British Journal of Nutrition (2009), **102**, 687–693
doi:10.1017/S0007114509289033

Supplemental zinc reduced intestinal permeability by enhancing occludin and zonula occludens protein-1 (ZO-1) expression in weaning piglets

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(Received 3 December 2008 – Revised 16 January 2009 – Accepted 26 January 2009 – First published online 9 March 2009)

The present study was carried out to evaluate the pharmacological effect of Zn in diarrhoea in relation to intestinal permeability. Seventy-two weaning piglets, aged 24 d, were allocated to three dietary treatments: (1) control diet without supplemental Zn; (2) control diet supplemented with 2000 mg Zn/kg from ZnO; (3) control diet supplemented with 2000 mg Zn/kg from tetrabasic zinc chloride (TBZC). At the end of a 14 d experiment period, piglets were weighed, feed consumption was measured, and mucosal barrier function was determined using the lactulose/mannitol test. Expression of mucosal tight junction protein was measured at RNA and protein level. Inclusion of TBZC or ZnO in the diet significantly increased average daily gain ($P<0·01$) and average daily feed intake ($P<0·05$), while leading to reduced feed conversion ratio ($P<0·05$) and faecal scores ($P<0·01$). TBZC reduced urinary lactulose:mannitol ratios of weaning piglets ($P<0·05$), while dietary supplementation with ZnO tended to reduce urinary lactulose:mannitol ratios ($P=0·061$). ZnO or TBZC significantly enhanced the mRNA and protein expression of occludin ($P<0·05$) and zonula occludens protein-1 (ZO-1) ($P<0·05$) in the ileal mucosa. Piglets fed the TBZC-supplemented diet had a higher level of occludin than pigs fed the ZnO-supplemented diet ($P<0·05$). The results indicate that Zn supplementation decreased faecal scores and the reduction was accompanied by reduced intestinal permeability, which was evident from the reduced urinary lactulose:mannitol ratios and increased expression of occludin and ZO-1. Therefore, the protective effect of pharmacological levels of dietary Zn in reducing diarrhoea might, at least partly, be associated with reduced intestinal permeability.

Dietary zinc: Piglets: Intestinal permeability: Occludin: Zonula occludens protein-1 (ZO-1)

Post-weaning diarrhoea is one of the most common causes of morbidity and mortality for weaning piglets[1], and hence the reduced performance in pigs. The mechanism(s) for post-weaning diarrhoea has not been completely elucidated. Feeding high levels of Zn to weaning piglets could decrease the incidence of post-weaning diarrhoea[2,3]. Our previous experiment demonstrated that feeding pharmacological levels of tetrabasic zinc chloride (TBZC) reduced the incidence and severity of diarrhoea, and improved faecal consistency after weaning[4,5]. However, the mechanism of the beneficial action remains unclear.

The results that weaning induced changes in permeability to macromolecules could partly explain post-weaning diarrhoea[6]. Intestinal permeability changes reflecting the integrity of the small-intestinal epithelium mucosa barrier had been reported in diarrhoea[7]. Intestinal permeability was increased in human and animal subjects with diarrhoeal disease[8,9]. The consequences of increasing intestinal permeability could be dramatic, as it could allow the indiscriminate entry of extracellular antigens and pathogenic micro-organisms. Oral Zn supplementation to children living in developing countries with diarrhoea reduced intestinal permeability[10] and diarrhoea[11]. Rodríguez *et al.* [12] reported that malnutrition was associated with increased intestinal paracellular permeability to small molecules, and pharmacological doses of Zn prevented such functional abnormality. However, little is known about the influence of Zn supplementation on the modulation of intestinal permeability in weaning piglets.

Tight junctions are one of the most important components of the intestinal mucosal barrier against macromolecular transmission[13]. They are made up of a complex of integral membrane proteins, which are tethered to cytoplasmic plaque proteins[14]. An increase in intestinal permeability induced by weaning might be attributed to the alterations of tight junction protein expression[15]. Therefore, it would be of interest to examine whether supplementation of pharmacological levels of Zn modulates the expression of tight junction proteins.

The purpose of the present experiment was to evaluate the beneficial role of pharmacological levels of Zn for post-weaning diarrhoea, intestinal permeability and expression of tight junction proteins, mainly occludin, zonula occludens protein-1 (ZO-1) and claudin-1.

Materials and methods

Pigs and diets

Seventy-two weaning piglets (Landrace × Large White) with an initial body weight of 6·7 (SEM 0·20) kg at 24 (SEM 1)

Abbreviations: TBZC, tetrabasic zinc chloride; ZO-1, zonula occludens protein-1.

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d of age were allocated to pens on the basis of similar body weight, ancestry and sex. Each treatment had six replications (pens) of four piglets (half castrated males and half females). Piglets were housed on plastic slotted floors (1·3 × 1·2 m per pen) with self-feeders and automatic stainless nipple waterers. Feed and water were available *ad libitum*. Temperatures (25–28°C) and a cycle of 16 h light and 8 h dark were controlled. Experimental protocols were approved by the China Agricultural University Animal Care and Use Committee. The basal diet (Table 1) with approximately 129·7 mg Zn/kg was formulated to meet or exceed the nutrient requirements recommended by the National Research Council (1998). The three experimental diets were: (1) basal diet (control group) without supplemental Zn; (2) basal diet supplemented with 2000 mg Zn/kg from ZnO (ZnO group); (3) basal diet supplemented with 2000 mg Zn/kg from TBZC (TBZC group). The TBZC ($Zn_5(OH)_8Cl_2{\cdot}H_2O$) contained 58 % Zn and ZnO contained 76 % Zn. TBZC or ZnO replaced wheat bran in the diet. The experiment lasted 14 d. Hahn & Baker[2] showed that ZnO can improve the daily gain of weaning pigs, while our previous studies demonstrated similar effects of ZnO and TBZC on the performance of weaning pigs[5,6]. Therefore, ZnO and TBZC were selected as two dietary Zn sources in the present study.

Table 1. Composition of the basal diets

	Composition
Ingredients (%)	
Maize	55
Soyabean meal (44 % crude protein)	14
Extruded soyabean meal	15
Fish meal	3
Sprayed dried plasma proteins	3
Soyabean oil	1
Whey	4·8
Limestone	0·7
Dicalcium phosphate	1·1
Sodium chloride	0·35
Colistin	0·06
Choline chloride	0·2
L-Lysine·HCl (78 %)	0·28
DL-Methionine	0·034
Threonine	0·04
Vitamin and mineral premix*	1·0
Wheat bran	0·436
Calculated composition	
Digestible energy (kJ/kg)	144 20
Crude protein (%)	20·02
Ca (%)	0·75
Total P (%)	0·68
Available P (%)	0·51
Lysine (%)	1·33
Methionine and cystine (%)	0·74
Threonine (%)	0·89
Zn (mg/kg)	129·7

*Mineral and vitamin premix supplied (per kg final diet): Zn, 100 mg ($ZnSO_4$); Cu, 10 mg ($CuSO_4.5H_2O$); Fe, 100 mg ($FeSO_4$); Mn, 10 mg ($MnSO_4{\cdot}H_2O$); Se, 0·3 mg (Na_2SeO_3); I, 0·5 mg (KI); vitamin A, 5 mg; vitamin D_3, 4·5 g; vitamin E, 60 mg; vitamin K, 4·4 mg; riboflavin, 8·8 mg; niacin, 33 mg; pantothenic acid, 30 mg, vitamin B_{12}, 22 μg; folic acid, 900 μg; thiamin, 2·5 mg; pyridoxine, 4·0 mg; biotin, 200 μg.

Measurements

Growth performance and faecal scores. Faecal scores were evaluated daily and expressed as percentages for a period of 2 weeks. The severity of scours (1–5) as previously described[4] were assigned daily by an individual unaware of the dietary treatments (1 = hard faeces, rarely seen; 2 = no scours, normal consistency of faeces formed; 3 = mild scours, soft, partially formed faeces; 4 = moderate scours, loose, semi-liquid faeces; 5 = watery faeces). Values of each pen were subsequently averaged for a period of 2 weeks. At the end of the experiment, piglets were weighed after overnight fasting, feed consumption was measured and the average daily gain, average daily feed intake and the feed conversion ratio were measured.

Intestinal permeability. On 14 d of the experiment, one barrow from each pen was selected randomly and then housed individually in metabolism cages. Intestinal permeability was assessed by the oral administration of lactulose and mannitol. After an overnight fast, a solution of 6 g lactulose (500 mg/kg body weight; Sigma, St Louis, MO, USA) and 0·6 g mannitol (50 mg/kg body weight; Sigma) in 20 ml distilled water was administered via the gastric tube. Piglets were fasted for the whole study period but were allowed to drink water after 30 min. Urine was collected over 6 h with 1 ml of 2 % chlorexidine as preservative after the gastric administration of lactulose and mannitol solution. Total urine volume was measured on completion of 6 h collection and a 20 ml sample was stored at −80°C until analysis. Urine was centrifuged at 3000 rpm for 10 min. Urinary lactulose and mannitol concentrations were simultaneously determined by HPLC coupled with ED50 electrochemical detection (Dionex, Idstein, Germany) with a platinum working electrode, according to the method of Generoso *et al.*[16]. The chromatography module was a 250 × 40 mm Carbopac PA-1 column (Dionex, Idstein, Germany), the eluent was 150 mmol NaOH and the flow was 0·5 ml/min. Sample injection (20 μl) occurred via an autosampler. The ratio between the 6 h recovery percentages of lactulose and mannitol was considered as an index of intestinal permeability[17].

Real-time polymerase chain reaction. At the end of the collection period, the piglets were euthanised with an intraperitoneal injection of pentobarbital sodium (60 mg/kg). The abdominal cavity was opened, and the distal 6–8 cm of ileum from the ileo-caecal orifice was removed. Ileal samples were cut longitudinally to expose mucosa and washed three times in ice-cold PBS to remove the mucus and digesta. The ileal mucosa was scraped gently, quickly frozen in liquid N_2, and stored at −80°C until use for mRNA and protein determination.

Total RNA was extracted from the ileal mucosa using TRIzol reagent (Invitrogen Company, Carlsbad, CA, USA) according to the manufacturer's instructions. The yield and quality of the RNA were determined spectrophotometrically using A_{260} and A_{280} measurements, and by electrophoresis on 1·3 % agarose gels. About 5 μl purified RNA was used as a template for cDNA synthesis in the presence of 1·25 μl Moloney murine leukaemia virus (M-MLV) RT (200 units) (Invitrogen Company), 1 μl of Oligo dT18 (30 mM), 13 μl RNAse-free water, 1·25 μl 10 mM-dNTP mix and 1 μl RNAsin® ribonuclease

inhibitor (50 units/μl). After incubation for 60 min at 37°C, the RT was inactivated at 70°C for 15 min.

The gene-specific primers for occludin, ZO-1, claudin-1 and β-actin were as follows: forward 5′-ATC AAC AAA GGC AAC TCT-3′, reverse 5′-GCA GCA GCC ATG TAC TCT-3′ for occludin (157 bp); forward 5′-GAG TTT GAT AGT GGC GTT-3′, reverse 5′-GTG GGA GGA TGC TGT TGT-3′ for ZO-1 (298 bp); forward 5′-TAC TTT CCT GCT CCT GTC-3′, reverse 5′-AAG GCG TTA ATG TCA ATC-3′ for claudin-1 (169 bp); forward 5′-CTG GAA CGG TGA AGG TGA-3′, reverse 5′-TTT GGA AAG GCA GGG ACT-3′ for β-actin (170 bp).

cDNA were amplified by real-time PCR using the Applied Biosystems ABI-PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA). Each PCR was on 2 μl of cDNA mixed with 0·25 μmol of each primer, 10 μl of SYBR Green PCR master mix (Applied Biosystems), 6 μl sterile super-stilled water for a final volume of 20 μl. Amplifications were performed as follows: 2 min at 55°C, and 10 min at 94°C followed by forty cycles of 30 s denaturalisation at 94°C, 30 s annealing at 62°C, and 45 s primer extension at 72°C. The relative standard curve methods were used for abundance of gene expression. Briefly, copy numbers were determined from three independent cDNA preparations of any sample. Copy numbers were calculated relative to a dilution series of the respective reference plasmids, comprising 10^3–10^8 copies. The reference plasmids contained the cloned RT-PCR products obtained with these primers. The housekeeping gene, β-actin, was used as the internal standard for the PCR reaction. The Ct-value (number of cycles halfway through the experimental phase) was determined and was used to calculate the relative expression level compared with β-actin.

Protein determination. Western blot analysis was performed by modifying the method of Kansagra *et al.* [18]. Briefly, 500 mg of pulverised frozen tissue was homogenised in 2 ml lysis buffer containing 50 mM-2-amino-2-hydroxymethyl-propane-1,3-diol (Tris)-HCl, 100 mM-phenylmethylsulfonyl fluoride, 0·5 mM-sodium dodecylsulfate and 1 mM-dithiothreitol. After the protein concentration of supernatant fractions was quantified by a standard bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA), equal protein amounts were separated on SDS-PAGE gels. Separated proteins were then transferred onto nitrocellulose membranes. After blocking with 3 % non-fat dry milk in Tris-buffered saline overnight, blots were incubated overnight at 4°C with specific primary antibodies. The following antibodies were used in our experiments: rabbit polyclonal anti-claudin-1 (1:300; Zymed, South San Francisco, CA, USA), rabbit polyclonal anti-occludin (1:450; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and rabbit polyclonal anti-zonula occludens-1 (1:400; Santa Cruz Biotechnology). After three washes with Tris-buffered saline containing 0·1 % Tween 20, blots were reacted for 60 min with a horseradish peroxidase-conjugated secondary antibody (anti-rabbit IgG; Santa-Cruz Biotechnology), and were washed again. Chemiluminescence detection was performed using the ECL Plus™ Western Blotting Detection System (Amersham, Arlington Heights, IL, USA) according to the manufacturer's instructions. Blots were also probed with anti-actin antibodies.

Statistical analysis

The experiment was designed as randomised complete-block designs. Data were subjected to Levene's test for homogeneity of variances before further statistical analysis, and expressed as mean values with their standard errors. Data were analysed by ANOVA using the general linear model (GLM) procedures of SAS (1999; SAS Institute, Inc., Cary, NC, USA). Each pen was set as the experimental unit for growth performance and faecal scores, but the individual piglet was the experimental unit for the urinary lactulose:mannitol ratio and tight junction proteins. Differences between treatments were detected by Duncan's multiple-range test. Differences were considered significant at $P<0·05$. Scouring data were analysed after being arcsin transformed. Actual scouring data are listed in Table 2, while SE values pertain to the transformed data.

Results

Growth performance and faecal scores

Inclusion of TBZC or ZnO at 2000 mg Zn/kg diet increased average daily gain ($P<0·01$) and average daily feed intake ($P<0·05$), and reduced feed conversion ratio ($P<0·05$),

Table 2. Growth performance and faecal scores of weaning piglets fed diets supplemented with zinc oxide or tetrabasic zinc chloride (TBZC)*
(Mean values with their standard errors)

	Group						
	Control		ZnO		TBZC		
	Mean	SE	Mean	SE	Mean	SE	*P*
ADG (g)	219·0[b]	3·6	266·5[a]	8·8	265·5[a]	8·0	<0·001
ADFI (g)	357·3[b]	4·4	380·7[a]	6·7	382·8[a]	8·3	0·029
FCR	1·63[a]	0·04	1·43[b]	0·04	1·45[b]	0·05	0·010
Faecal scores (%)†	17·8[a]	0·4	7·3[b]	0·5	3·1[b]	0·3	0·001
Faecal consistency	2·41[a]	0·09	2·12[b]	0·04	2·14[b]	0·09	0·018

Control, control diet without Zn supplemented; ZnO, supplemented with 2000 mg Zn/kg from ZnO; TBZC, supplemented with 2000 mg Zn/kg from TBZC; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio, namely feed/gain.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* Each value represents the mean of six pens of four piglets (n 6).
† Faecal scores (%) were calculated as the percentage of the total number of days that signs of scours were evident within the pen on the total number of days (56 d).

faecal scores ($P<0{\cdot}01$) and faecal consistency ($P<0{\cdot}05$) of weaning piglets compared with the control group during weeks 1–2 (Table 2). There was no difference in growth performance or faecal scores between piglets fed TBZC- and ZnO-supplemented diets during the whole experimental period.

Intestinal permeability

Intestinal permeability was assessed by measuring 6 h urinary excretion of lactulose and mannitol in piglets fed any of the study diets (Table 3). Piglets fed the TBZC-supplemented diet had a reduced ($P<0{\cdot}05$) urinary lactulose:mannitol ratio and recovery of lactulose compared with piglets fed the control diet. Dietary supplementation with ZnO tended ($P=0{\cdot}061$) to reduce the urinary lactulose:mannitol ratio when compared with the control group. No differences in urinary lactulose:mannitol ratios and recovery of lactulose between the TBZC group and ZnO group were observed. ZnO or TBCZ supplementation in the diet for weaning piglets did not affect the urinary recovery of mannitol ($P>0{\cdot}05$).

mRNA and protein levels for occludin, zonula occludens protein-1 and claudin-1

Added ZnO or TBZC to the diet of weaning piglets significantly enhanced ($P<0{\cdot}05$) mRNA levels for occludin and ZO-1 in the ileal mucosa (Fig. 1). However, ZnO or TBCZ supplementation in the diet for weaning piglets did not affect the mRNA levels of claudin-1 ($P>0{\cdot}05$).

For estimating protein levels, Western blot analysis revealed that ZnO or TBZC significantly increased ($P<0{\cdot}05$) the protein expression of occludin and ZO-1 in the ileal mucosa when compared with the piglets fed the control diet (Fig. 2). Piglets fed the TBZC-supplemented diet exhibited a higher ($P<0{\cdot}05$) level of occludin than piglets fed the ZnO-supplemented diet.

Discussion

Supplementation of TBZC or ZnO to a diet for weaning piglets enhanced growth performance and reduced faecal scores of weaning piglets for weeks 1–2. This result was consistent with previous reports that high levels of dietary Zn improved growth performance and reduced the incidence and severity of diarrhoea after weaning[3,4,19]. However, the underlying mechanism, by which the high dietary level of Zn decreased the incidence of post-weaning diarrhoea, is still controversial. The integrity of the intestinal barrier was fundamental to the proper functioning of the epithelial cells and to preventing the entry of pathogenic bacteria[20]. The fact that intestinal permeability was increased with diarrhoea[8,9] was confirmed in our piglet study.

The measurement of intestinal permeability, by testing the urinary excretion of lactulose and mannitol, was a reliable, standard, non-toxic and non-invasive method to investigate the function of the intestinal mucosa barrier[21,22]. It is known that lactulose traverses the intestinal wall by paracellular pathways via the intercellular tight junctions of epithelial crypts, whereas mannitol passes predominantly by the transcellular pathways of epithelial villi. When lactulose and mannitol are combined at a fixed concentration ratio, the influence of variables, such as rate of ingestion, gastric emptying, intestinal motility, and excretion and bladder emptying, would apply equally to both[23]. Changes in intestinal permeability would be reflected in changes of the urinary lactulose:mannitol excretion ratio. Therefore, the reduced lactulose:mannitol ratio supports the idea of a tightening intestinal permeability for piglets supplemented with TBZC or ZnO. This was consistent with the report of Roselli *et al.* [24] that a high dietary level of Zn might prevent the increase in tight junction permeability induced by enterotoxigenic *Escherichia coli*, as indicated by both the high transepithelial electrical resistance values and the absence of [^{14}C]inulin transfer.

Zn is essential for DNA, RNA and protein synthesis. Dietary supplementation of Zn has been shown to enhance intestinal mucosal repair in patients suffering from acrodermatitis enteropathica. Some authors reported that Zn played a role in maintaining epithelial barrier integrity and function. For example, Zn supplementation might improve mucosal repair and paracellular permeability in rats with experimental colitis[9], and reduce intestinal permeability in Bangladeshi children with acute diarrhoea and persistent diarrhoea syndrome[10]. Furthermore, Tran *et al.* [25] indicated that dietary supplementation of a pharmacological dose of Zn reduced methotrexate-induced intestinal damage and enhanced recovery. Thus, the present results are consistent with these findings.

The impact of pharmacological levels of Zn supplementation on the tight junction proteins was examined in the

Table 3. Urinary recovery (% of administered dose) of lactulose and mannitol, and urinary lactulose:mannitol ratios of weaning piglets fed diets supplemented with zinc oxide or tetrabasic zinc chloride (TBZC)*

(Mean values with their standard errors)

	Group						
	Control		ZnO		TBZC		
	Mean	SE	Mean	SE	Mean	SE	*P*
Lactulose recovery	1·41[a]	0·27	1·01[a,b]	0·07	0·86[b]	0·09	0·097
Mannitol recovery	10·27	1·09	10·00	0·91	10·40	0·93	0·958
Lactulose:mannitol ratio	0·135[a]	0·014	0·104[a,b]	0·009	0·087[b]	0·012	0·040

Control, control diet without Zn supplemented; ZnO, supplemented with 2000 mg Zn/kg from ZnO; TBZC, supplemented with 2000 mg Zn/kg from TBZC.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0{\cdot}05$).
* Each value represents the mean of six pens of four piglets (n 6).

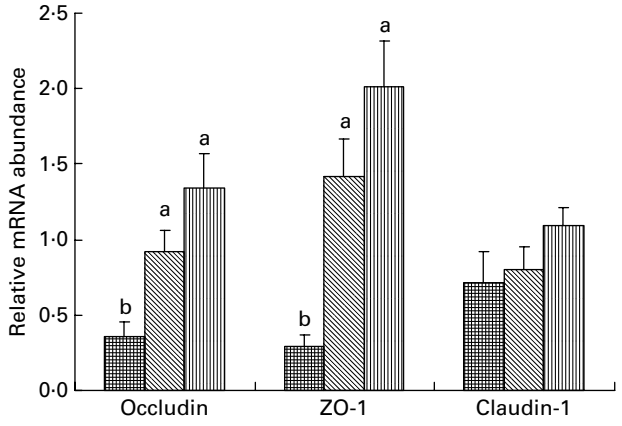

Fig. 1. Relative mRNA levels of occludin, zonula occludens protein-1 (ZO-1) and claudin-1 in ileac mucosa of piglets fed diets supplemented with ZnO or tetrabasic zinc chloride (TBZC). (▦), Control diet without Zn supplemented; (▧) diet supplemented with 2000 mg Zn/kg from ZnO; (▥), diet supplemented with 2000 mg Zn/kg from TBZC. Values are means, with standard errors represented by vertical bars. Five piglets were tested in each group (n 5). [a,b] Mean values with unlike letters were significantly different ($P<0·05$).

present study for the first time. Intestinal permeability had long been considered as an indicator of intestinal epithelial barrier function. This barrier was primarily regulated by a well-organised system of an epithelial junctional complex referred to as the tight junction[26,27]. The tight junction is comprised of several unique proteins, including the transmembrane protein occludin[28,29], junctional adhesion molecule[30], members of the claudin family[31,32], linker proteins such as ZO-1[33], and so on. Occludin, ZO-1 and claudin-1 are the most important and critical components in the structural and functional organisation of the tight junctions[34]. Occludin is an integral membrane protein of the epithelial tight junction, having functional importance in maintaining the integrity and barrier function of the tight junction[29,35,36]. ZO-1 is an important linker protein in tight junctions, binding to C-terminal sequences of occludin and β-actin[34] and acting as a bridge between the plasma membrane and cytoskeleton proteins. Claudin-1 appears fairly tightly localised to the expression of ZO-1 in the small intestine[37]. To better clarify the molecular mechanism for the reduction of intestinal permeability in weaning piglets fed high levels of Zn, we determined the changes in the expression of occludin, ZO-1 and claudin-1 at the mRNA level. The result of our work indicated that high dietary levels of Zn increased occludin and ZO-1 mRNA expression. Having observed that TBZC or ZnO enhanced mRNA levels of occludin and ZO-1, we wanted to determine whether TBZC or ZnO influenced protein expression of occludin and ZO-1. Occludin and ZO-1 protein expression in ileal epithelium from piglets fed the control, ZnO or TBZC diet were performed by Western blots. Consistent with the above observations, TBZC or ZnO stimulated the protein expression of occludin and ZO-1.

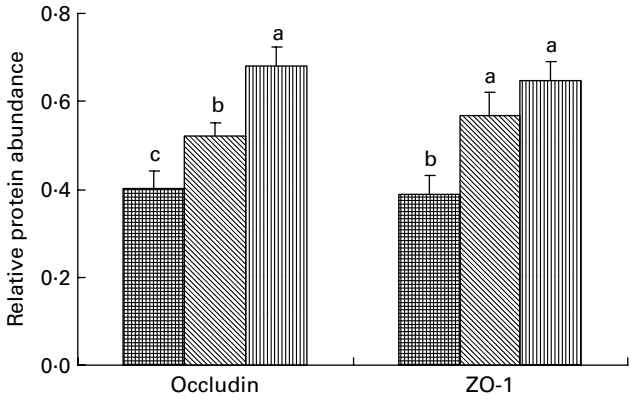

Fig. 2. Relative protein expression levels of occludin and zonula occludens protein-1 (ZO-1) in ileac mucosa of piglets fed diets supplemented with ZnO or tetrabasic zinc chloride (TBZC). (▦), Control diet without Zn supplemented; (▧), diet supplemented with 2000 mg Zn/kg from ZnO; (▥), diet supplemented with 2000 mg Zn/kg from TBZC. The value of protein expression = densitometry units of selected protein/densitometry units of β-actin detected by Western blotting. Values are means, with standard errors represented by vertical bars. Five piglets were tested in each group (n 5). [a,b,c] Mean values with unlike letters were significantly different ($P<0·05$).

Weaning piglets are exposed to numerous stresses (for example, changing from a liquid to a solid diet) and frequently suffer infections, mainly caused by enterotoxigenic *E. coli* [38]. These combined factors increase epithelial permeability by disassembling tight junction proteins or reducing their expression[39]. The increased intestinal permeability in turn enhances antigenic exposure to immune cells and leads to local inflammation, further compromising barrier function[39–41], and leading to the occurrence of post-weaning diarrhoea. Kansagra *et al.* [18] also implicated that the changes in intestinal permeability during weaning might be due to a breakdown in tight junction integrity. The present study demonstrated that dietary supplementation with high levels of ZnO or TBZC could in part prevent alterations in intestinal permeability during the weaning period by enhancing the expression and production of occludin and ZO-1. Previous animal experiments confirmed that intestinal permeability was closely related to occludin levels[36,42]. *In vitro* experiments also have shown that occludin has the ability for sealing the tight junction[36,42]. There was more of a tendency for TBZC than for ZnO to ameliorate intestinal permeability; this might contribute to the higher protein levels of occludin induced by TBZC.

In conclusion, high dietary levels of Zn supplementation led to a marked decline in faecal scores, and the reduction was accompanied by reduced intestinal permeability, which was evident from the reduced urinary lactulose:mannitol ratios and increased expression of occludin and ZO-1. Therefore, the protective effect of pharmacological levels of dietary Zn in reducing diarrhoea might, at least partly, be associated with reduced intestinal permeability.

Acknowledgements

The present study was financially supported by the National Basic Research Program (project no. 2004 CB 117504) and by the Scientific Research Fund of China Agricultural University (project no. 2008029).

B. K. Z. was responsible for the design of the study, collection of data, analysis of data, and writing of the manuscript, whilst Y. M. G. was responsible for the conception and design of the study, and supervised all aspects of its implementation.

There is no conflict of interest that should be disclosed.

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