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Prophylaxis of post-weaning diarrhoea in piglets by zinc oxide and sodium humate

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ABSTRACT: The high prophylactic doses of ZnO commonly used to control post-weaning diarrhoea (PWD) in piglets have become an environmental problem. The possibility of partial replacement of ZnO by sodium humate (HNa) in PWD treatment was investigated in this study. Weaned piglets (32) were challenged with two enterotoxigenic *E. coli* strains (ETEC/O149/F4/LT and ETEC/O147/F18/LT) and allocated into four treatments maintained for three weeks: C (no supplementation); ZnO2.5 (2.5 g ZnO/kg diet); HNa + ZnO1.0; HNa + ZnO1.5; HNa + ZnO1.7 (20 g HNa and 1.0, 1.5 and 1.7 g ZnO/kg diet, respectively). The effects on incidence, severity and duration of diarrhoea, faecal shedding of total *E. coli* and both ETEC strains, growth rate and selected blood parameters were investigated. In contrast with ZnO2.5 and HNa + ZnO1.7, high daily diarrhoea scores, incidence and duration and mortality due to severe dehydration were seen in C, HNa + ZnO1.0 and HNa + ZnO1.5 groups. The administration of ZnO and HNa did not affect the faecal shedding of the challenged ETEC strains for eght days, even in clinically healthy piglets in ZnO2.5 and HNa + ZnO1.7 groups. Signs of growth depression were found in C; HNa + ZnO1.0 and HNa + ZnO1.5 groups during the first week. No difference in growth performance was observed in ZnO2.5 and HNa + ZnO1.7 piglets. Most of the selected biochemical and haematological parameters did not differ significantly among the treatments. However, a significantly higher serum Zn as a result of high dietary ZnO intake in the ZnO2.5 group compared to the control and HNa groups was detected. Significantly lower serum P in ZnO2.5; HNa + ZnO1.7 and HNa + ZnO1.0 groups compared to the control group was most likely induced by the increased Zn in serum. The results indicate the possibility of reducing the high pharmacological levels of ZnO in the prophylaxis of PWD through partial replacement with HNa. Such a treatment maintains the favourable prophylactic effect while lowering the Zn content in faeces.

Keywords: humic; enterotoxigenic *E. coli*; faecal shedding; growth performance; blood chemistry

List of abbreviations:

ALP = alkaline phosphatase, **ALT** = alanine transaminase, **ANOVA** = analyses of variance, **AST** = aspartate aminotransferase, **BWG** = body weight gains, **CFU** = colony forming units, **DDS** = daily diarrhoea score, **ETEC** = enterotoxigenic *Escherichia coli*, **FCR** = feed conversion ratio, **FI** = feed intake, **HNa** = sodium humate, **HS** = humic substances, **PWD** = post-weaning diarrhoea

Post-weaning diarrhoea (PWD), which is caused by enterotoxigenic *Escherichia coli* (ETEC), is by far the most common disease in weaned pigs and is considered to be a major problem in the swine industry. Antibiotics have been used as feed additives for elimination or reduction of pathogenic bacteria and as growth promoters in animal production for many years. However, their use has recently been banned in the EU due to increased concerns surrounding bacterial resistance and residual risk in animal products (Regulation (EC) No. 1831/2003). Therefore, there is a pressing need to find antibiotic alternatives for use in animal production, which are harmless to human health. Zinc oxide (ZnO) has become a universal remedy in controlling PWD, not least because it is relatively cheap and readily available (Heo et al. 2013). However, high prophylactic doses of ZnO in pig diets (2.5 to 3.0 g/kg) result in

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the production of zinc-enriched slurry, which may become an environmental problem.

Humic substances (HS) are natural bioactive agents primarily decomposed from organic matter by bacteria in the soil. Major components of HS are humus, humic, fulvic and ulmic acids and trace minerals. HS have been described to possess antibacterial, anti-diarrhoeal, anti-allergic, antitoxic, immunomodulatory and anti-inflammatory properties (Joone and van Rensburg 2004; Zraly et al. 2008; van Rensburg and Naude 2009), and humic acid and its sodium salt are permitted for oral use in horses, ruminants, swine and poultry for the treatment of diarrhoea, dyspepsia and acute intoxications (EMEA 1999). HS as a feed additive in the pig diet could also improve growth performance and meat quality and reduce ammonia emission from manure (Ji et al. 2006; Wang et al. 2008).

The aim of the present study was to investigate the possibility of partial replacement of a high dose of ZnO (2.5 g/kg) with HNa, with the goal of maintaining a favourable prophylactic effect in PWD treatment while simultaneously reducing the Zn content in pig faeces.

MATERIAL AND METHODS

Animal management and dietary treatment. Testing of ZnO and HNa supplementation was performed on 32 weaned piglets (equal numbers of barrows and gilts). Piglets (LW × (P × Du)), originated from a specific pathogen-free herd, were transported to the experimental animal facility of the Veterinary Research Institute, Brno, Czech Republic on the day of weaning (28th day of life). Animal handling followed EU directive 86/609/EEC concerning animal care. The animal care protocol for this experiment followed the Czech guidelines for animal experimentation and was approved by the Branch Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic (Permission No. MZe 50-2011). Piglets were identified by individual ear tags and housed in indoor pens at a temperature of 21 to 23 °C and humidity of 51% to 62%.

Weaned piglets with an average initial body weight of 7.79 ± 1.10 kg were allotted to four treatments. Each group was kept in one pen with eight piglets per pen. Dietary treatments were as follows: C = control with no supplementation; ZnO2.5 = supplemented with high prophylactic dose of ZnO (2.5 g/kg diet); HNa + ZnO1.0; HNa + ZnO1.5; HNa + ZnO1.7 = supplemented with 20.0 g HNa and 1.0, 1.5, 1.7 g ZnO/kg diet, respectively. The basal diet (Table 1) was formulated according to animal requirements (National Research Council 1998). No antibiotics were included in the diets. Pigs were fed partly *ad libitum* twice a day at 7.00 and 16.00 h. The refusals were removed before the subsequent feeding, weighed and taken into account in the calculations of feed consumption. Water was provided by automatic waterers. The dietary treatment was maintained for three weeks.

Table 1. Composition of the diet for weaned piglets (as-fed basis)

Ingredient (g/kg)	Basal diet
Wheat	400.2
Barley	298.0
Soybean meal, 47% CP	186.0
Dry whey and soy protein concentrate	50.0
Soybean oil	23.0
Limestone, ground	12.0
Dicalcium phosphate	11.0
Salt	3.0
Sodium carbonate	1.0
L-Lysine HCl	0.2
L-Threonine	0.3
DL-Methionine	0.3
Vitamin and mineral premix[1]	15.0
Calculated chemical composition	
ME (MJ/kg)	12.27
Crude protein (g/kg)	189.00
Lysine (g/kg)	11.95
Methionine (g/kg)	4.00
Ca (g/kg)	7.00
Na (g/kg)[2]	1.90
Zn (g/kg)[3]	140.0
P (g/kg)	4.55

[1]Provided per kg diet: 12 000 IU of vitamin A, 2000 IU of vitamin D3, 100 IU of vitamin E, 152 mg of Cu (as $CuSO_4$), 22 mg of Zn (as ZnO), 88 mg of Zn (as $ZnSO_4$), 32 mg of Mn (as MnO), 110 mg of Fe (as $FeSO_4$), 1.0 mg of I (as $Ca(IO_3)_2$), 0.20 mg of Co (as $Co_2O_3 \times 7\ H_2O$), and 0.3 mg of Se (as $Na_2SeO_3 \times 5\ H_2O$)

[2]Experimental diets were supplemented with 0 (ZnO2.5); 0.58 (HNa + ZnO1.7), 0.58 (HNa + ZnO1.5) and 0.58 (HNa + ZnO1.0 diets) g Na/kg from HNa

[3]Experimental diets were supplemented with 2.5 (ZnO2.5); 1.7 (HNa + ZnO1.7), 1.5 (HNa + ZnO1.5) and 1.0 (HNa + ZnO1.0 diets) g Zn/kg from ZnO

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ETEC challenge. All piglets were orally challenged by ETEC/O149/F4/LT (12549) and O147/F18/LT (12524) with a single dose of 1.5×10^{11} colony forming units (CFU) per piglet on Day 4 after weaning. The ETEC strains used for infection were grown in medium containing 12.5 g of acid casein hydrolysate, 12.5 g of enzymatic casein hydrolysate and 0.5 g of yeast extract (Oxoid) per litre and incubated at 37 °C for 16 h. For individual administration, the culture was condensed by centrifugation and subsequently incorporated into semolina porridge paste.

Diarrhoea evaluation. Piglets were clinically examined individually once per day. The severity of diarrhoea was assessed visually and evaluated by individual scoring of the consistency of the faeces: 0 normal, 1 pasty, 2 mushy, 3 liquid, 4 liquid with blood. Diarrhoea incidence was evaluated by the ratio (%) of scouring piglets in group. Mean daily diarrhoea score (DDS) was calculated as the group sum divided by the number of piglets in the group. The duration of diarrhoea was recorded individually and the mean duration for the group was calculated. Mortality rate throughout the monitoring period was also measured.

Faecal total *E. coli* and ETEC shedding. Bacteriological examination of faecal samples was performed on Day 4, 5, 7, 9, 12, and 14 to evaluate intestinal colonisation by the challenged ETEC strains. Faecal samples were taken individually from the rectum of piglets. They were plated on MacConkey and Columbia agar containing 5% lamb blood (LabMediaServis, Czech Republic) and cultured at 37 °C for 16 h. In faecal cultures, the total *E. coli* and the haemolytic CFU were recorded. Haemolytic colonies of bacterial cells (CFU < 5 per sample) were isolated and after incubation tested for virulence factors. O-serogroups were serologically detected using anti-O rabbit sera (Salajka et al. 1992). F4, F18 adhesin and LT enterotoxin analyses were performed using PCR as described previously (Alexa et al. 1997; Zajacova et al. 2012).

Growth performance of piglets. Piglets were weighed on Day 1, 7, 14 and 21. Individual body weight gains (BWG) were calculated. Average daily feed intake (FI) of the groups was recorded. Feed conversion ratio (FCR) was calculated from FI and BWG. The weights of the dead piglets were used to adjust BWG.

Haematological and serum biochemical profile. At the end of the trial, 10 ml of blood were drawn from the *vena cava cranialis* to monitor the haematological and serum biochemical profile of the animals. Haematological parameters (haematocrit, erythrocyte and leukocyte counts) were measured using the Coulter Counter M4 apparatus (Coulter Cientifica S.A., Mostoles, Spain), whereas the differential leukocyte count in blood smears was detected using the Nikon Eclipse E600 fluorescence microscope (Nikon, Tokyo, Japan). In serum, biochemical parameters (total protein, albumin, creatinine, urea, triacylglycerol, cholesterol, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), calcium, phosphorus, magnesium, iron and zinc) were measured on a BS200 chemistry analyser (Mindray, Shenzhen, China).

Zn levels in faeces. On Day 20, individual faecal samples (5 g) from the rectum of piglets were taken. Before Zn determination, samples were mineralised using nitric acid in an autoclave with microwave heating (ETHOS SEL, Milestone, Italy). Zn levels in faeces were determined using flame atomic absorption spectrometry (spectrometer AA-30, Varian, Palo Alto, USA).

Statistical analyses. All data were subjected to statistical analysis using the program GraphPadInstat 3.0. The individual pig served as the experimental unit. For performance analyses, initial body weight was used as a covariate. The normality of the data, except mortality rate, was tested with the Kolmogorov-Smirnov test and the homogeneity of variances with Levene's test. When data passed the normality test, statistical significance of the differences among the group means was determined by the analyses of variance (ANOVA) in conjunction with the Tukey-Kramer test. When normality of the data was not validated ($P < 0.05$) Kruskal-Wallis nonparametric ANOVA was used. Mortality data were analysed by the Chi-squared test. Differences between means with $P < 0.05$ were accepted as being statistically significant. The results were expressed as means and mortality as a sum.

RESULTS AND DISSCUSION

Diarrhoea evaluation

The piglets were challenged with two pathogenic strains, ETEC O149/F4/LT and O147/F18/LT, which are commonly present in the environment and frequently cause diarrhoeal infection in weaned piglets. Fimbriae-designated F18 and F4 are the most frequently detected ETEC types in PWD piglets

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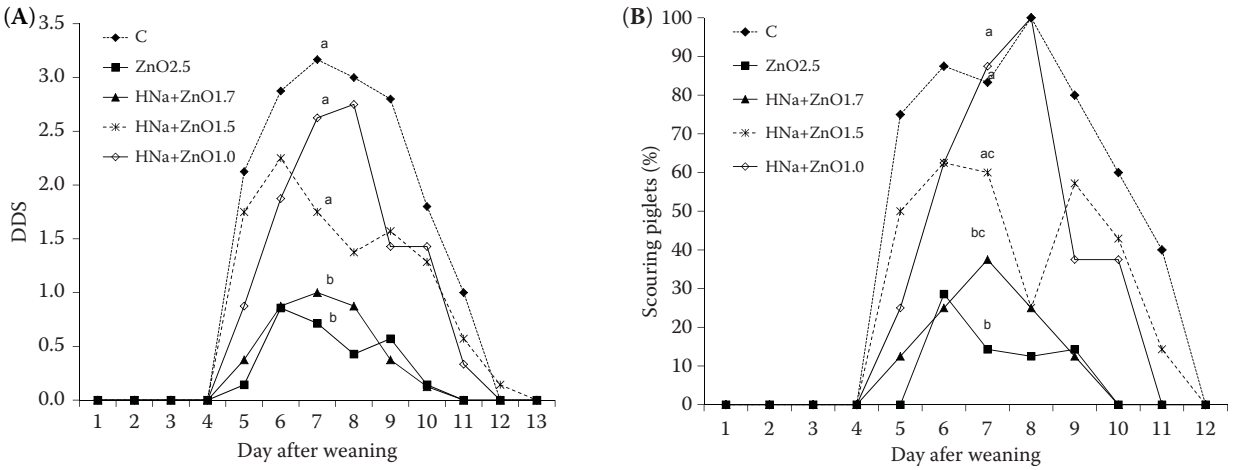

Figure 1. Daily diarrhoea scores (DDS; **A**) and diarrhoea incidence (**B**) of piglets challenged with ETEC O149:F4/LT and O147:F18/LT on Day 4 after weaning. Treatments: C = control with no supplementation; ZnO2.5 = supplemented with high prophylactic dose of ZnO (2.5 g/kg diet); HNa + ZnO1.7, HNa + ZnO1.5, HNa + ZnO1.0 = supplemented with HNa (20.0 g/kg diet) and 1.7, 1.5 and 1.0 g ZnO/kg diet, respectively. The results are expressed as means. Statistical significance of the differences among the groups was determined by ANOVA in conjunction with the Tukey-Kramer multiple comparisons test. *P*-values lower than 0.05 were considered statistically significant. Superscripts denote statistically significant differences among groups (groups sharing the same superscript are not significantly different from each other, groups that have no superscript in common are significantly different from each other)

(Frydendahl 2002; Fairbrother et al. 2005). However, some pigs lack the receptor for the F18 or F4 fimbriae and are therefore resistant to colonisation by F18- or F4-positive ETEC (Frydendahl et al. 2003). According to our experiences (unpublished data) and an experimental model published by Madec et al. (2000), the conjunction of two serotypes with different fimbrial adhesins strains is able to induce severe disease in piglets. Therefore, we used the composite inoculum in the experimental challenge.

In pigs fed the C diet, severe cases of diarrhoea were already observed on the following days after experimental challenge. The overall course of diarrhoeal infection in the group was very intense with a high incidence of scouring piglets (up to 100%) and liquid faeces (Figure 1). Due to severe diarrhoea with resultant dehydration, five piglets died in the first week after challenge (Table 2). In survivors, clinical signs of diarrhoea lasted on average 5.0 days.

Scouring was also seen in piglets supplemented with HNa + ZnO1.5 and HNa + ZnO1.0 (Figure 1). One and two piglets died from these groups, respectively (Table 2). Mean diarrhoea duration in these two groups was 2.7 and 3.5 days, respectively, with a range of 0–6.0 days in individual piglets.

Supplementation of piglets with a high ZnO dose (2.5 g/kg diet) or a lower ZnO dose (1.7 g/kg diet) supplemented with HNa (20.0 g/kg diet) was in both cases effective in the prophylaxis of PWD. No severe clinical signs of diarrhoea were observed in either group (Figure 1). When exceptional mild cases of diarrhoea (pasty or mushy faeces) appeared, the pigs of ZnO and HNa + ZnO1.7 groups recovered within a short period of 0.57 and 1.0 days, respectively (mean diarrhoea duration). No mortality was observed throughout the monitoring period in these two groups (Table 2).

Humic acids are recommended for the treatment of diarrhoea, dyspepsia and acute intoxications in horses, swine and poultry. They exert a protective action on the intestinal mucosa and have antiphlogistic, adsorptive, anti-toxic and antimicrobial properties (EMEA 1999). Veterinary pharmaceuticals containing humic acid have worked well in the treatment of digestive disorders and diarrhoea in cats and dogs (Kuhnert et al. 1991). However, there are no data on the use of HS in the prophylaxis of diarrhoea in piglets. According to our experiences (unpublished data), supplementation of 1% and 2% HNa was insufficient for the treatment of diarrhoea in piglets challenged with ETEC. The results of the present study indicate that in the case of severe diarrhoeal infections caused by pathogenic *E. coli* strains, administration of HNa to piglets can be

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Table 2. Growth performance of piglets challenged with ETEC O149/F4/LT and O147/F18/LT

Parameter	Period	Treatments[1]				
		C (n = 8)[2]	ZnO2.5 (n = 8)	HNa + ZnO1.7 (n = 8)	HNa + ZnO1.5 (n = 8)[2]	HNa + ZnO1.0 (n = 8)[2]
Body weight gain (kg)	1st week	–0.78[a]	0.71[b]	0.60[b]	–0.58[ab]	–0.11[ab]
	2nd week	1.33	2.00	1.91	1.59	0.99
	3rd week	1.31	2.83	3.18	2.01	1.86
	total	1.86	5.54	5.69	3.02	2.74
Feed intake (kg/pig)	1st week	1.574	2.198	2.106	1.674	1.693
	2nd week	3.333	2.875	2.813	3.037	3.154
	3rd week	5.417	5.358	5.563	4.179	4.248
	total	10.324	10.430	10.481	8.890	9.095
Feed conversion ratio	total	5.54	1.88	1.84	2.94	3.32
Mortality (pig)	total	5[a]	0[b]	0[b]	1[b]	2[b]

[1]C = control with no ZnO or HNa supplementation; ZnO2.5 = supplemented with high prophylactic dose of ZnO (2.5 g/kg diet); HNa + ZnO1.7, HNa + ZnO1.5, HNa + ZnO1.0 = supplemented with HNa (20.0 g/kg diet) and 1.7, 1.5 and 1.0 g ZnO/kg diet, respectively

[2]The weights of the dead piglets were used to adjust body weight gain

The individual pig served as the experimental unit. The results are expressed as means, mortality as sum. Statistical significance of the differences among the groups was determined by ANOVA in conjunction with the Tukey-Kramer multiple comparisons test. Mortality data were analyzed by the Chi-squared test. *P*-values lower than 0.05 were considered statistically significant. Superscripts denote statistically significant differences among groups (groups sharing the same superscript are not significantly different from each other, groups that have no superscript in common are significantly different from each other)

effective only when combined with an adequate dose of ZnO (1.7 g/kg diet).

Faecal total *E. coli* and ETEC shedding

The results of screening faeces for *E. coli* and ETEC shedding are shown in Figure 2. The dietary supplementation of ZnO and HNa did not affect the shedding of the challenged strains in piglet faeces. ETEC strains were shed by all piglets, and shedding in all treatments persisted for eight days (until Day 12). Interestingly, shedding of pathogenic strains was detected also in clinically healthy piglets with no signs of diarrhoea in ZnO2.5 and HNa + ZnO1.7 groups. Differences in total *E. coli* and ETEC CFU in the faeces of piglets fed a prophylactic dose of ZnO and the lower doses (1.7; 1.5 and 1.0) with HNa were non-significant (Figure 2).

This lack of response in intestinal colonisation or faecal concentration of coliforms or other bacterial groups after administration of 2.5 and 3.0 g/kg ZnO, which is in contrast to reduced diarrhoea incidence, has also been reported by other authors (Jensen-Waern et al. 1998; Katouli et al. 1999; Li et al. 2001). In contrast, Slade et al. (2011) showed that dietary supplementation of 3.1 g/kg ZnO reduced faecal ETEC shedding in challenged piglets. These findings suggest that the suppression of PWD seen with high levels of ZnO supplementation in pigs may not be associated only with the bactericidal effect of ZnO. Some studies indicate that ZnO can reduce the expression of PWD through improving intestinal barrier function, maintaining the diversity and stability of the intestinal microflora and reducing ETEC adhesion and intestinal colonisation (Katouli et al. 1999; Li et al. 2001; Roselli et al. 2003; Kim et al. 2012). Additionally, Crane et al. (2007) and Crane et al. (2011) reported that Zn inhibited the expression of several important virulence factors in enteropathogenic (EPEC) and shiga-toxigenic *E. coli* (STEC) strains and reduced intestinal damage and fluid secretion in rabbit intestinal loops *in vivo*. Therefore, we can assume that Zn exerts inhibitory effects on virulence factors in ETEC strains similar to those observed in EPEC and STEC strains, which could suppress PWD in challenged piglets.

HNa could play a role in the mucus resistance to ETEC. HS are able to form a protective film on

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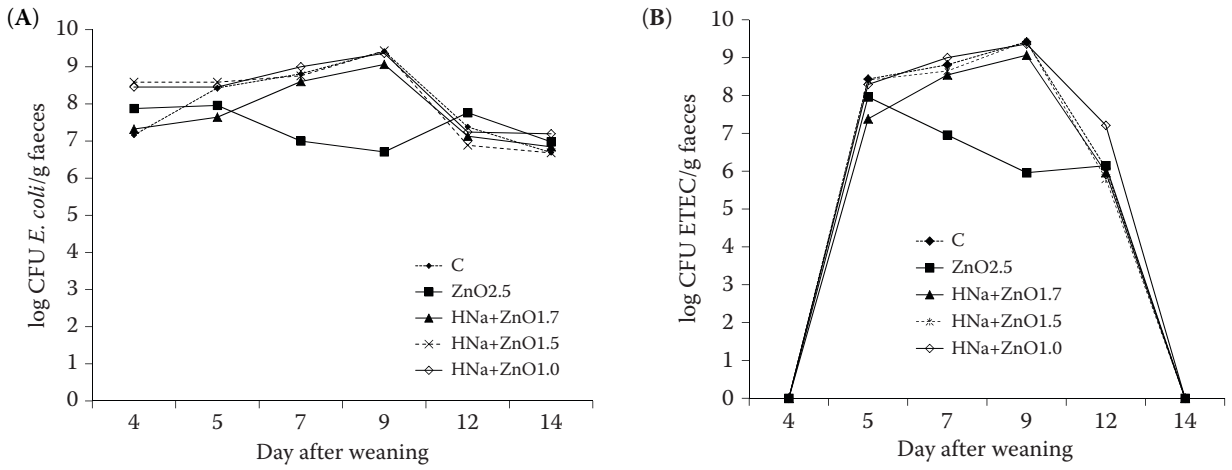

Figure 2. Faecal total *E. coli* (**A**) and ETEC (**B**) shedding in piglets challenged on Day 4 after weaning. Treatments: C = control with no supplementation; ZnO2.5 = supplemented with high prophylactic dose of ZnO (2.5 g/kg diet); HNa + ZnO1.7, HNa + ZnO1.5, HNa + ZnO1.0 = supplemented with HNa (20.0 g/kg diet) and 1.7, 1.5 and 1.0 g ZnO/kg diet, respectively. The results are expressed as means. Statistical significance of the differences among the groups was determined by ANOVA. No significant differences between treatments were observed

the mucosa of the gastrointestinal tract and can so reduce or completely prevent the resorption of toxic metabolites (Kuhnert et al. 1991). The stabilisation of the intestinal microflora and the maintenance of optimum pH in the gut of animals have been reported as results of HS treatment (Islam et al. 2005). The *in vitro* study of Parent and Velegol (2004) showed that HS could alter the adhesion of *E. coli*. However, *in vitro* studies are often not sufficient to explain bacterial adhesion events *in vivo* due to a lack of numerous biological interactions (e.g. presence of other organic substances or the complex nature of the intestinal microbiota).

To the authors' knowledge, there are currently no data available on the effect of HS on *E. coli* populations in weaned piglets. Contradictory findings are described regarding the effect of HS on *E. coli* in the gastrointestinal system of chickens. Aksu and Bozkurt (2009) found that HS-supplemented diet reduced the number of *E. coli* in the digesta of broilers. However, Shermer et al. (1998) reported that dietary HNa promoted the growth of *E. coli* in the caecal content of chickens probably due to changes in microbial metabolism.

Growth performance

Diarrhoea which developed after ETEC challenge negatively affected growth performance of piglets in C, HNa + ZnO1.5 and HNa + ZnO1.0 groups; signs of growth depression were observed already during the first week after weaning. The animals of the ZnO2.5 and HNa + ZnO1.7 groups gained weight in this period and significantly higher BWG values ($P < 0.05$) were calculated in comparison with C (Table 2). Over the subsequent two weeks, recovery was observed in the negatively affected groups. Differences in total BWG for the whole experimental period were not quite significant among treatments. FI was not affected by dietary treatments. Differences in total FCR in control and supplemented groups reflected the different health status of piglets (Table 2).

It has been confirmed in many studies that dietary ZnO supplementation after weaning improves growth rate in piglets (Jensen-Waern et al. 1998; Kim et al. 2012). Likewise, HS has been reported to improve pig performance probably through stabilisation of the intestinal microflora and the subsequent improvement in nutrient absorption, especially protein digestion and trace element utilisation (Islam et al. 2005; Wang et al. 2008). However, the better growth rates observed in the ZnO2.5 and HNa + ZnO1.7 piglets in the present study resulted rather from maintenance of gut health and elimination of the nutrient loss through diarrhoea than a direct growth promoting effect of ZnO and/or HS. In any case, the results confirmed that HNa (20 g/kg) supplemented with ZnO (1.7 g/kg) could give the same performance as that achieved by high dietary ZnO intake (2.5 g/kg).

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Zn levels in faeces

A positive consequence of the lower ZnO dietary uptake in piglets was the significant reduction ($P < 0.01$) of Zn content in the faeces in comparison with the ZnO2.5 treatment (Table 3). No significant differences were found between HNa + ZnO1.0, HNa + ZnO1.5 and HNa + ZnO1.7 treatments.

Haematological and serum biochemical profile

Most of the selected biochemical and haematological parameters, which are related directly to piglet health, did not differ significantly among the treatments (Table 4). Significant differences were found only in serum Zn and P concentrations. Significantly higher ($P < 0.01$) serum Zn as a result of high dietary ZnO intake in the ZnO2.5 group compared to the control and HNa groups with lower supplementation of ZnO is in agreement with previous reports (Jensen-Waern et al. 1998; Windisch et al. 1998; Martinez et al. 2005; Walk et al. 2013). Significantly lower ($P < 0.01$) serum P in ZnO2.5, HNa + ZnO1.7 and HNa + ZnO1.0 compared to C was most likely a result of the increased Zn in serum. The antagonistic nature of Zn and P is well known in soil and plants; however, their relationship and absorption in the small intestine of the pig is less clear. The decrease of serum P in pigs as the result of increased dietary Zn was also observed by Walk et al. (2013). The authors speculated that Zn and phosphate may have precipitated within the small intestine, which may have resulted in reduced serum P as ZnO supplementation increased. In contrast, according to Martinez et al. (2005), administration of Zn did not affect P in plasma and Shelton et al. (2011) even observed that plasma P increased with increasing dietary Zn in weaned pigs.

HS were reported to reduce serum minerals (P, Ca, Mg and Fe) in broilers (Rath et al. 2006; Celik et al. 2008; Demeterova et al. 2009; Samudovska and Demeterova 2010), and rabbits (Mista et al. 2012). A potential metal-chelating effect might be exerted by many reactive functional groups located in the structures of HS (Rath et al. 2006). This effect was not proven in the present study with dietary supplementation of HNa at a dose of 20.0 g/kg diet for three weeks. The serum levels of Ca, Mg and Fe were not affected by HNa treatment.

Poulsen (1995) observed increased ALP activity in weaned piglets fed high levels of ZnO. They suggested that it might be an effect of a higher circulating level of Zn in serum. This assumption is in accordance with findings of significantly reduced ALP activity and Zn concentrations in the serum of Zn-deficient pigs (Hartman et al. 1995). Herzig et al. (2009) reported non-significantly higher ALP activity in chickens after treatment with a diet containing Zn and humic acid. In contrast, dietary humic acids tended to decrease ALP activity in broilers (Rath et al. 2006; Celik et al. 2008; Demeterova et al. 2009). In the present study, slightly higher ALP activity was detected in the serum of piglets fed ZnO and HNa diets compared to C, but the difference was non-significant.

HS were described to decrease serum triacylglycerols and cholesterol levels in animals (Herzig et al., 2009; Samudovska and Demeterova 2010; Mista et al. 2012; Ozturk et al. 2012). After three weeks of HNa dietary treatment we observed only slightly (non-sig-

Table 3. Zn levels in piglet faeces

Parameter		Treatments[1]				
		C ($n = 3$)	ZnO2.5 ($n = 8$)	HNa + ZnO1.7 ($n = 8$)	HNa + ZnO1.5 ($n = 7$)	HNa + ZnO1.0 ($n = 6$)
Zn (mg /kg faeces)	$\bar{x}$	203^{A}	2.446^{B}	1.622^{C}	1.472^{C}	1.077^{C}
	SD	25	665	119	254	447

[1]C = control with no supplementation; ZnO2.5 = supplemented with high prophylactic dose of ZnO (2.5 g/kg diet); HNa + ZnO1.7, HNa + ZnO1.5, HNa + ZnO1.0 = supplemented with HNa (20.0 g/kg diet) and 1.7, 1.5 and 1.0 g ZnO/kg diet, respectively
The individual pig served as the experimental unit. The results are expressed as means and standard deviation. Statistical significance of the differences among the groups was determined by the Kruskal-Wallis test followed by the Dunn's multiple comparisons test. *P*-values lower than 0.05 were considered statistically significant. Superscripts denote statistically significant differences among groups (groups sharing the same superscript are not significantly different from each other, groups that have no superscript in common are significantly different from each other)

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Table 4. Serum biochemical and haematological parameters of piglets on day 21 after weaning

Parameter	Treatments[1]				
	C (n = 3)	ZnO2.5 (n = 8)	HNa + ZnO1.7 (n = 8)	HNa + ZnO1.5 (n = 7)	HNa + ZnO1.0 (n = 6)
Total protein (g/l)	51.37	50.03	48.26	52.64	46.81
Albumin (g/l)	32.6	31.17	30.65	31.95	30.40
Triacylglycerols (mmol/l)	0.53	0.53	0.48	0.48	0.45
Cholesterol (mmol/l)	2.70	2.24	2.72	2.70	2.33
Creatinine (µmol/l)	81.88	76.52	77.88	77.46	76.70
Urea (mmol/l)	5.67	5.80	4.86	5.20	4.71
ALT (µkat/l)	0.95	0.95	0.98	1.06	0.84
AST (µkat/l)	0.76	0.78	0.71	1.00	0.91
ALP (µkat/l)	4.79	5.52	5.37	5.99	5.32
Ca (mmol/l)	3.51	3.43	3.70	3.95	3.56
P (mmol/l)	3.44^{A}	2.46^{B}	2.42^{B}	2.82^{AB}	2.42^{B}
Mg (mmol/l)	0.88	0.92	0.89	0.97	0.90
Zn (mmol/l)	16.05^{A}	44.78^{B}	24.12^{A}	22.62^{A}	18.86^{A}
Fe (µmol/l)	19.13	18.08	18.23	19.04	19.00
Erythrocytes ($\times 10^{12}$/l)	5.59	6.49	6.39	5.70	5.92
Leukocytes ($\times 10^{9}$/l)	20.48	19.26	19.72	18.56	15.32
Lymphocytes (%)	53.58	54.93	56.56	55.07	60.42
Neutrophils (%)	44.58	43.43	41.81	43.64	38.08
Monocytes (%)	0.67	0.43	0.75	0.36	0.75
Eosinophils (%)	1.00	0.93	0.50	0.71	0.67
Basophils (%)	0.17	0.29	0.38	0.21	0.08
Hematocrit (%)	31.05	37.28	36.03	33.07	33.94
Haemoglobin (g/l)	102.0	107.85	102.13	100.33	100.40

[1]C = control with no supplementation; ZnO2.5 = supplemented with high prophylactic dose of ZnO (2.5 g/kg diet); HNa + ZnO1.7, HNa + ZnO1.5, HNa + ZnO1.0 = supplemented with HNa (20.0 g/kg diet) and 1.7, 1.5 and 1.0 g ZnO/kg diet, respectively
The individual pig served as the experimental unit. The results are expressed as means. Statistical significance of the differences among the groups was determined by the Kruskal-Wallis test followed by the Dunn's multiple comparisons test. *P*-values lower than 0.05 were considered statistically significant. Superscripts denote statistically significant differences among groups (groups sharing the same superscript are not significantly different from each other, groups that have no superscript in common are significantly different from each other)

nificant) lower levels of triacylglycerols in serum of HNa groups compared to the C and ZnO2.5 groups. Cholesterol levels were not affected by the treatments.

The present study found no significant effect of ZnO and HNa treatment on blood cells, haematocrit and haemoglobin. Rupic et al. (1998) reported that inorganic Zn in the diet of fattening pigs appears to be responsible for the increase in the values of erythrocytes, haemoglobin and haematocrit and Kim et al. (2004) observed significantly increased erythrocyte values in pigs supplemented with HS. We observed non-significantly higher erythrocytes and haematocrit in ZnO2.5 and HNa + ZnO1.7 as well as non-significantly higher haemoglobin levels in the ZnO2.5 group after three weeks of treatment.

The lymphocytes content did not significantly differ among the groups in the study. However, the slightly higher values in HNa-supplemented groups could be due to the beneficial effect of HS on the immune system as was also described in finishing pigs fed diets with 5% and 10% HS after eight weeks (Wang et al. 2008).

The results described here indicate the possibility of reducing the high pharmacological levels of ZnO in the prophylaxis of PWD through partial replacement with HNa in the piglet diet. While main-

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taining a favourable prophylactic effect, a positive consequence of lower ZnO dietary uptake in the HNa + ZnO1.7 group was the significant reduction of Zn content in faeces.

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