Dietary supplementation of zeolite can also suppress NH₃ emissions from both the digestive tract and bedding (Meisinger et al., 2001). Moreover, the reduction of NH₃ and H₂S emissions may be attributable to the reduced faecal E. coli levels (Arakawa et al., 2000). Overall, the results of the present study indicate an increased IgG level, reduced faecal E. coli concentration and suppressed faecal noxious gas emissions. Additionally, AZ was found to be an ideal growth medium for nitrifying bacteria that ultimately oxidized NH₄⁺ to nitrate and controlled the viscosity and nutrient retention of the manure.

Conclusions

Based on the results of this study, dietary supplementation with 0.5% AZ had no effect on growth performance, carcass quality or conformation grade. However, an improvement in IgG level and reduction in faecal E. coli concentrations was detected. Moreover, dietary AZ significantly reduced faecal noxious gas emissions. Accordingly, further research with different levels of AZ should be conducted to better understand the effects of AZ on growth performance of grower to finisher pigs.

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