Experimental study on the effect of zeolite (clinoptilolite) on the growth performance, nutrient digestibility, and faecal microbiota of finishing pigs

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ABSTRACT

The aim of this study was conducted to determine the clinoptilolite in diets as a growth promoter on the growth performance, nutrient digestibility, and faecal microflora in finishing pigs. A total of 180 finishing pigs [(Landrace × Yorkshire) × Duroc; 50.20 ± 1.92 kg] were used in 84 days in experimental feeding trials. There were 12 replication pens in each treatment, with 5 pigs/pen (three barrows and two gilts). Dietary treatment groups were as follows: (1) basal diet (CON), (2) CON + 0.05% clinoptilolite (T1), (3) CON + 0.1% clinoptilolite (T2). On day 84, there was a linear increase in body weight (BW) (\(P < 0.05\)) of finishing pigs fed diets supplemented with clinoptilolite. During day 35–84 and overall experimental period, increasing the concentration of clinoptilolite linearly improved the average daily gain (ADG) (\(P < 0.05\)) and gain:feed ratio (G/F) (\(P < 0.05\)). The clinoptilolite treatments reduced \textit{Escherichia coli} (\(P < 0.05\)) counts compared with CON treatment. And, no significant effect was found in any treatments on nutrient digestibility (\(P > 0.05\)). In conclusion, the dietary supplementation with clinoptilolite has been shown to improve growth performance and gut health in finishing pigs.

1. Introduction

Antibiotic growth promoters have been banned by the European Union in food animal production since 2006 (Valpotić et al. 2016). Therefore, looking for a cost-effective method becomes a very rewarding issue, especially natural materials capable of maintaining animal health and improving growth performance is widely accepted.

Clinoptilolite is a member of the naturally occurring zeolite family of minerals. On the other hand, clinoptilolite tuffs are the most abundant natural zeolite with a zeolite of approximately 0.76–0.95% (Defang and Nikishov 2009). Clinoptilolite has found widespread environmental applications due to important properties of adsorption, catalysis and ion exchange such as purification of water, soil improvement, cleaning of fish pond, food supplement and radioprotection etc. (Wu et al. 2016). Previous studies of zeolite were more concerned about its ion exchange capacity and the ability to absorb ammonium ion (Hedström 2001). Many studies suggested that clinoptilolite can adsorb some mycotoxins, and may improve growth performance in an animal such as pig, sheep, poults, and dairy cows (Forouzani et al. 2004; Papaioannou et al. 2004; Katsoulos et al. 2006; Hcini et al. 2018), and alter the blood and/or tissue mineral concentrations in growing pigs, dairy cows and broilers, etc. (Alexopoulos et al. 2007).

However, the beneficial effects of natural zeolite on the host have a great relationship with the differences in type, geographical source, and additional level used in a diet. Therefore, the principal objective of this study was to evaluate the mycotoxin binder clinoptilolite in gut health improvement and improvement in growth performance in finishing pigs.

2. Materials and methods

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Dankook University (DK-2-1827).

2.1. Source of clinoptilolite

The experimental clinoptilolite was obtained from Calsnbtt Co. Ltd., Korea. The main components of this product were, 45.86% SiO\textsubscript{2}, 25.44% Al\textsubscript{2}O\textsubscript{3}, 10.77% Fe\textsubscript{2}O\textsubscript{3}, 8.52% Na\textsubscript{2}O, 4.48% CaO, 2.28% MgO, 1.32% TiO\textsubscript{2}, 0.71% SO\textsubscript{3}, 0.30% K\textsubscript{2}O, 0.14 P\textsubscript{2}O\textsubscript{5}, and others 0.18%.

2.2. Animal management, experimental design, and diets

A total of 180 finishing pigs [(Landrace × Yorkshire) × Duroc; 50.20 ± 1.92 kg] were used in an 84-day trial to evaluate the growth performance, nutrient digestibility, and faecal microbiota which were randomly allotted into three treatments.
There were 12 replication pens in each treatment, with 5 pigs/pen (three barrows and two gilts). Dietary treatment groups were as follows: (1) basal diet (CON), (2) CON + 0.05% clinoptilolite (T1), (3) CON + 0.1% clinoptilolite (T2). The experimental period was divided into 2 phases: Phase 1: 1–42 days and Phase 2: 43–84 days. The diets were formulated to meet or exceed NRC (2012) recommendations for all nutrients (Table 1). Pigs were housed in an environmentally controlled facility with slatted plastic flooring and a mechanical ventilation system. Each pen was equipped with a single face self-feeder and a nipple drinker to allow the pig ad libitum access to feed and water throughout the experimental period.

### 2.3. Growth performance and nutrient digestibility

Pigs were weighed at day 0 and day 35, and day 84 of the experimental period. Feed consumption was recorded per pen during the experiment to calculate the average daily gain (ADG), average daily feed intake (ADFI), and gain: feed ratio (G/F).

On day 78, chromium oxide (Cr₂O₃, 2 g/kg) was added to the diet as an indigestible marker to measure digestibility. Fresh faecal samples were collected directly via rectal massage from at least two pigs in each pen at the end of the experiment to determine dry matter (DM) and nitrogen (N). All faecal and feed samples were stored at −20°C until analysed. Prior to chemical analysis, the faecal samples were thawed and dried for 72 h at 60°C, after which they were ground to pass through a 1 mm screen. The feed and faecal samples were analysed for DM and N according to AOAC (2005). Chromium was analysed by UV absorption spectrophotometry (Shimadzu, UV-1201, Kyoto, Japan). The apparent total tract digestibility was then calculated using the following formula: Digestibility (%) = \[ \frac{(N_f \times C_d)/(N_d \times C_f)}{1} \times 100 \] where N_f = nutrient concentration in faeces (% DM), N_d = nutrient concentration in diet (% DM), C_d = chromium concentration in diet (% DM), and C_f = chromium concentration in faeces (% DM).

### Table 1. Composition of the experimental finishing pig diets (as-fed basis).

| Items                              | phase 1 (1-42 days) | phase 2 (43-84) |
|------------------------------------|---------------------|-----------------|
| Ingredients %                      |                     |                 |
| Corn                               | 37.98               | 36.15           |
| Wheat                              | 24                  | 29              |
| Rice bran                          | 2                   | 2               |
| Parm kernell meal                   | 3                   | 3               |
| Soybean meal                       | 3                   | 3               |
| Dehulled Soybean meal              | 11.34               | 8.12            |
| Rape seed meal                     | 4                   | 4               |
| Sesame meal                        | 2                   | 2               |
| Brown rice                         | 5                   | 5               |
| Animal fat                         | 3.26                | 2.89            |
| Mollases                           | 2                   | 2               |
| Limestone                          | 1.08                | 1.1             |
| Monocalcium phosphate              | 0.1                 | 0.09            |
| Salt                               | 0.3                 | 0.3             |
| Methionine 98%                     | −                   | 0.01            |
| Threonine 98%                      | 0.01                | 0.05            |
| Lysine 25%                         | 0.49                | 0.79            |
| Choline chloride 50%               | 0.09                | 0.1             |
| Vitamin/mineral mixture*           | 0.35                | 0.4             |
| Chemical composition               |                     |                 |
| Digestible energy (kcal/kg)        | 3540                | 3510            |
| Metabolic energy (kcal/kg)         | 3260                | 3250            |
| Crude protein (%)                  | 16.00               | 15.00           |
| Crude fat (%)                      | 5.90                | 5.50            |
| Crude ash (%)                      | 4.20                | 4.10            |
| Crude fiber (%)                    | 3.90                | 3.90            |
| Total lysine (%)                   | 0.88                | 0.86            |
| Calcium (%)                        | 0.65                | 0.65            |
| Phosphorus                         | 0.39                | 0.39            |

Notes: CON = basal diet; T1 = basal diet + 0.05% clinoptilolite; T2 = basal diet + 0.10% clinoptilolite; SEM = Standard error of means.

### 2.4. Faecal microbial

Faecal samples were collected directly by massaging the rectum of 2 pigs randomly selected from each pen (1 gilt and 1 barrow) on day 84, and pooled, and transported to the laboratory, where the microbial analysis was immediately carried out. The obtained faecal sample (1 g) from each pen was diluted with 9 mL of 10 g/L peptone broth (Becton, Dickinson, and Co., Rutherford, NJ, USA), and homogenized. Viable counts of bacteria in the faecal samples were then conducted by plating serial 10-fold dilutions (in 10 g/L peptone solution) onto MacConkey agar plates and lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate the *Escherichia coli* (*E. coli*) and *Lactobacillus*, respectively. The *lactobacilli* medium III agar plates and MacConkey agar plates were then incubated for 48 h at 39°C and 24 h at 37°C under anaerobic conditions, respectively. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator.

### 2.5. Statistical analyses

The data were analysed using the GLM procedure of SAS (2013) in a randomized complete block design using pen as the experimental unit. The initial BW was used as a covariate for the ADFI and ADG. Linear and quadratic polynomial contrasts were used to examine responses to supplemental graded levels of clinoptilolite at 0%, 0.05% and 0.1% in the basal diet. Variability in the data was expressed as the standard error of means (SEM). A probability level of *P* < 0.05 was considered to be statistically significant and 0.05 < *P* < 0.10 indicating trends.

### 3. Result

#### 3.1. Growth performance and nutrient digestibility

The effect of clinoptilolite supplementation into the diet of finishing pigs on the growth performance, nutrient digestibility of finish pigs is presented in Table 2. On day 84, there was a linear increase in BW (*P* < 0.05) of finishing pigs fed diet supplemented with graded levels of clinoptilolite. Increasing the concentration of clinoptilolite linearly improved the ADG and G/F (*P* < 0.05) during day 36 - 84 and the overall experimental period. In addition, the BW at day 35, the ADFI during day 36–84 and during the overall experimental period showed a linear increasing trend (0.05 < *P* < 0.1) associated with the clinoptilolite supplementation. But, no difference (*P* > 0.05) on
nutrient digestibility was observed compared with CON treatment.  

**3.2. Faecal microbial**

The effect of clinoptilolite feed supplementation on the faecal microbial of finish pigs is presented in Table 3. The dietary supplementation of clinoptilolite to finishing pigs reduced *E. coli* (*P* < 0.05) counts compared with pigs fed with CON diet, and showed a linear decreased trend associated with the clinoptilolite.

**4. Discussion**

In the present study, supplementation of clinoptilolite has shown a significant increase in ADG and a trend in the increase in ADFI and G/F in finishing pigs. This is in agreement with Alexopoulos et al. (2007), who indicated that clinoptilolite-rich tu

| Items | CON | T1 | T2 | SEM | Linear | Quadratic |
|-------|-----|----|----|-----|--------|-----------|
| Body weight, kg | 50.20 | 50.20 | 50.19 | 0.006 | 0.198 | 0.771 |
| Day 35 | 76.65 | 77.23 | 77.69 | 0.40 | 0.053 | 0.896 |
| Day 84 | 116.01 | 117.68 | 119.07 | 0.80 | 0.018 | 0.891 |
| Day 0–35 | 756 | 772 | 786 | 10 | 0.052 | 0.899 |
| ADFI, g | 2241 | 2252 | 2282 | 18 | 0.124 | 0.684 |
| G/F | 0.337 | 0.343 | 0.344 | 0.002 | 0.053 | 0.454 |
| Day 36–84 | 803 | 826 | 845 | 11 | 0.015 | 0.905 |
| ADFI, g | 2702 | 2726 | 2756 | 19 | 0.058 | 0.896 |
| G/F | 0.297 | 0.303 | 0.306 | 0.002 | 0.031 | 0.756 |
| Overall | 783 | 803 | 820 | 10 | 0.017 | 0.885 |
| ADG, g | 2510 | 2529 | 2559 | 17 | 0.060 | 0.791 |
| G/F | 0.312 | 0.317 | 0.320 | 0.002 | 0.012 | 0.553 |
| Nutrient digestibility, % | | | | | |
| Dry matter | 71.15 | 71.53 | 71.64 | 0.66 | 0.599 | 0.871 |
| Nitrogen | 70.62 | 70.63 | 71.01 | 0.74 | 0.716 | 0.841 |
| Notes: Values represent the means of 12 pens with 5 pigs per replicate pen (*n* = 60) per treatment for body weight, and 12 pens (*n* = 12) per treatment for ADG, ADFI, G/F, Dry matter and Nitrogen. CON = basal diet; T1 = basal diet + 0.05% clinoptilolite; T2 = basal diet + 0.10% clinoptilolite; SEM = Standard error of means.

The results of this study also showed that the ATTD of DM and N were not affected by clinoptilolite supplementation. Similarly, Fokas et al. (2004) also found that in pigs given 2% clinoptilolite in the diet, the apparent digestibility of the other constituents remained unaffected except ether extract. In another study, Shurson et al. (1984) observed different results that increasing graded levels of clinoptilolite in the diet linearly reduced N digestibility in piglets, but the biological value of protein was increased. The variation in the results of these studies can be ascribed to several factors, including the age of the pigs, housing conditions, presence of mycotoxins or other antinutritional factors in animal feeds, etc.

The results of faecal microbial have shown that the faecal *E. coli* counts were significantly reduced in finishing pigs fed clinoptilolite supplemented diet. Islam et al. (2014) reported that the concentration of *E. coli* decreased in both grower and finisher pigs fed zeolite supplemented the diet. The results observed in this study have also been proved by Hu et al. (2013). Wu et al. (2013) reported a significant reduction of *E. coli* counts in broilers supplemented with the clinoptilolite diet. Usually, in a well-balanced microbial environment, the

Table 3. Effect of clinoptilolite supplementation on faecal microbial in finishing pigs.

| Items, log10 CFU/g | CON | T1 | T2 | SEM | Linear | Quadratic |
|--------------------|-----|----|----|-----|--------|-----------|
| Lactobacillus      | 7.45 | 7.53 | 7.51 | 0.04 | 0.355 | 0.372 |
| Escherichia coli   | 6.11 | 6.09 | 6.04 | 0.02 | 0.042 | 0.592 |
| Notes: Values represent the means of 12 pens with 2 pigs per replicate pen (*n* = 24) per treatment. CON = basal diet; T1 = basal diet + 0.05% clinoptilolite; T2 = basal diet + 0.10% clinoptilolite; SEM = Standard error of means.
numbers of E. coli are lower. The decrease in E. coli in this study may be due to the ion exchange, adsorption and catalytic properties of clinoptilolite could favour the physiological status of the intestinal epithelium enhance enzyme activity altering the gastrointestinal pH and the ionic composition of the lumen contents and affecting microbiota ecosystem. In addition, the improved microbiota ecosystem might be another reason for better growth performance.

5. Conclusions
In the context of this study, dietary supplementation with clinoptilolite has shown to improve the overall BW, ADG, and G:F. Moreover, it decreased the faecal E. coli counts. These results indicated that the clinoptilolite improves growth performance and gut health in finishing pigs.

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Disclosure statement
No potential conflict of interest was reported by the author(s).

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