Iowa State University
Department of Animal Science

Zinc oxide does not alter ex vivo intestinal integrity or active nutrient transport in nursery pigs

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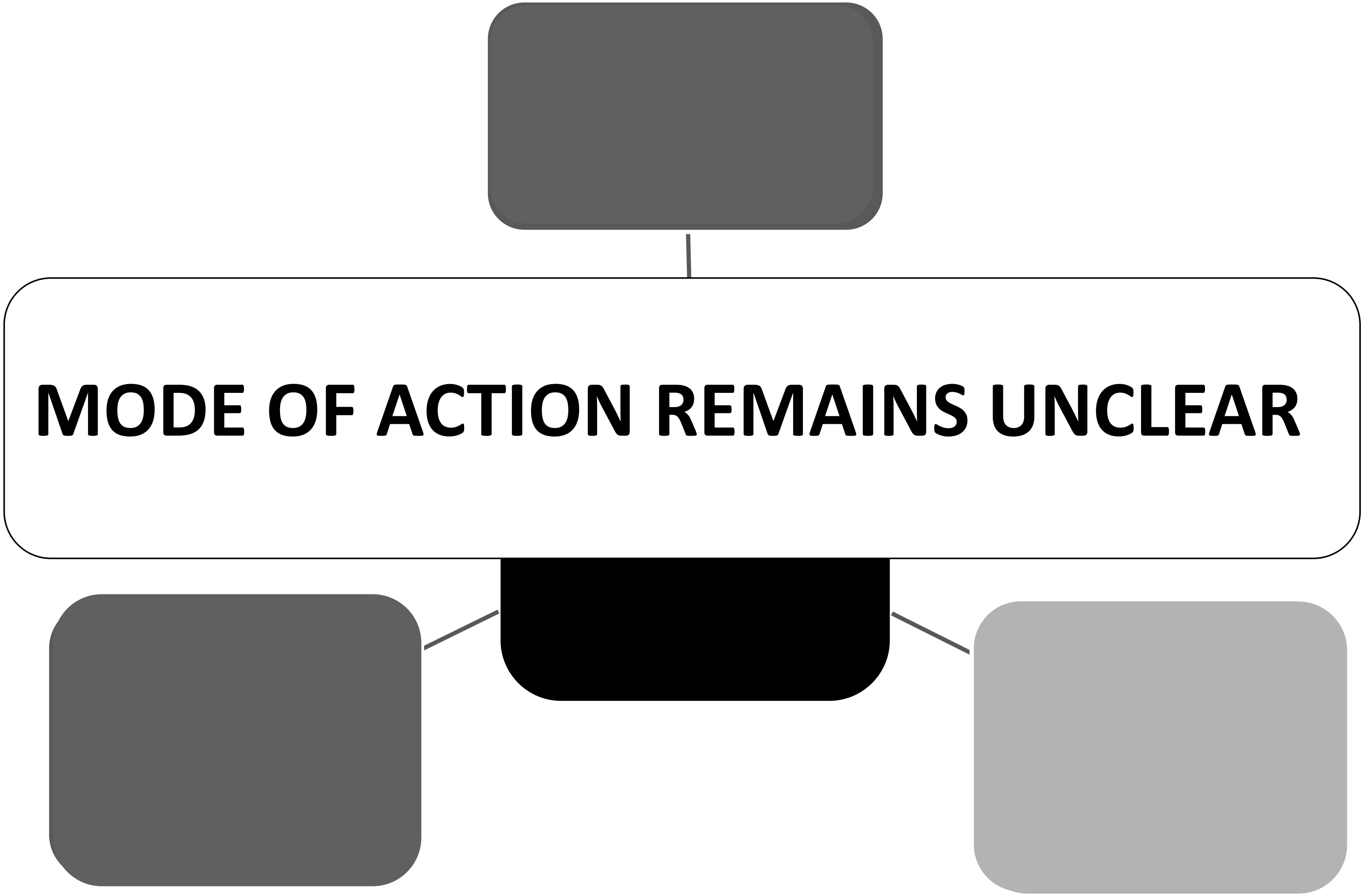
Background

- The Dietary Requirement for a nursery pig (5-11kg) is 100 mg/kg diet (NRC, 2012)

- Pharmacological levels of ZnO (2,000 – 3,000 ppm) (Carlson et al., 1999, Hill et al., 2000, Poulsen, 1995)
  - Increase growth
  - Reduce incidence of enteric bacterial disease
  - Reduce post-weaning diarrhea

- Concerns
  - Environmental & AMR concerns (Romeo et al., 2014, EFSA, 2014, Cavaco et al., 2011)
  - Issues with prolonged feeding (Burrough et al., 2019)



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Objective & Hypothesis

- Evaluate the effect of pharmacological ZnO on performance and intestinal function of recently weaned pigs

- We hypothesized that improved growth is related to improved intestinal integrity and active nutrient transport

Materials and Methods

- Experiment 1 = Growth study (4.37 ± 0.21 kg BW)
- Experiment 2 = Serial necropsy study to assess *ex vivo* intestinal function and integrity (5.45 ± 0.23 kg BW)
- In both experiments 19-21 d old freshly weaned pigs were utilized (F25 sows x 6.0 sires; Genetiporc, PIC, Hendersonville, TN)

Experiment 1

Ingredient, %	Phase 1 d 0-7	Phase 2 d 7-21
Corn, yellow dent	59.36	64.49
Soybean meal, 48%	15.00	18.00
Casein	8.50	5.17
Whey, dried	10.00	5.00
Fish meal, menhaden	2.00	2.00
Soybean oil	1.50	1.62
L-lysine HCl	0.35	0.35
DL-methionine	0.13	0.11
L-threonine	0.10	0.10
L-valine	0.34	0.21
Limestone	1.15	0.87
Monocalcium phosphate, 21%	0.85	1.38
Salt	0.40	0.40
Vitamin premix	0.15	0.15
Trace mineral premix	0.15	0.15
Calculated Composition		
CP, %	22.65	20.79
ME, kcal/kg	3,395	3,395
NE, kcal/kg	2,526	2,511
Lys, SID %	1.53	1.36

Experiment 2

Ingredient, %	Phase 1 d 0-14	Phase 2 d 14-21
Corn, yellow dent	57.26	64.91
Soybean meal, 48%	15.00	20.00
Casein	6.17	3.01
Oats	2.00	-
Whey, dried	10.00	5.00
Fish meal, menhaden	5.00	3.00
Soybean oil	1.72	1.00
L-lysine HCl	0.30	0.34
DL-methionine	0.09	0.09
L-threonine	0.07	0.07
L-valine	0.22	0.10
Limestone	0.79	0.91
Monocalcium phosphate, 21%	0.78	0.97
Salt	0.40	0.40
Vitamin & Trace Mineral premix	0.10	0.10
Calculated Composition		
CP, %	22.65	20.79
ME, kcal/kg	3,395	3,395
NE, kcal/kg	2,526	2,511
Lys, SID %	1.47	1.30

- Phase 1 ZnO = 3,000 ppm
- Phase 2 ZnO = 2,000 ppm

Statistics

- Completely randomized design
- MIXED Procedure of SAS v 9.4
- Experimental unit = pig
- Expt 1
  - Fixed effect of treatment
- Expt 2
  - Fixed effects of treatment, day, and treatment*day
- $P \leq 0.05$ is considered significant and $0.05 < P \leq 0.1$ is considered a tendency

Growth Performance Results

	Treatment			
Ingredient	NC	ZN	SEM	*P*-Value
Phase 1				
ADG, kg/d	-0.01	0.00	0.02	0.634
ADFI, kg/d	0.05	0.07	0.01	0.395
GF	-2.52	-2.38	0.02	0.949
Phase 2				
ADG, kg/d	0.23[b]	0.31[a]	0.03	0.050
ADFI, kg/d	0.26	0.30	0.03	0.428
GF	0.80	0.93	0.06	0.122
Overall				
ADG, kg/d	0.15	0.21	0.02	0.095
ADFI, kg/d	0.22	0.26	0.02	0.182
GF	0.67	0.79	0.06	0.146
Ileum Morphology				
Villus, µm	286	272	16.8	0.570
Crypt, µm	159	154	5.62	0.565
V:C	1.90	1.81	0.07	0.426

Are these improvements in performance related to changes in intestinal integrity and permeability?

Intestinal Integrity and Nutrient Transport (expt. 2)

- Serial Necropsy – 4 pigs/trt/d

Day 2 → Day 7 → Day 21

- Fresh ileum and colon were collected from each pig and mounted on modified Ussing chambers.
  - Transepithelial Resistance (TER; higher is better).
  - Macromolecule permeability (PaPP; lower is better). of 4 kDa Dextran (mucosal to serosal flux; FD4).
  - Active glucose and glutamine transport (ileum).

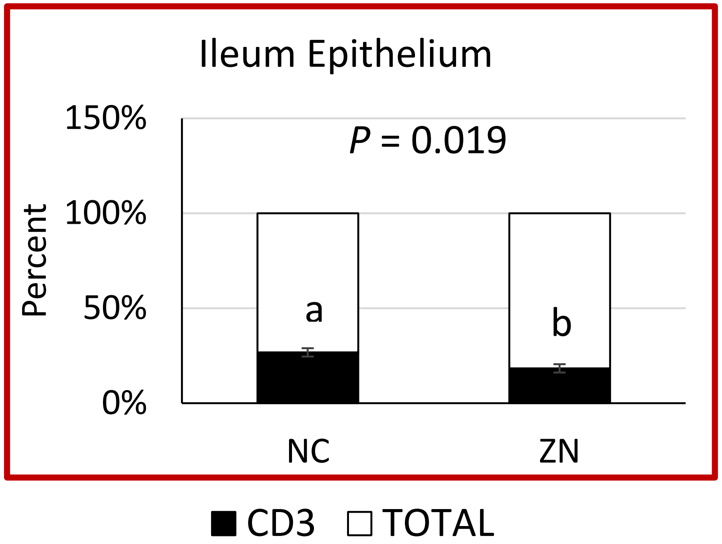
Intestinal Integrity Results

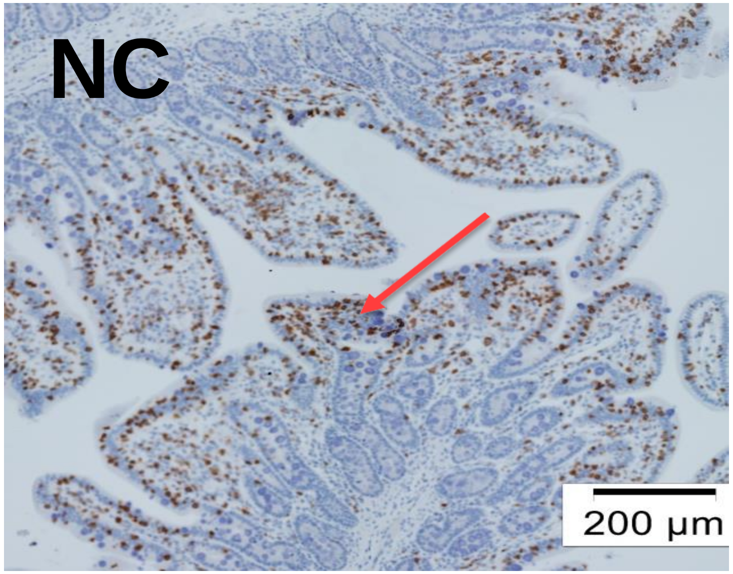
Item	day			SEM	P-Value		
	2	7	21		Trt	Day	Trt*Day
Ileum							
TER, $\Omega \times cm^2$							
NC	51.5	40.9	67.6	15.12	0.677	0.068	0.632
ZN	61.4	55.6	94.2				
Glucose, μA^2							
NC	17.7	54.3	42.4	24.56	0.861	0.558	0.730
ZN	29.5	68.3	50.9				
Glutamine, μA^2							
NC	3.5	20.2	8.3	5.74	0.427	0.982	0.220
ZN	6.3	15.0	14.1				
PaPP[3]							
NC	241	172	116	90.82	0.479	0.912	0.333
ZN	271	101	85				

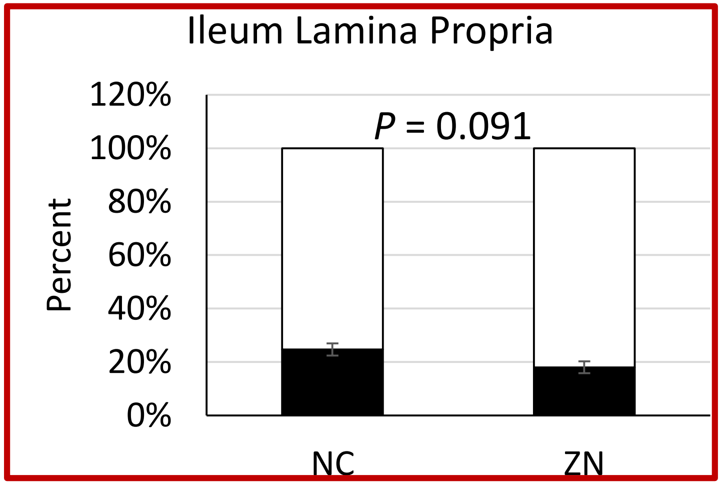
Intestinal Integrity Results

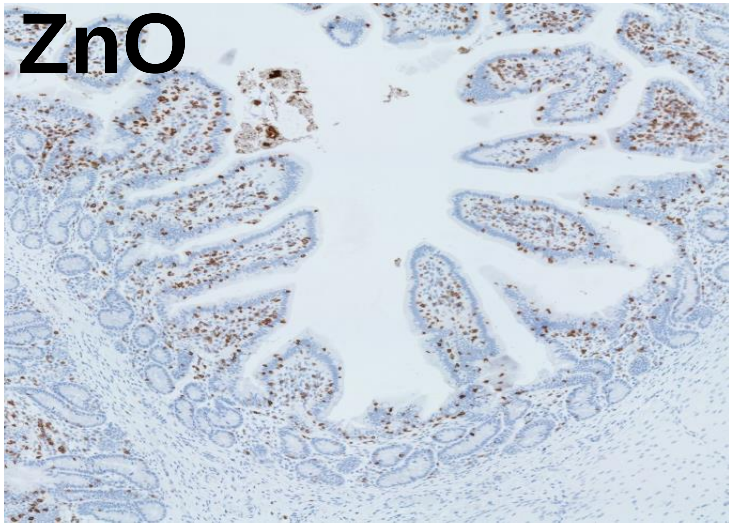
	day				*P*-Value		
Item	**2**	**7**	**21**	**SEM**	**Trt**	**Day**	**Trt*Day**
Colon							
TER, $\Omega \times cm^2$							
NC	63.9	62.8	82.7	10.71	0.847	0.088	0.077
ZN	66.7	68.2	55.5				
PaPP[3]							
NC	354	132	137	78.29	0.795	0.719	0.464
ZN	133	186	62				

Local T-Cell Population – preliminary data









Summary and Conclusions

- Improvement in growth rates due to ZnO (Smith, et al. 1995, Carlson et al., 1999, Hill et al., 2000, Hu et al., 2013)
- This growth promoting effect was not associated with improved intestinal integrity or active nutrient transport (Hu et al., 2013, Wang et al., 2019)
- Altogether, the results suggest that the mode of action by which ZnO promotes growth is less targeted towards the intestinal epithelium, contrary to our hypothesis.
- The mode of action may be associated with changes in immune or metabolic processes, altered microbiota, or post-absorptive metabolism.

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