# The effect of kaolin feeding on efficiency, health status and course of diarrhoeal infections caused by enterotoxigenic *Escherichia coli* strains in weaned piglets

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**ABSTRACT**: The purpose of the present study was to assess the effect of kaolin feeding on health status, body weight gain (BWG), course of diarrhoeal infections caused by enterotoxigenic strains of *Escherichia coli* (ETEC) and the level of mycobacterial contamination in weaned piglets. The testing was performed in two experiments involving 40 weaned piglets at the age of 28 days. In the infection-free experiment, piglets were fed a diet without (C0) or with 1% content of kaolin (K0) for 20 days. Subsequently, all of them were fed the same diet without kaolin supplementation for 39 days. Identical diets were fed during the infection experiment, and moreover, both groups (CI and KI) were orally infected with ETEC (O141:F18ac, STa+) on Day 1 of experiment. The short-term feeding of kaolin to weaned piglets had a significant positive effect on their BWG. During the period of feeding the kaolin-containing diets, BWG in C0 and K0 were 0.20 and 0.29 kg, respectively ($P < 0.05$), and in CI and KI 0.13 and 0.19 kg, respectively ($P < 0.05$). There was no evidence of side effects to their health, neither was there any change in biochemical and haematological profiles. In the infection experiment, a protective effect of kaolin on the course of ETEC infection was evident. Colonization and shedding of ETEC by piglets fed the kaolin diet were milder and had a shorter duration in comparison with control piglets. The culture examination of pure kaolin and kaolin containing diets for mycobacteria were negative. Potentially pathogenic mycobacteria occurring in the environment were isolated from faeces and tissues of pigs. According to these results, supplementation of diets with 1% kaolin to prevent diarrhoea in piglets and to support their growth in the critical post-weaning period could be recommended.

**Keywords**: kaolinite; aluminosilicate; clay; feed additives; enterosorbents; pig; growth; serum biochemistry; haematology; diarrhoea; *Mycobacterium avium* complex; feed safety

Infections which cause diarrhoea in weaned piglets are one of the most common causes of serious economic loss in swine herds (Madej et al., 1999; Hedemann and Jensen, 2004; Melin et al., 2004). Among pathogens, *Escherichia coli* infections caused by enterotoxinogenic strains of *E. coli* (ETEC) are found most frequently (Alexa et al., 1995, 2001; Bertschinger, 1999; Melin et al., 2004). Until recently antibiotic treatment and vaccination were primarily used to prevent enteric diseases in piglets. In 2002, the European Commission proposed new safety measures for the use of supplements in animal diets. An absolute ban on the use of antibiotic growth stimulators came into force in 2006 (Anonymous, 2002; Castro, 2005; Chen et al., 2005), mainly due to the potential occurrence of cross resistance to antimicrobial agents that are used in human medicine (Barton, 2000; Castro, 2005; Chen et al., 2005). Likewise, the requirements for health safety and safety of food of animal origin

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force the breeders to restrict their use of antibiotics in the prevention of animal diseases.

Recently, the use of various alternative feed supplements for pigs such as clays (kaolin, bentonites, zeolites, etc.) has been investigated. The effect of these natural materials in the prevention of enteric diseases is above all based on their ability to absorb enterotoxins and significant mycotoxins (Abdel-Wahhab et al., 1999; Phillips, 1999; Boranic, 2000; Dominy et al., 2004; Trckova et al., 2004).

Kaolin is a plastic raw material, largely containing the clay mineral kaolinite. In systematic mineralogy, it is classified as a phyllosilicate clay. Due to its adsorption qualities and absence of primary toxicity (Anonymous, 1998), the use of kaolin is considered to be a simple and effective prevention of the effects exerted by a number of toxic materials not only in the environment, but also in living organisms. Kaolin-based medicaments are commonly used for the treatment of diarrhoeal and digestive disorders in humans (Heimann, 1984; Kasi et al., 1995; Knezevich, 1998; Gebesh et al., 1999). When kaolin is added to animal diets, it reduces resorption of harmful toxins present in the feed through intestinal mucosa to the organism because it binds them firmly and selectively, and thus eliminates their toxic effects. A number of studies have demonstrated the excellent ability of kaolin to decontaminate enterotoxins which cause diarrhoea (Dominy et al., 2004), aflatoxins (Schell et al., 1993; Abdel-Wahhab et al., 1999; Phillips, 1999), plant metabolites (alkaloids, tannins), pathogenic microorganisms, heavy metals (Hassen et al., 2003; Katsumata et al., 2003), and poisons (Knezevich and Tadic, 1994). Kaolin added to farm animal diets can also influence their efficiency and perhaps also quality of meat (Savory 1984; Sakata, 1986; Kolacz et al., 2005).

Potential contamination of kaolin with potentially pathogenic mycobacteria (PPM) poses a threat to the health of animals, even if the original raw material is sterile. PPM are often found in surface water (Kazda, 2000; Matlova et al., 2003, 2004), which is used for kaolin floating during production. If the final product is damp and the temperature used for its drying is not sufficient to enable safe devitalisation of PPM (Pavlas et al., 1984), there exists a considerable risk of tuberculous lesion formation in pig lymph nodes. It is likely that *Mycobacterium avium* subsp. *hominissuis* and other non-identified slowly growing mycobacterial species were, due to this contamination factor, isolated from kaolin fed to pigs as a supplement (Matlova et al., 2003, 2004). However, other sources of PPM kaolin contamination, such as dust, ground above kaolin, bird faeces etc., should also be taken into consideration (Beerwerth and Schurmann, 1969; Dawson, 1971; Beerwerth and Kessel, 1976).

The purpose of the present study was to assess the effect of kaolin feeding on the health status and body weight gain of weaned piglets, on the occurrence and course of diarrhoeal *E. coli* infections caused by ETEC and the level of PPM contamination.

## MATERIAL AND METHODS

The effect of kaolin feeding was tested in an infection-free and infection experiment. Forty Pietrain × (Duroc × Large White × Landrace) weaned piglets at the age of 28 days were used.

### Infection-free experiment

A total of 20 weaned piglets were used in the experiment. Piglets were allocated at random to two groups of 10 animals each (numbers of barrows and gilts were equal). From day one after weaning, different groups were fed for a period of 20 days as follows:

– control group (C0) was fed with the feed mixture COS without kaolin supplementation;

– experimental group (K0) was fed the feed mixture COS supplemented with 1% of kaolin (Sedlec by kaolin, mine in Unanov, Znojmo, Czech Republic).

In the subsequent period, i.e., from Day 21 of experiment, the animals were fed a diet without kaolin for 39 days (up to reaching a body weight of about 30 kg). The experiment was completed by the slaughter of the animals. The content of basic nutrients (AOAC, 2001) in the aforementioned diets was analyzed. Their composition was in agreement with the requirements for different body weight categories of pigs. The used kaolin was preventatively treated with anti-fungal preparations (130°C, 45 min).

### Monitoring of health status and growth efficiency of pigs

The health status of animals in different groups was monitored during the experiment, above all the occurrence and intensity of diarrhoea, mortality, feed intake (FI) and body weight gain (BWG). Piglets were weighed at the beginning and on Days 20 and 59 of the trial. Feed conversion (FCR) was calculated from FI and BWG. Relative growth rate ($q$) was calculated according to Karakoz (1968):

$$q = [(y_t - y_o) / y_o] \times 100$$

where:
$y_o$ = body weight at the beginning
$y_t$ = body weight at the end of a particular period

Blood samples (10 ml) were drawn from *v. cava cranialis* for evaluation of the biochemical profile and clinical status of animals on Day 20 and 59 of experiment. The following biochemical parameters were assessed: glucose, total protein, albumin, triacylglycerols, cholesterol, activities of aspartate aminotransferase (AST) and alanin aminotransferase (ALT), alkaline phosphatase (ALP), calcium, phosphorus and iron. Blood tests (hematocrit, erythrocyte and leukocyte counts) were performed on samples collected on Day 20 of experiment.

### Histological examination

The effect of kaolin feeding on the histomorphology of intestinal mucosa and its protective effect against experimental *E. coli* infection was assessed. Histopathological examination of different parts of the intestine (duodenum, jejunum, ileum and colon) was performed on two piglets from each group, which were slaughtered on Day 20 of the experiment. The collected samples were fixed in 10% neutral buffered formalin for 24 h. Paraffin embedded sections (6 µm; Leica SM2000R) were mounted on silanized slides, and after deparaffinization, the sections were routinely stained with haematoxylin-eosin. Histopathological examination of the intestines was used for qualitative assessment of intestinal mucosa and for quantification of selected parameters (height of villi, numerosity of inflammatory cell infiltrate) using computer-assisted image analysis system LUCIA G (Laboratory Imaging, Prague, Czech Republic).

### Mycobacterial and serological examinations

Before the experiment was initiated, 42 kaolin samples were collected at different stages of the technological process of kaolin purification (mine, surface water used for raw material levigation, suspension of extracted material, levigation, cleaning, separation, coagulation, baling, drying and adjustment) and their culture examination for the presence of mycobacteria was performed according to methods described below. All experimental feed mixtures and samples from the pigsty environment were collected before and during the experiment (on Days 14 and 51 of experiment). Individual samples of pig faeces were collected on Days 1, 14, 20 and 59 of experiment. Lymph nodes (*lymphonodes submandibulares*, *pulmonales*, *tracheobronchiales*, *inquinales*, *jejunales*, *ileales,* and *hepatici*) and organ samples (liver, spleen, kidney, jejunum and ileum) were collected after feeding the experimental diets (on Day 20) and at the end of experiment (on Day 59) from two slaughtered pigs from each group.

Conventional culture methods were used for mycobacterial examination. After collection, kaolin and environmental samples were cultured at 37°C and 25°C in four media (solid Herrold's medium, Herrold's medium with Mycobactin J, Stonebrink's medium and liquid medium according to Sula). Organ samples and rectal swabs from pigs were cultured in the same media at 37°C. Before culture, the above mentioned samples were examined by microscopy, using the Ziehl-Neelsen staining method, for the presence of acid-fast rods (AFR), characteristic of mycobacterial infections.

Blood was collected from all pigs on Day 20 and 59 of experiment. The blood sera of pigs were tested by direct agglutination method, which consisted in the visual detection of the presence of a specific antigen-antibody reaction. Blood sera were tested for the presence of antibodies against mycobacteria from the *M. avium* complex (*M. avium* subsp. *avium*, *M. a.* subsp. *hominissuis* and *M. intracellulare*).

Bacterial isolates from culture were identified as mycobacteria by a conventional PCR as described previously (Moravkova et al., 2008). The species of isolates identified as *Mycobacterium* sp. were determined by the sequencing of the gene for 16S rRNA as described previously (Harmsen et al., 2003).

### Infection experiment

Having finished the infection-free experiment, the challenge experiment was performed on 20 weaned piglets, allocated into two groups with 10 animals in each. The experimental model was the same as in the infection-free experiment, with the difference that the groups were orally infected with ETEC No. 11732 (O141:F18ac, STa+) on Day 1. The infection was performed by individual oral administration of a paste containing ETEC, in a dose of $1.5 \times 10^{11}$ CFU (colony forming units) per head. On the following day, the animals were repeatedly infected by ETEC in feed in a dose of $3 \times 10^{10}$ CFU per head. The ETEC strain intended for the infection was cultured in casein hydrolysate medium for 16 h at 37°C. For individual administration, the culture was condensed by centrifugation and subsequently incorporated into semolina porridge paste. Control group CI was fed with the feed mixture COS I without kaolin. Experimental group KI was fed the feed mixture COS supplemented with 1% of kaolin. From Day 21 of experiment, the animals were fed the feed mixture without kaolin for 39 days (up to reaching the live body weight of about 30 kg) and the experiment was completed by slaughter of the animals.

### Bacteriological examination

Confirmation of intestinal colonization of the piglet intestines with the challenge strain was performed by bacteriological examination of rectal swabs from piglets collected before infection and on Days 1, 2, 4, 6, 8, 10, 13 and 15 after infection. The percentage of the administered ETEC strain among the *E. coli* strains shed in faeces was calculated (Alexa et al., 2002). Diluted strains were cultured on blood agar. In cultures, the number of suspected CFU was estimated. Suspected CFU were examined serologically. If found to belong to serogroup O141, the presence of colonization factors F18 was serologically identified.

### Monitoring of the health status and parameters of growth efficiency, histological, mycobacterial and serological examinations

Monitoring of the other parameters was the same as with the non-challenge experiment: the same amounts of samples, blood, faeces and organs were collected at the same time for different analyses.

## RESULTS

### Analysis of feed mixtures

The feed mixtures used in the present study were analyzed for essential nutrients (AOAC, 2001) and their composition was in agreement with the nutrient requirements for different weight categories of piglets (Simecek et al., 1993). Supplementation of the feed mixtures with 1% kaolin caused a slight reduction in the content of nitrogenous substances.

Table 1. Nutrient content in the diets

| Nutrients (g/kg) | Diet | | | | | |
|---|---|---|---|---|---|---|
| | COS[1] | | COS + kaolin[2] | | A1[3] | |
| Dry matter | 856.8 | 1 000.0 | 873.3 | 1 000.0 | 886.8 | 1 000.0 |
| Nitrogenous substances | 204.2 | 238.3 | 195.0 | 223.3 | 172.5 | 194.5 |
| Fat | 35.0 | 40.9 | 24.8 | 28.4 | 15.4 | 17.4 |
| Crude fibre | 31.9 | 37.2 | 27.6 | 31.6 | 34.6 | 39.0 |
| NDF | 118.8 | 138.7 | 94.2 | 107.9 | 122.6 | 138.2 |
| ADF | 57.5 | 67.1 | 59.5 | 68.1 | 61.4 | 69.2 |
| Ash | 52.6 | 61.4 | 56.5 | 64.7 | 55.8 | 62.9 |
| Nitrogen-free extracts | 533.1 | 622.2 | 569.4 | 652.0 | 608.5 | 686.2 |
| Organic matter | 804.2 | 938.6 | 816.8 | 935.3 | 831.0 | 937.1 |
| MEp (MJ/kg) | 13.2 | 15.4 | 13.2 | 15.2 | 12.9 | 14.6 |

[1]diet for weaned piglets without kaolin supplementation
[2]diet for weaned piglets supplemented with 1% of kaolin
[3]diet for pre-fattening pigs without kaolin supplementation

The content of metabolizable energy was almost identical in both mixtures (Table 1).

## Body weight parameters

Average live body weight (LWG), BWG and q in different groups are presented in Table 2. At the beginning of the experiment, no significant differences in LWG were detected between control and experimental piglets. After the 20-day feeding of a kaolin-containing feed mixture, significantly higher ($P < 0.05$) LWG was found in experimental piglets in comparison with the control group fed a diet without kaolin supplementation. The protective effect of kaolin was observed in both the infection-free and infection experiment. The BWG of piglets fed the feed mixture containing kaolin was significantly higher ($P < 0.05$) in comparison with piglets fed the feed mixture without kaolin supplementation. In the subsequent period from Day 21 to Day 59 of experiment, all pigs were fed the same feed mixture A1 without kaolin supplementation. In this period, no significant differences in BWG of piglets were noted (Table 2).

## Feed intake and conversion

Pigs readily consumed the experimental feed mixture containing kaolin. Over 20 days of feeding, its consumption was slightly larger in comparison with the control feed mixture. It was comparable in both infection and infection-free experiments (Table 2). Due to significantly higher BWG in experimental piglets in this period, higher feed conversion of the experimental feed mixture with kaolin was obtained in comparison with the feed mixture without kaolin supplementation. These results were similar in both the infection-free and infection experiments. However, due to the ETEC infection, higher feed conversion was observed in the infection experiment as opposed to the infection-free experiment, where the same feed mixtures were used (Table 2).

## Health, biochemical and haematological examinations

Monitoring of health status in animals by observation did not reveal any serious health prob-

Table 2. Average live body weight (LBW), body weight gain (BWG), relative growth rate ($q$) of pigs and feed intake (FI) and feed conversion (FCR) of diets

| Parameter | Day | Infection-free experiment | | Infection experiment | |
|---|---|---|---|---|---|
| | | C0[1] | K0[2] | CI[3] | KI[4] |
| LBW (kg) | 1 | 7.98 ± 0.77 | 8.39 ± 0.86 | 8.86 ± 0.66 | 9.40 ± 0.74 |
| | 20 | 11.93[a] ± 1.92 | 14.26[b] ± 2.48 | 11.48[a] ± 1.51 | 13.04[b] ± 0.95 |
| | 59 | 30.48 ± 4.16 | 31.99 ± 5.40 | 33.16 ± 3.40 | 33.59 ± 3.01 |
| BWG (kg/pig/day) | 1–20 | 0.20[a] ± 0.08 | 0.29[b] ± 0.10 | 0.13[a] ± 0.06 | 0.18[b] ± 0.03 |
| | 20–59 | 0.51 ± 0.09 | 0.48 ± 0.08 | 0.56 ± 0.06 | 0.53 ± 0.06 |
| | 1–59 | 0.39 ± 0.07 | 0.40 ± 0.08 | 0.41 ± 0.05 | 0.41 ± 0.04 |
| $q$ (%) | 1–20 | 52.42 ± 21.87 | 69.59 ± 22.21 | 29.55 ± 13.27 | 38.96 ± 7.51 |
| | 1–59 | 294.49 ± 57.25 | 265.68 ± 48.46 | 276.85 ± 31.99 | 264.06 ± 14.75 |
| FI (kg/pig/day) | 1–20 | 0.44 | 0.52 | 0.44 | 0.49 |
| | 20–59 | 1.34 | 1.00 | 1.27 | 1.27 |
| FCR | 1–20 | 2.14 | 1.78 | 3.37 | 2.68 |
| | 20–59 | 2.62 | 2.10 | 2.27 | 2.38 |

[1]control group fed a diet without kaolin supplementation
[2]experimental group fed a diet supplemented with 1% of kaolin
[3]control group infected with ETEC and fed a diet without kaolin supplementation
[4]experimental group infected with ETEC and fed a diet supplemented with 1% of kaolin
[a:b] = $P < 0.05$

Table 3. Selected biochemical characteristics of blood plasma of pigs

| Parameter | | Infection-free experiment | | | | Infection experiment | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | C0[1] | | K0[2] | | CI[3] | | KI[4] | |
| | | 20* ($n$ = 9) | 59* ($n$ = 6) | 20* ($n$ = 10) | 59* ($n$ = 6) | 20* ($n$ = 10) | 59* ($n$ = 6) | 20* ($n$ = 10) | 59* ($n$ = 6) |
| Total protein (g/l) | $\bar{x}$ | 52.30 | 52.35 | 50.91 | 51.75 | 50.85 | 56.83 | 53.64 | 59.05 |
| | SD | 6.89 | 6.53 | 3.54 | 4.22 | 4.48 | 4.41 | 4.53 | 5.62 |
| Albumin (g/l) | $\bar{x}$ | 27.22 | 26.17 | 28.20 | 27.67 | 26.70 | 29.50 | 28.20 | 28.83 |
| | SD | 2.95 | 2.71 | 3.55 | 3.27 | 2.98 | 1.38 | 2.10 | 2.86 |
| Glucose (mmol/l) | $\bar{x}$ | $4.67^{a}$ | 4.92 | $3.86^{b}$ | 5.48 | 2.65 | 6.35 | 2.50 | 6.67 |
| | SD | 0.53 | 0.31 | 0.98 | 0.69 | 0.55 | 0.81 | 0.58 | 1.20 |
| Triacylglycerols (mmol/l) | $\bar{x}$ | $0.62^{A}$ | 0.46 | $0.45^{B}$ | 0.37 | 0.43 | 0.18 | 0.47 | 0.24 |
| | SD | 0.13 | 0.20 | 0.11 | 0.10 | 0.15 | 0.08 | 0.10 | 0.04 |
| Cholesterol (mmol/l) | $\bar{x}$ | 1.63 | 2.10 | 1.39 | 2.00 | 1.80 | 1.93 | 1.93 | 1.97 |
| | SD | 0.30 | 0.33 | 0.40 | 0.51 | 0.21 | 0.17 | 0.31 | 0.20 |
| ALT (µkat/l) | $\bar{x}$ | $1.10^{A}$ | 0.76 | $0.74^{B}$ | 0.83 | 1.25 | 1.11 | 1.25 | 1.25 |
| | SD | 0.33 | 0.16 | 0.11 | 0.14 | 0.26 | 0.12 | 0.34 | 0.18 |
| AST (µkat/l) | $\bar{x}$ | 0.89 | 0.65 | 0.84 | 0.60 | 0.84 | 0.62 | 0.89 | 0.95 |
| | SD | 0.31 | 0.25 | 0.22 | 0.11 | 0.16 | 0.07 | 0.13 | 0.43 |
| ALP (µkat/l) | $\bar{x}$ | 4.33 | 3.75 | 5.14 | 3.45 | 4.43 | 3.80 | 4.37 | 3.48 |
| | SD | 1.14 | 0.72 | 1.63 | 0.55 | 0.67 | 0.51 | 0.30 | 0.60 |
| Ca (mmol/l) | $\bar{x}$ | 2.53 | 2.34 | 2.44 | 2.36 | 2.18 | 2.59 | 2.26 | 2.66 |
| | SD | 0.16 | 0.14 | 0.07 | 0.14 | 0.15 | 0.11 | 0.10 | 0.08 |
| P (mmol/l) | $\bar{x}$ | 2.88 | 3.37 | 3.12 | 3.31 | 2.99 | 3.13 | 2.98 | 3.30 |
| | SD | 0.22 | 0.27 | 0.32 | 0.23 | 0.26 | 0.20 | 0.19 | 0.16 |
| Fe (mmol/l) | $\bar{x}$ | 39.30 | 18.18 | 41.31 | 17.75 | 17.17 | $36.25^{a}$ | 14.97 | $27.05^{b}$ |
| | SD | 15.17 | 5.48 | 18.02 | 3.63 | 4.37 | 5.99 | 5.28 | 3.95 |

*day of experiment
[1]control group fed a diet without kaolin supplementation
[2]experimental group fed a diet supplemented with 1% of kaolin
[3]control group infected with ETEC and fed a diet without kaolin supplementation
[4]experimental group infected with ETEC and fed a diet supplemented with 1% of kaolin
$^{a:b}$ = $P < 0.05$, $^{A:B}$ = $P < 0.01$

lems. During experimentation, two piglets from the control infection-free group (C0) died on Day 11 and 27 due to congenital heart and renal defects, respectively.

Results of biochemical analyses of blood sera from piglets collected on Day 20 and 59 of experiment are presented in Table 3. Levels of selected biochemical parameters in piglets were within physiological ranges (Tluchor, 2001) and pointed to the good health of all pigs. Significant differences were detected between the infection-free control group and the experimental group in glucose ($P < 0.05$), triacylglycerol ($P < 0.01$) and ALT ($P < 0.01$) levels as compared to all the remaining groups. Significantly lower concentrations of Fe ($P < 0.05$) in the experimental group KI were found on Day 59. On Day 20 of experiment, Fe concentrations in piglets from control and experimental groups were comparable. No significant differences were found for Fe concentrations between the control group and experimental group during the infection-free experiment (Table 3).

Blood samples for haematological tests were collected from all piglets after a 20-day feeding of piglets with the feed mixture containing kaolin. No significant differences in the investigated parameters (hematocrit, erythrocyte and leukocyte counts) were observed between control and experimental piglets in both infection-free and infection experiments (Table 4).

## Bacteriological examination

Colonization of piglets and shedding of ETEC of serogroup O141:F18 is presented in Figures 1 and 2. Due to the fact that the weaned piglets originated from a conventional herd, the intestines of some piglets from both groups had already been colonized with ETEC from serogroup O141:F18 before the initiation of the experiment. This was the identical ETEC type to that used for experimental infection. In the control group, ETEC already rapidly predominated in rectal swabs taken on the first days after infection (Figure 1). The percentage of ETEC among the *E. coli* shed during four days reached almost 100% in all but one piglet from the control group. On Day 8 after infection, ETEC pre-

Table 4. Haematological parameters in piglets on day 20 of experiment

| Parameter | | Infection-free experiment | | Infection experiment | |
|---|---|---|---|---|---|
| | | C0[1] ($n$ = 9) | K0[2] ($n$ = 10) | CI[3] ($n$ = 10) | KI[4] ($n$ = 10) |
| Erythrocytes ($\times 10^{12}$/1 l) | $\bar{x}$ | 5.37 | 5.08 | 5.91 | 5.76 |
| | SD | 0.46 | 0.33 | 0.33 | 0.20 |
| Leukocytes ($\times 10^{9}$/1 l) | $\bar{x}$ | 21.25 | 19.24 | 14.57 | 15.37 |
| | SD | 4.67 | 4.97 | 2.09 | 2.63 |
| Lymphocytes (%) | $\bar{x}$ | 65.56 | 68.01 | 63.75 | 69.40 |
| | SD | 7.97 | 9.85 | 11.58 | 9.30 |
| Neutrophils (%) | $\bar{x}$ | 32.74 | 29.64 | 35.45 | 29.20 |
| | SD | 8.11 | 9.84 | 11.71 | 9.44 |
| Monocytes (%) | $\bar{x}$ | 0.99 | 1.70 | 0.45 | 1.00 |
| | SD | 0.87 | 1.30 | 0.64 | 0.62 |
| Eosinophils (%) | $\bar{x}$ | 0.71 | 0.50 | 0.35 | 0.50 |
| | SD | 0.57 | 0.53 | 0.41 | 0.41 |
| Hematocrit (%) | $\bar{x}$ | 31.67 | 30.80 | 36.60 | 37.50 |
| | SD | 3.00 | 3.05 | 1.90 | 1.84 |

[1]control group fed a diet without kaolin supplementation
[2]experimental group fed a diet supplemented with 1% of kaolin
[3]control group infected with ETEC and fed a diet without kaolin supplementation
[4]experimental group infected with ETEC and fed a diet supplemented with 1% of kaolin

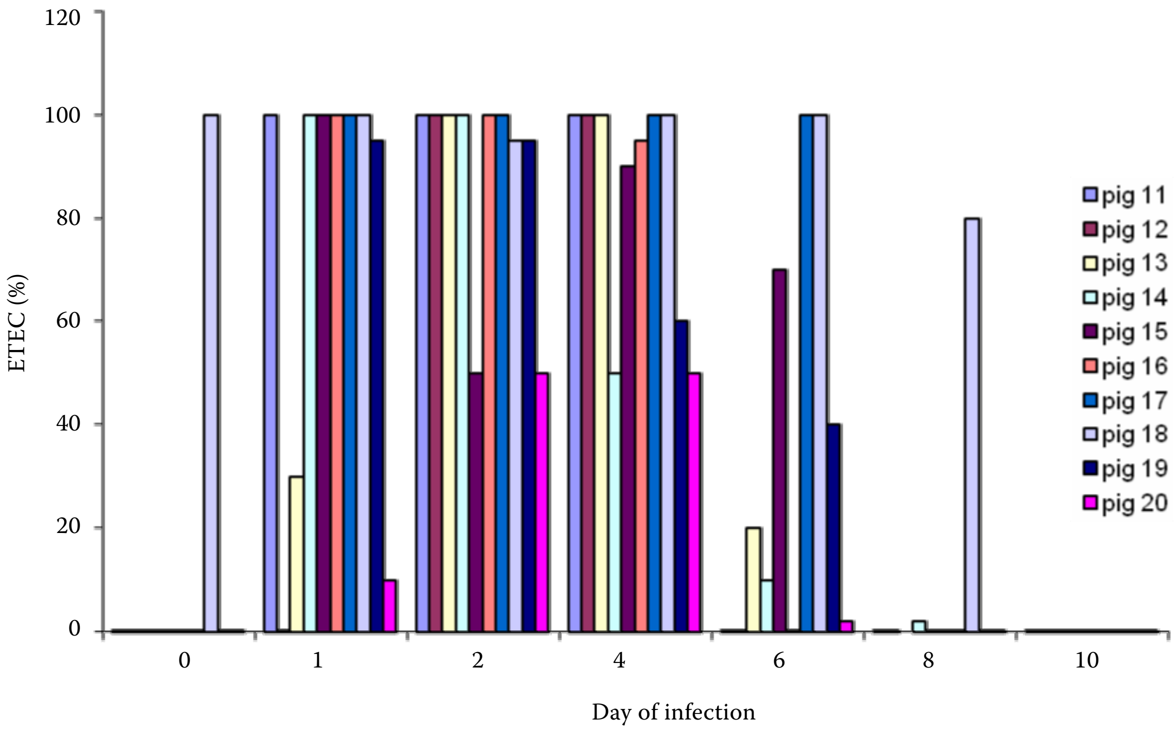


Figure 1. Shedding of ETEC O141:F18 in individually collected rectal swabs of piglets from control group CI fed a diet without kaolin supplementation

dominated in rectal swabs from one piglet only and from Day 10 onwards it was undetectable.

In the experimental group, ETEC O141:F18 also predominated amongst *E. coli* found in rectal swabs. However, in comparison with the control group, the increase was slower and ETEC predominated for only a short period. Shedding of ETEC in experimental groups detected on Day 2 after infection was significantly lower ($P < 0.05$) as compared with control. Out of a total *E. coli* count, the ETEC percentage in swabs was generally lower in the experimental group of piglets.

## Histological examination of intestines

After a 20-day feeding of experimental feed mixture containing kaolin to piglets, its effect on different parts of intestinal mucosa (duodenum, jejunum, ileum and colon) was investigated. No histopathological lesions were apparent in the intestines of experimental piglets (K0) and the findings were comparable with control piglets (C0). Occasional minor desquamations of the intestinal epithelium and a slightly increased presence of eosinophils were found, but were within the range of physiological values.

The findings in the small intestines of control piglets after experimental infection with *E. coli* (CI) gave evidence of the late stages of a process of reparation in mucosa, evidently after a preceding acute form of inflammation. In duodenum, jejunum and ileum, local epithelial cell destructions were seen. In some cases, the entire upper parts of villi were affected. Increased numbers of loose epithelial cells, *lamina propria* cells and inflammatory cells, including lymphocytes, macrophages and plasma cells, were found at a high frequency in the lumen of the intestines. Increased numbers of eosinophil granulocytes were detected in mucosa (Figure 3). Colonic mucosa were without visible gross lesions. No gross lesions indicative of previous inflammation were diagnosed in the intestines of infected piglets fed the diet containing kaolin. Occasional minor intestinal epithelial cell destructions and slightly increased numbers of eosinophils were within physiological ranges (Figure 4).

## Mycobacterial examination of kaolin and feed mixtures

The results of mycobacterial examination showed that the contamination of kaolin was low during the

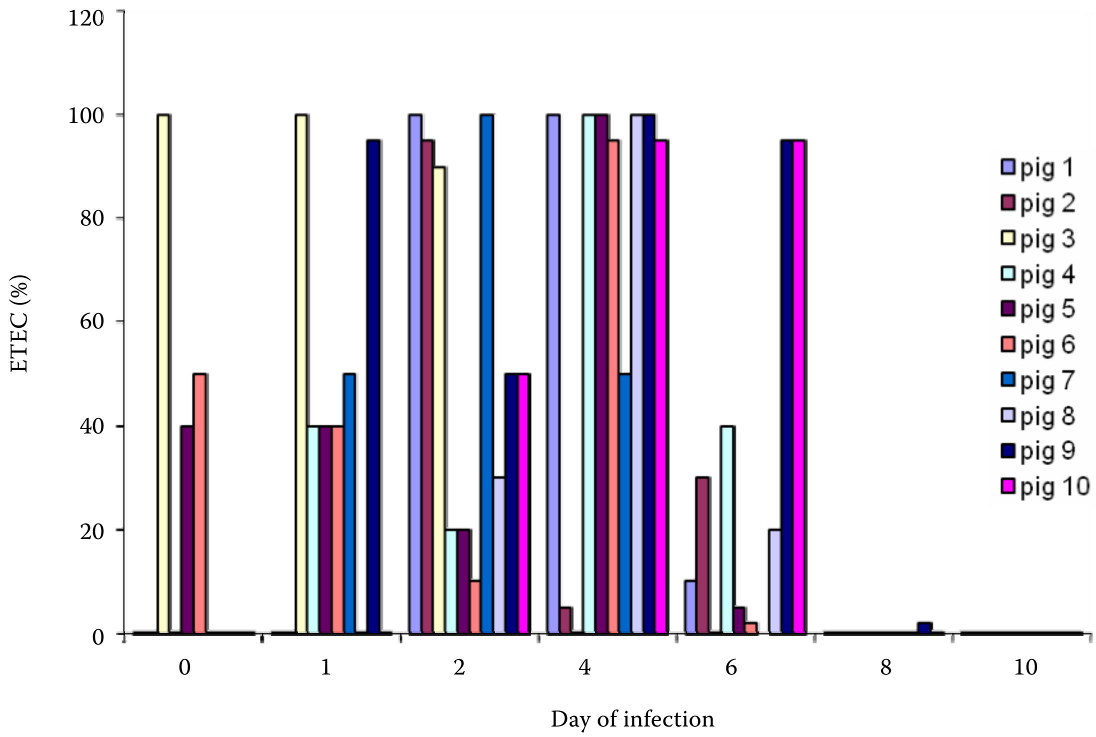


Figure 2. Shedding of ETEC O141:F18 in individually collected rectal swabs of piglets from experimental group KI, fed a diet supplemented with 1% of kaolin

entire production process. Direct microscopic examination for mycobacteria was positive in three (7%) of 42 samples, i.e., water from surface lakes Nos. 2 and 3 and fine, wet sand separated from extracted raw kaolin. However, *Mycobacterium* sp. was isolated by culture in only one sample. Culture examination of all other 39 samples was negative for mycobacteria. Culture examinations of pure kaolin used for supplementation of the experimental feed mixture and all the other feed mixtures (COS, COS + kaolin and A1) were negative for mycobacteria ($n$ = 17).

## Mycobacterial examination of pigsty environment

AFR were detected in 10 (14.7%) of 68 samples from the pigsty environment. In the infection-free experiment, *M. chelonae* was isolated in one case from the floor in a pen used to house the control group of piglets, from a sample taken at the end of the experiment. *M. peregrinum* and *M. gordonae* were isolated from the floor in the feed store and from water in the water main, respectively. In the pigsty environment where the infection experiment was performed, *M. chelonae* was isolated from a swab taken from the grate in the pen, and in the control group, from a swab taken in the space under the grate; these were taken before initiation of the experiment. We failed to isolate mycobacteria in the other four cases.

## Mycobacterial examination of rectal swabs

In the infection-free experiment, individual rectal swabs from piglets were negative. In the infection experiment on Day 20, AFR were present in six of 20 samples. Mycobacteria were isolated from five of them: these included three cases of piglets fed the feed mixture supplemented with kaolin and two cases of piglets from the control group. In all cases, these were PPM, which were not members of the *M. avium* complex. Swabs collected on Day 59 of the experiment from both control and experimental group of piglets were negative.

## Mycobacterial examination of organs

Culture examination of body organs of slaughtered piglets from both experiments showed that AFR were present in 6 of 192 samples. PPM which were not members of the *M. avium* complex were

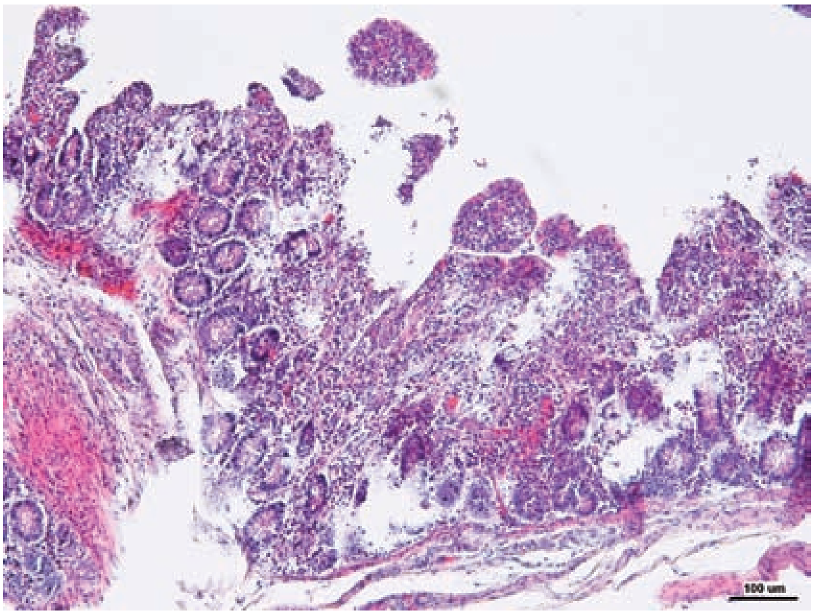


Figure 3. Histology of ileum of a piglet from group CI, infected with ETEC fed a diet without kaolin supplementation. Local destruction of intestinal epithelium and apical parts of villi and increasing presence of inflammatory cells in *lamina propria* gave evidence of late inflammatory process (Photo V. Kummer)

isolated from five samples taken from the infection-free group of piglets. In the control group, the hepatic lymph node from one piglet tested positive for mycobacteria on Day 59 of the experiment. In the experimental group, positive results were obtained from the kidney of one piglet on Day 20 and the ileal and hepatic lymph node of another piglet on Day 59 of experiment. In the experiment dealing with ETEC infection, member of the *M. avium* complex (*M. avium* subsp. *avium*) was isolated from a pig fed with kaolin (inguinal lymph node) slaughtered on Day 20 of the experiment.

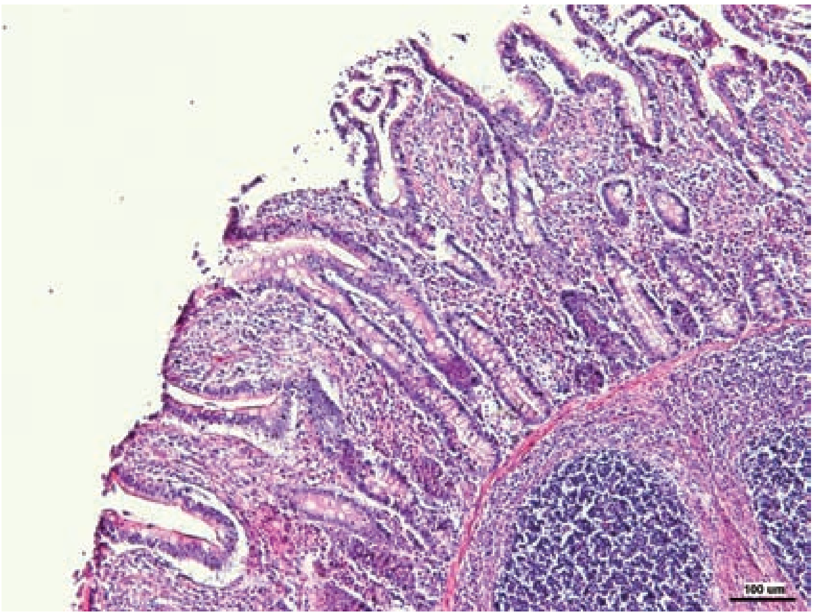


Figure 4. Histology of ileum of a piglet from group KI, infected with ETEC fed a diet with 1% kaolin supplementation. No gross lesions indicative of a present or previous inflammation can be seen in the intestinal mucosa (Photo V. Kummer)

### Examination of sera for mycobacteria

On Day 20 of the infection-free experiment, two (10.5%) serum samples ($n$ = 19) from piglets fed the kaolin-containing diet were positive for antibodies against *M. intracellulare*. On Day 59 of experiment, a positive response was observed in four control and four experimental piglets ($n$ = 14). On Day 20 of the infection experiment, the sera of all control and experimental piglets ($n$ = 20) were negative, but on Day 59, all sera ($n$ = 16) were positive for antibodies against *M. intracellulare.*

## DISCUSSION

Piglets readily ingested the feed mixture supplemented with 1% of kaolin. The supplementation caused a slight decrease in the content of nitrogen substances, metabolizable energy and some nutrients in the feed mixture (Table 1). This increased intake of the feed mixture by piglets compensated for the dilution effect of kaolin. While the animals consumed higher amounts of feed, its conversion was more efficient (Table 2). The 20-day feeding of weaned piglets with the diet containing 1% of kaolin had a significant ($P < 0.05$) positive effect on BWG in both non-infected and ETEC-infected piglets (Table 2).

There is a paucity of current studies dealing with the effect of kaolin feeding on the efficiency of farm animals. Some of them are focused on the effect of feed supplementation with other clay minerals (e.g., in groups of clinoptilolites, halloysites, montmorillonites, smectites, etc.). The structure and physical-chemical characteristics of these clay minerals and kaolin, which is a phyllosilicate mineral, are similar (Trckova et al., 2004). A significant increase in the body weight gain and improved conversion of feed containing clay minerals has been shown by numerous studies (Pond et al., 1981, 1988; Vrzgula et al., 1982; Bartko et al., 1983; Pond and Yen, 1987; Castro and Iglesias, 1989; Cabezas et al., 1991; Papaioannou et al., 2004; Chen et al., 2005; Kolacz et al., 2005; Papaioannou et al., 2005; Alexopoulos et al., 2007; Prvulovic et al., 2007). Naturally, their effect on the efficiency of animals is associated with the type, physicochemical and structural qualities, purity and proportion of these minerals in the diet (Pond and Yen, 1987; Pond et al., 1988; Papaioannou et al., 2004, 2005), and by the age of animals (Papaioannou et al., 2004; Alexopoulos et al., 2007).

Supplementation of diets with 1% to 3% of clay adsorbents is generally recommended. Animals have also been observed to readily consume feed containing higher proportions of absorbents (Castro and Elias, 1978; Vrzgula et al., 1982; Bartko et al., 1983; Savory, 1984; Sakata, 1986; Castro and Mas, 1989). Nevertheless, the efficiency of animals can be reduced if the low content of organic matter, nitrogenous substances and energy are not compensated by increased feed intake (Savory, 1984; Poulsen and Oksbjerg, 1995). However, a majority of studies have reported that higher adsorbent proportions in diets do not adversely affect either the animals' growth or efficiency (Castro and Elias, 1978; Castro and Iglesias, 1989; Castro and Mas, 1989).

The effect of clay adsorbents on the efficiency of animals can be explained by several different mechanisms. It is assumed that clay minerals reduce the speed of feed passage along the intestines, which results in better nutrient conversion and higher water resorption (Castro and Elias, 1978; Papaioannou et al., 2005). Whilst Poulsen and Oksbjerg (1995) did not observe any changes in protein retention using a diet supplemented of with 3% of zeolite, other studies have reported improved digestibility of nutrients (Castro and Elias, 1978; Kyriakis et al., 2002; Chen et al., 2005; Ly et al., 2007), and protein and energy retention (Ly et al., 1996, 2007; Parisini et al., 1999). Some authors put the improvement in protein and energy retention down to the increased activity of pancreatic enzymes due to the supplementation of diets with clay minerals. Pancreatic enzymes bind to the surface of the adsorbents and form complexes that are active within a wide pH range in the digestive tract (Cabezas et al., 1991; Parisini et al., 1999; Papaioannou et al., 2005).

Other authors have suggested that growth and efficiency were improved due to the reduced microbial production of ammonium in the intestines (Shurson et al., 1984; Poulsen and Oksbjerg, 1995; Papaioannou et al., 2005). This effect is comparable with antibiotics, which also reduce microbial production of ammonium and attenuate intestinal cell damage. This lowers the nutritional demands for cell renewal and thereby the amount of available nutrients for growth (Yen and Pond, 1990; Poulsen and Oksbjerg, 1995).

Health status and efficiency could also be improved by the considerable adsorption abilities of these substances. They can firmly and selectively bind harmful substances from feeds in the intestines and thus reduce their absorption across the intestinal mucosa and resulting toxic effect (Knezevic and Tadic, 1994; Phillips, 1999; Abdel-Wahhab et al., 1999; Hassen et al., 2003; Katsumata et al., 2003; Dominy et al., 2004). Bartko et al. (1983) and Boranic (2000) emphasized the high selective ion exchange capacity of clay minerals. This is retained during their passage through the gastrointestinal tract and can influence the resorption capacity of the intestinal tract and thereby the metabolism of macro- and microelements.

Natural clay minerals improve the function of the intestinal barrier and are effective in treating gastroenteric diseases in humans and animals (Castro and Elias, 1978; Said et al., 1980; Vrzgula et al., 1982; Brouillard and Rateau, 1989; Ramu et al., 1997; Narkeviciute et al., 2002; Dominy et al., 2004; Castro, 2005). Their use retards the rate of digestive passage through the intestines and their ability to absorb water results in more compact and better shaped faeces. The amelioration in the clinical manifestations of diarrhoeal diseases can also be explained by the elimination of various factors that are associated with the occurrence of these diseases, e.g., in piglets, primarily at the period of weaning. These factors include intestinal hypersensitivity to feed antigens or weaning-induced changes leading to the manifestation of malabsorption syndrome caused by a reduction in the digestive enzyme activity; both can predispose to infectious enteritis (Wilson et al., 1989; Papaioannou, et al., 2004, 2005).

The inactivation of diarrhoea-causing enterotoxins by hydrogen bond implicated interactions with the surface of the layered crystalline structure of mineral clays or with their between-layer space (Brouillard and Rateau, 1989; Papaioannou et al., 2004) markedly reduces the duration and severity of diarrhoea (Castro and Elias, 1978; Bartko et al., 1983; Papaioannou et al., 2004). Brouillard and Rateau (1989) and Ramu et al. (1997) confirmed a high adsorption ability of clay adsorbents to act against *E. coli* enterotoxins under *in vivo* conditions. Martinez et al. (2004) reported a comparable effectiveness of zeolite and erythromycin in the treatment of diarrhoea caused by *E. coli*. The pH, dosage, and time of exposure, as well as enterotoxin type play an important role in the activity of adsorbents. Brouillard and Rateau (1989) observed that smectite was more active than kaolin, because it was immediately active, particularly at the pH in the intestine chymus. Novakova (1968) observed that kaolin had a marked effect on the growth curve of *E. coli*, prolonged its lag phase and diminished the cell yield.

The results of our bacteriological examination confirmed the protective effect of kaolin on the course of ETEC infection in piglets (Figures 1 and 2). The colonization and shedding of ETEC of serogroup O141:F18 in piglets fed with the kaolin-containing feed mixture were reduced and persisted for a shorter period in comparison with control piglets. Bartko et al. (1983) reported that supplementation of feed mixture with 5% clinoptilolite had a positive effect on the health status of piglets and alleviated the symptoms of diarrhoeal diseases. Even though it failed to fully prevent diarrhoea in piglets, its signs were milder, and bacteriological examinations of faeces from experimental piglets failed to detect *Vibrio dysenteriae* in contrast to the control group. Feeding a diet supplemented with 2% of clinoptilolite to piglets, Papaioannou et al. (2004) also observed a significant reduction in the occurrence and duration of clinical signs of diarrhoeal diseases and mortality. However, this effect was not as marked as was the effect of antibiotic treatment or a combination of antibiotics and clinoptilolite.

The question of mineral supplementation/deficiency (above all iron) has often been discussed in association with the consumption of clay minerals. Some authors consider the habit of eating clay or earth (geophagia) as an important source of minerals in the diet of animals and humans (Vermeer, 1966; Hunter, 1973; Hunter and de Kleine, 1984; Johns and Duquette, 1991; Abrahams, 1997; Geissler et al., 1998). In contrast, other studies have documented associations between geophagia and anaemia or deficiency in some other mineral substances (Halstead, 1968; Patterson and Staszak, 1977; Hooda et al., 2002). Investigating the effect of supplementation of a diet for pregnant rats with 20% of kaolin on the blood and embryonic development, Patterson and Staszak (1977) observed a significant reduction in haemoglobin/hematocrit levels and erythrocyte counts and a marked reduction in the weight of neonates, symptoms of maternal anaemia. Supplementing the kaolin-containing diet with iron ensured that haemoglobin/hematocrit levels, erythrocyte counts and the weight of neonates were within normal ranges.

On the other hand, Wiles et al. (2004), who supplemented a diet for pregnant rats with relatively high doses of clay (2% w/w) did not observe significant changes in the intake and utilization of mineral substances. Other studies dealing with clinoptilolite feeding to pigs failed to find any changes in haematological parameters (hematocrit, erythrocyte and leukocyte counts, haemoglobin, differential blood picture; Vrzgula et al., 1982; Alexopoulos et al., 2007). The ion-exchange properties and adsorption capacity of clay minerals are above all affected by particle size in different types of soils, crystallite size and the degree of aggregation as well as by the porosity of different particles (Papaioannou et al., 2002; Alexopoulos et al., 2007). These properties and the amount of a clay mineral added to the diet influence the final binding effect of the clay-based adsorbent in the animal or human organism.

No adverse effects of 1% kaolin feeding on the clinical status of piglets were observed. The values of selected biochemical (Table 3) and haematological parameters (Table 4) in blood serum of pigs ranged within physiological values (Sova et al., 1981; Drazan et al., 1987; Tluchor, 2001) and gave evidence of good health in all pigs. In piglets fed the kaolin-containing diet, lower glucose, triglyceride and ALT levels were detected on Day 20 in comparison with control piglets. These results were in agreement with similar studies focused on the feeding of clay minerals to pigs. These studies documented that even long-term feeding did not affect levels of total serum protein, albumin and mineral elements in pigs (Kyriakis et al., 2002; Papaioannou et al., 2002; Alexopoulos et al., 2007). Some studies have reported changes in glucose, cholesterol and AST levels (Concepcion-Rosabal et al., 1997; Alexopoulos et al., 2007; Prvulovic et al., 2007). The present study did not find any changes in Fe distribution and haematological parameters in piglets. Haematological parameters and Fe concentration in experimental and control piglets from both experiments were comparable at the end of the 20-day period of kaolin feeding. The significantly lower Fe concentration in ETEC-infected piglets detected more than five weeks after kaolin feeding had ceased was likely not associated with kaolin intake.

In addition to the effect on the composition of intestinal microflora, feeding clay minerals can cause morphological changes in the intestinal mucosa. Montmorillonite added to a diet for pigs or broilers improved the morphology of small intestine mucosa (Xia et al., 2005; Ma and Guo, 2008). Long-term feeding resulted in an increased height of villi related to the depth of crypts in the pig jejunum (Xia et al., 2004, 2005). Our experiments documented that a 20-day feeding of piglets with a diet supplemented with 1% kaolin did not cause any visible changes in histopathological features of mucosa in small and large intestines of piglets. The protective effect of kaolin on the intestines of piglets was observed in the ETEC infected group. In contrast to infected control piglets where apparent reparation processes in the mucosa were taking place following a mild form of acute inflammation as shown by the findings in small intestines, no processes indicative of previous inflammation were diagnosed in piglets fed the kaolin-containing diet (Figures 3 and 4).

Samples for culture examinations of kaolin, intended for supplementation of the experimental feed mixture, were taken at different stages of the kaolin production. Our results confirmed that PPM can be found in surface water used for levigation during kaolin purification (Matlova et al., 2004). Nevertheless, the risk of contamination of final product seems to be low. The occurrence PPM in the pigsty environment (*M. chelonae* and *M. peregrinum*) and in the water main (*M. gordonae)* can be regarded as common according to our previous research (Matlova et al., 2003).

Non-specified PPM (not belonging to the *M. avium* complex) were detected in rectal swabs from five ETEC-infected piglets. Causing immunosuppression in the piglets, the infectious agents could predispose them to become susceptible to these mycobacteria. The results of serological examination can support the hypothesis that ETEC infection facilitates colonization of the pig organism with atypical mycobacteria. After completing the infection-free experiment, we found that eight (57%) piglets tested positive for antibodies against *M. intracellulare*, whilst in the infection experiment, the sera of all pigs ($n$ = 16, 100%) were positive. We could similarly speculate about the occurrence of *M. avium* complex members in the lymph node of a pig after overcoming the ETEC infection. Nevertheless, we also found non-specified PPM in the organs of non-infected pigs. Therefore, the weakening of the immune system by ETEC infection was not necessarily the main factor that facilitated colonization with atypical mycobacteria.

## CONCLUSIONS

From these results we can conclude that the supplementation of feed mixtures for the short-term feeding of weaned piglets with 1% of kaolin has a positive effect on their growth and efficiency, without substantial adverse effects on their health status and biochemical and haematological parameters. Also, kaolin added to a diet for piglets can attenuate diarrhoea caused by ETEC strains and reduce its duration.

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