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Zinc methionine supplementation effect on performance and intestinal epithelium morphology in pigs reared in hot or cool environments

Efecto de la suplementación con metionina de zinc en el desempeño productivo y morfología del epitelio intestinal en cerdos criados en ambiente caluroso o fresco

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ABSTRACT

To determine the effect of zinc-methionine supplementation during the gestation-lactation (GL) and growing-finishing (DF) period in the performance and during DF on the epithelium intestinal morphology of fattening pigs under hot and cool condition, two experiments were carried out. The experiment (Exp.) 1 was carried out during the hot season and Exp. 2 during the cool season of the year. 192 pigs were used (96 per Exp.) with an average age of 79 days and 26.39±SD4.97 kg of body weight, piglets of sows that received or not feed added with 100 mg of Zn/kg, from 80 to 114 days of gestation and during 21 d of lactation (GL). In each experiment, the pigs were assigned to one of four treatments in a completely random design with a 2 x 2 factorial arrangement. The treatments were T1 (control, n = 24), non-supplemented mothers-non-supplemented pigs; T2 (Zn FD; n = 24), non-supplemented mothers, pigs supplemented with 100 mg of Zn/kg feed; T3 (Zn GL; n = 24), supplemented mothers – non-supplemented pigs and, T4 (Zn GL + Zn DF; n = 24), supplemented mothers + supplemented pigs. Supplementation with 100 mg of Zn / kg of feed during GL and DF did not modify the productive performance of the pigs during the study period. However, the villus height: crypt depth ratio was higher ($p < 0.01$) in pigs supplemented with Zn (3.36 vs. 2.77) during the hot season. An interaction ($p < 0.02$) between climate and zinc, methionine supplementation was observed in the depth of the crypt and the V:C ratio. Supplementation during GL tended ($P = 0.06$) to lower the mortality of developing-finishing pigs in the cool season. According to results, it is concluded that the addition of zinc methionine to the diet improves the integrity of the intestinal epithelium in developing-finishing pigs reared under a hot environment, and supplementation during the gestation and lactation period reduces mortality during fattening.

Keywords: Pig, Zinc methionine, Intestinal epithelium, Productive response.

RESUMEN

Con el objetivo de determinar el efecto de la suplementación con metionina de zinc durante el periodo de gestación-lactación (GL) y desarrollo-finalización (DF) en el desempeño productivo y durante DF en la morfología del epitelio intestinal en cerdos criados en ambiente caluroso o fresco, se realizaron dos experimentos (Exp. 1, en la época calurosa; Exp. 2, en la época fresca del año). Se utilizaron 192 cerdos (96 por Exp.) con una edad promedio de 79 d y 26.39±DE4.97 kg de peso, hijos de cerdas que recibieron o no, alimento adicionado con 100 mg de Zn/kg, a partir de los 80 a 114 días de gestación y durante 21 d de lactación (GL). En cada experimento, los cerdos fueron asignados a uno de cuatro tratamientos en un

diseño completamente al azar con arreglo factorial 2 x 2. Los tratamientos fueron: T1 (Testigo; n = 24), madres no suplementadas-cerdos no suplementados; T2 (Zn DF; n = 24), madres no suplementadas-cerdos suplementados con 100 mg de Zn/kg alimento; T3 (Zn GL; n = 24), madres suplementadas-cerdos no suplementados y, T4 (Zn GL + Zn DF; n = 48), madres suplementadas + cerdos suplementados. La suplementación con 100 mg de Zn/kg de alimento durante GL y DF no modificó el desempeño productivo de los cerdos durante el periodo completo de estudio. La relación altura de la vellosidad: profundidad de la cripta fue mayor (p <0.01) en los cerdos suplementados con Zn (3.36 vs. 2.77) durante la época de calor. Se observó una interacción (p<0.02) entre el clima y la suplementación con metionina de zinc en la profundidad de la cripta y en la relación V: C. La suplementación durante GL tendió (P = 0.06) a bajar la mortalidad de los cerdos en desarrollo-finalización en la época fresca. Los resultados permiten concluir que la adición de metionina de zinc a la dieta mejora la integridad del epitelio intestinal en los cerdos en desarrollo-finalización criados bajo ambiente caluroso, y la suplementación durante el periodo de gestación y lactación reduce la mortalidad durante la engorda.
Palabras clave: Cerdo, Metionina de Zinc, Epitelio intestinal, Respuesta productiva.

INTRODUCTION

Animal production is seriously affected by heat stress (HS); the United States pork industry is estimated to lose more than $ 300 million a year; while global losses amount to billions of dollars (St-Pierre *et al*., 2003). HS -induced economic losses are the result of poor sow performance, reduced and inconsistent growth of fattening pigs, lower carcass quality, and increased veterinary costs (St-Pierre *et al*., 2003; Renaudeau *et al*., 2011). The consequences of HS in the low productive performance of the pig may be related to the negative effects on intestinal integrity (Pearce *et al*., 2013a; Sanz-Fernandez *et al*., 2014).

Mammals in HS redistribute blood to the periphery to maximize radiant heat dissipation, resulting in less blood and nutrient flow in the intestine; compromising the intestinal barrier and increasing its permeability (Pearce *et al*., 2013b); in addition, HS can antagonize digestibility and anabolic pathways in pigs (Mani *et al*., 2012; Rakhshandeh *et al*., 2012). Because Zn requirements increase during heat stress (Lagana *et al*., 2007), it has been suggested that Zn supplementation could be used to attenuate the serum Zn decrease during periods of high ambient temperatures (Chand *et al*., 2014; Li *et al*., 2015). Zinc in the form of a divalent metal ion, Zn^{2+}, is nutritionally essential for all living organisms (Maret, 2013); is a trace mineral with proven importance for the function of more than 300 enzymes (Chasapis *et al.,* 2012). Pig diets are generally supplemented with inorganic Zn ($ZnSO_4$ or ZnO), to ensure the required contribution, being the inorganic source of $ZnSO_4$ the one with the highest bioavailability (NRC, 2012); however, under normal physiological conditions and with adequate intake, only 5 to 15% of the Zn from the diet is apparently absorbed (McDowell, 2003). It has been suggested that organic sources of Zn are more bioavailable than inorganic forms, and the bioavailability of organic versus inorganic forms increases in the presence of antagonists, such as Ca, P, phytic acid, and crude fiber (Schlegel *et al.,* 2013; Richards *et al.*, 2015). Ming-Zhe *et al.* (2016), observed that the biological value of organic zinc from zinc methionine (ZnMet) was 64% higher than that of $ZnSO_4$.

The objective of the present study was to determine the effect of zinc methionine supplementation during the gestation-lactation period and the development-completion period, on the productive performance and during the development-completion period on the morphology of the intestinal epithelium of fattening pigs, raised under a warm or cool environment.

MATERIAL AND METHODS

Location of the study area. The study was carried out in the experimental area for fattening pigs of the Faculty of Veterinary Medicine and Zootechnics-UAS, located in the “La Huerta” pig farm, located in Culiacán municipality, Sinaloa, Mexico. The study consisted of two experiments; Experiment 1 was carried out during the months of July to October (warm period), and Experiment 2 in the months of December to March (cool period). The histological analysis of the small intestine samples (jejunum, duodenum and ileum), taken in the trace, were processed in the Pathology Laboratory of the Faculty of Veterinary Medicine and Zootechnics of the Autonomous University of Sinaloa, according to the proposed procedure by Prophet *et al.* (1995).

Experimental design. 192 pigs were used (96 in each experiment), with an average age of 79 days and 26.39 ± SD4.97 kg of b.w, to which one of four treatments were assigned in a completely randomized experimental design, with factorial arrangement 2 x 2. In each experiment, the treatments consisted of T1 (Control; n = 24), non-supplemented dams-non-supplemented pigs; T2 (ZnDF; n = 24), non-supplemented dams-pigs supplemented with 100 mg of Zn/kg DM; T3 (ZnGL; n = 24), dams supplemented with 100 mg of Zn/kg DM-non-supplemented pigs and, T4 (ZnGL + ZnDF; n = 24), supplemented dams + supplemented pigs.

The diets used are shown in table 1. The pigs used in the experiment were animals from a previous work, piglets of sows (PIC® Lines) that received or did not receive food added with zinc methionine (100 mg/kg DM) during gestation (80-114 d) and lactation (1-21 d); the diets provided were based on corn-soybean meal (table 2). The organic zinc was provided as zinc methionine (ZnMet) from the Zinpro120 premix (Zinpro® 120; contains 12% Zn and 27.3% methionine; Zinc methionine US Patent No. 4.764.633 and 5.430.164; Mexico release note: B00. 02.08.02.02.0398/11).

Handling of animals. The pigs of each experiment, previously weighed and identified, were housed in 12 pens, each one with a space of 9 m^2 (6 x 1.5 m), with a concrete floor, equipped with a hopper-type feeder and integrated nipple drinker; 8 pigs (4 males and 4 females) were housed in each pen. During the test period, which lasted 90 days in each experiment, the pigs had permanent access to free access drinking water and food. The food served in each pen was recorded and at the end of each experiment, the pigs were weighed and sent to the slaughterhouse, where they were slaughtered in the TIF slaughterhouse in accordance with NOM-033-SAG/ZOO-2014. After slaughter, tissue samples were taken from the small intestine, from the portions of the duodenum, jejunum and ileum, 15 pigs from each experiment, which received or did not receive feed supplemented with 100 mg of zinc methionine/kg of feed during the test period.

Table 1. Composition and nutritional information of the diets used in the development and completion stage

Ingredients	Development (30-60 kg body weight)	Ending (60-100 kg body weight
Corn	749	810
Soybean paste	217	165
Oil	9	5
Mineral premix	25	20
	Nutritional contribution	
E.M.(Mcal Kg^{-1})	3.351	3.353
Protein (%)	16.702	14.688
Lysine (%)	1.052	0.875
Fiber (%)	2.524	2.520
Phosphorus (%)	0.520	0.439
Calcium (%)	0.570	0.457
*Zinc (mg kg^{-1})	120.28	105.31

*Zn content of the control diet, provided by the mineral premix as ZnO. Supplementation with 100 mg of zinc was provided as zinc methionine (ZnMet) from the Zinpro120 premix (833.33 g/ton of feed on a dry basis). The diet was developed based on the tables of nutritional requirements for pigs (NRC, 2012)

Table 2. Composition and nutritional information of the diets used in pregnancy and lactation

Ingredients	Pregnancy	Lactation
Corn	793	692
Soybean paste	160	254
Oil	5	18
Mineral premix	42	36
	Aporte nutrimental	
E.M.(Mcal Kg^{-1})	3.272	3.351
Protein (%)	14.165	17.953
Lysine (%)	0.866	1.081
Fiber (%)	2.463	2.492
Phosphorus (%)	0.596	0.699
Calcium (%)	0.980	0.915

Supplementation with 100 mg of zinc was as zinc methionine (ZnMet) provided from the Zinpro120 premix (833.33 g/ton of feed on a dry basis). The diet was based on the tables of nutritional requirements for pigs developed (NRC, 2012)

Measurements: The food served in each pen was recorded weekly. At the end of each experiment, the pigs in each pen were weighed and with the information on feed consumption and weight gain, the feed conversion was obtained. Pig mortality was in each pen recorded and the mortality rate was determined based on this. In the case of the productive behavior test, the experimental unit was the pen.
At the end of each experiment, the pigs were to the slaughterhouse sent, where samples were taken from each segment of the small intestine (duodenum, jejunum and ileum). Fifteen representative pigs from each experiment (hot and cool season) received or not food supplemented with zinc methionine; without considering whether they were the offspring of sows that received feed or not added with zinc methionine during the gestation and lactation period. The samples were in bottles with 10% buffered formalin preserved until histological analysis.

Determination of THI. The temperature (t °C) and relative humidity (RH,%) data were taken with a thermo-hygrometer, located inside the experimental unit, and were recorded daily during the experimental period (tables 3 and 4). The temperature and humidity index (THI) was calculated using the formula THI = [0.8 x room temperature] + [(% RH/100) x (room temperature –14.4)] + 46.4 (Mader *et al*., 2006).

Table 3. Temperature and humidity index (THI), to which the pigs were during the warm period exposed (July 11 to October 8, 2015)

Week	Average. RH	Temp. Average. (°C)	Max Temp. (°C)	Min Temp. (°C)	THI[1] Average.	Max THI.	Min THI.
1	64.6	30.6	38.6	25.8	81.34	92.91	74.40
2	77.3	28.0	31.9	25.1	79.31	85.44	74.75
3	59.8	32.6	38.1	28.3	83.36	91.05	77.35
4	64.1	31.7	36.5	27.5	82.84	89.76	76.79
5	72.9	28.8	34.3	25.0	79.93	88.34	74.12
6	76.4	28.7	34.1	25.6	80.28	88.73	75.43
7	73.2	29.8	36.9	24.9	81.51	92.39	73.76
8	75.2	29.5	36.2	25.1	81.35	91.75	77.18
9	78.1	29.0	35.3	24.8	81.39	95.54	74.36
10	77.9	28.9	35.0	24.8	80.81	90.44	74.34
11	73.4	30.3	37.4	25.8	82.31	93.20	75.40
12	72.0	30.6	39.0	25.5	82.54	84.94	74.79
13	79.1	26.1	32.3	22.3	76.53	86.40	71.28
Average	72.61	29.58	35.81	25.42	81.03	90.06	74.92

[1]Temperature and humidity index (THI) = 0.8 × Room temperature + [(% relative humidity ÷ 100) × (Room temperature − 14.4)] + 46.4. THI ranges (normal THI <74; alert 75 a 79; danger 79 a 84; and emergency >84).

Intestinal histology. The samples were sent to the Pathology Laboratory of the Faculty of Veterinary Medicine and Zootechnics of the Autonomous University of Sinaloa, for processing using the routine paraffin embedding technique and subsequent staining with the hematoxylin and eosin technique. For the histological evaluation, a Motic® model BA 201 brand digital binocular microscope was used, equipped with a camera and software to photo-document images. Images of 30 fields were taken from each intestinal section, the length (in microns) of 10 villi was measured; as well as the depth (in microns) of the crypts, and based on this, the average height of the villi of each intestinal section was calculated; as well as the depth of the crypt. Based on this information, the villus height, crypt depth (V:C) ratio was determined; which was used for the corresponding statistical analysis. It is worth mentioning that the samples from each section of the small intestine that did not meet the requirements for histological analysis were discarded and were not considered in the statistical analysis.

Table 4. Temperature and humidity index (THI), to which the pigs were during the cool period exposed (December 2, 2015 to March 1, 2016)

Week	Average. RH	Average Temp. (°C)	Max Temp. (°C)	Min Temp. (°C)	THI Average[1]	Max THI.	Min THI.
1	64.6	22.7	33.3	15.3	70.36	85.24	59.22
2	74.8	19.3	27.9	12.5	65.50	78.81	54.90
3	72.7	19.0	26.8	13.1	64.94	76.85	55.93
4	64.9	17.2	26.6	9.8	61.97	75.59	51.25
5	66.8	17.3	26.1	10.0	62.17	75.09	48.00
6	64.4	20.0	29.7	13.6	66.00	80.01	56.76
7	72.2	20.1	27.5	14.6	66.59	77.85	58.22
8	72.7	19.6	27.5	13.8	65.86	77.92	57.00
9	76.2	28.8	28.9	14.9	80.41	80.57	58.70
10	74.4	20.0	30.2	12.7	66.56	82.31	55.29
11	80.6	21.4	28.5	16.8	69.16	80.56	62.24
12	77.3	22.0	31.0	15.5	69.87	84.03	59.65
13	74.3	20.1	29.2	13.1	66.71	80.75	55.58
Average	72.0	20.57	28.70	13.5	67.39	79.66	56.36

[1]Temperature and humidity index (THI) = 0.8 × Room temperature + [(% relative humidity ÷ 100) × (Room temperature − 14.4)] + 46.4. THI ranges (normal THI <74; alert 75 a 79; danger 79 a 84; and emergency >84).

Statistical analysis. To the data obtained from daily feed consumption (DFC), daily weight gain (DWG), feed conversion (FC), total weight gain per stage (TWG) and mortality during the experiment; as well as, to the relation villi, crypt. An analysis of variance was applied to them for a completely randomized design, with factorial arrangement (Steel and Torrie, 1985); setting a maximum alpha of 0.05 to accept statistical difference.

For the analysis of productive performance, the mathematical model was the following:

$$Y_{ijk} = \mu + Zn_i + A_j + ZnA_{ij} + E_{ijk;}$$

where
Y_{ijk} = is the response variable, µ = is the general mean from the experiment, Zn_i = is the effect of the *i-th* Zinc level, A_j = the effect of the *j-th* feeding factor, $Zn*A_{ij}$ = effect of the interaction and E_{ijk}= the random error.

For the intestinal morphology analysis, the mathematical model was the following:

$$Y_{ijk} = \mu + Zn_i + C_j + ZnC_{ij} + E_{ijk};$$

where: Y_{ijk} = is the response variable, µ = is the general mean of the experiment, Zn_i = is the effect of the *i-th* level of Zinc, C_j = the effect of the *j-th* climate factor, $Zn*C_{ij}$ = effect of the interaction and E_{ijk} = is the random error.

RESULTS AND DISCUSSION

Experiment 1. The results of the effect of the consumption of feed added with Zn methionine (ZnMet) on the productive performance of pigs during the development-completion stage, under conditions of high caloric load, are shown in table 5. In the completion stage (days 50 to 90, of the experimental period), the DWG tended (p = 0.07) to improve. The DFC was higher (p = 0.03) in the pigs of sows supplemented with Zn during GL; observing a trend (P = 0.10) of improvement in total weight gain during this stage. However, when analyzing the entire study period (90 d), supplementation with zinc methionine did not improve the productive performance of pigs.
The results of the effect of the consumption of feed added with ZnMet on the epithelial morphology of the small intestine during the hot and cool season are shown in Tables 7 and 8. The results indicate that the pigs that consumed diets supplemented with 100 mg of Zn/kg of feed, from zinc methionine, during the hot season, had a higher (p <0.01) V:C ratio (3.36 *vs* 2.77), compared to those that did not consume additional zinc during said period. Consumption of additional zinc decreased (p <0.05) the depth of the crypts in all segments (duodenum, jejunum and ileum) of the small intestine.

Experiment 2. During the cool season of the year the treatments did not modify the productive response of the pig (table 6), nor the morphology of the intestinal epithelium (tables 7 and 8). However, the additional consumption of zinc methionine during the gestation-lactation period tended (p = 0.06) to decrease mortality during the fattening period. In this regard, it has been reported that supplementation with Zn in the sow's diet during gestation and lactation reduces pre-weaning mortality (Payne *et al*., 2006; Romo *et al*., 2017) and improves the immune function of weaned piglets (Romo *et al*., 2017); which agrees with the results obtained in the present study.

The amount of organic Zn (ZnMet) supplemented to the pigs in the present study was 100 mg/kg of feed, in addition to the inorganic Zn content provided by the diets (120.20 mg/kg in the development diet and 105.31 mg/kg in completion), an amount higher than recommended by the NRC (2012). However, these levels are not toxic and are routinely used in commercial swine diets. The pigs of exp. 2 were exposed to an average temperature of 20.57 °C and relative humidity of 72.0% (table 4; CIAD, 2015, 2016); remaining at a THI of 67.39, environmental conditions under which the pig does not suffer physiological stress derived from the physical environment (Mader *et al.*, 2006). While the pigs of experiment 1, they remained in a THI of 81 points (in physiological danger, see table 3) with an average environmental temperature of 29.58 °C and 72.61% relative humidity. The difference in environmental temperature and relative humidity between the pigs in experiment 1, compared to those in experiment 2, was 9.01 °C and 0.61%.

Table 5. Effect of the consumption of feed added with 100 ppm of organic zinc on the productive performance of developing-finishing pigs, during hot season (July-October)

Variable	Treatments				SEM[1]	Main effects		Interaction
						Values of P		
	Control	Zn DF	Zn GL	Zn GL + Zn DF		Zn GL	Zn DF	Zn GL * Zn DF
Observations (n)	3	3	3	3				
Pigs (n)	24	24	24	24				
Period (days)	90	90	90	90				
Initial weight (kg)	21.63	22.33	21.73	21.50	0.7445			
Weight to 49 d (kg)	54.16	55.70	56.67	52.13	1.9154	0.90	0.73	0.50
Gaining of weight to 49 d (Kg)	32.533	33.333	34.933	30.633	1.3550	0.96	0.57	0.41
DWG[2] (kg)	0.663	0.681	0.713	0.626	0.0277	0.96	0.58	0.41
DFC[3] (kg)	1.603	1.596	1.650	1.570	0.0539	0.93	0.73	0.77
FC[4]	2.426	2.353	2.310	2.533	0.0395	0.66	0.32	0.07
Weight to 90 d (kg)	79.50	79.17	83.07	79.67	1.9142	0.65	0.68	0.73
Gaining of weight to 50-90 d (kg)	25.667	23.467	26.400	27.533	0.7091	0.10	0.69	0.23
DWG[2] (kg)	0.591	0.568	0.628	0.696	0.0222	0.07	0.57	0.27
DFC[3] (kg)	1.907	1.773	2.033	1.990	0.0416	0.03	0.23	0.52
FC[4]	3.230	3.133	3.240	2.923	0.0910	0.62	0.31	0.58
Gaining of weight in the whole period (kg)	57.700	56.800	61.333	58.167	1.4036	0.44	0.52	0.72
DWG[2] (kg)	0.641	0.631	0.681	0.646	0.0156	0.44	0.52	0.72
DFC[3] (kg)	1.763	1.706	1.850	1.783	0.0402	0.37	0.50	0.95
FC[4]	2.760	2.706	2.713	2.756	0.227	0.97	0.92	0.36
Dead	0.33	0.667	0	0.667	0.1930	0.69	0.25	0.69

Treatments: Control = unsupplemented dams-unsupplemented pigs, ZnDF = unsupplemented dams-supplemented pigs; ZnGL = supplemented dams-unsupplemented pigs; ZnGL + ZnDF = supplemented dams-supplemented pigs. Supplement of 100 mg of Zn/kg of feed, provided from zinc methionine (Zinpro 100; Zinpro, Eden Praire, MN). Two Factors: supplementation method = ZnGL and ZnCF, Zn addition level (0 and 100 mg/kg food); [1]Standard error of the mean; [2]Daily body weight gain; [3] Daily food intake and [4]Diet conversion

The results of the morphological analysis of the duodenum, jejunum and ileum samples show that the pigs that consumed diets supplemented with 100 mg of Zn/kg of feed, from zinc methionine, during the hot season, had a higher (p <0.01) V: C ratio (3.36 *vs* 2.77), with respect to those who did not consume additional zinc during said period. Consumption of additional zinc decreased (p <0.05) the depth of the crypts in all segments (duodenum, jejunum and ileum) of the small intestine. An interaction (p <0.02) was observed between the climate and zinc methionine supplementation in the depth of the crypt and in the V:C ratio. The effect of climate and zinc methionine supplementation on the epithelial morphology of each segment of the small intestine analyzed is described below:

Table 6. Effect of the consumption of feed added with 100 ppm of organic zinc on the productive performance of developing-finishing pigs, during the cool season (December to March)

Variable	Treatments				SEM[1]	Main effects		Interaction
						Values of P		
	Control	Zn DF	Zn GL	Zn GL + Zn DF		Zn GL	Zn DF	Zn GL * Zn DF
Observations (n)	3	3	3	3				
Pigs (n)	24	24	24	24				
Period (days)	90	90	90	90				
Initial weight (kg)	32.13	30.00	29.93	31.86	0.8132			
Weight to 49 d (kg)	69.60	71.40	67.36	72.03	1.4980	0.81	0.35	0.67
Gaining of weight to 49 d (Kg)	37.467	41.400	37.433	40.133	1.2656	0.81	0.25	0.82
DWG [1] (kg)	0.764	0.844	0.764	0.818	0.0258	0.81	0.26	0.82
DFC [2] (kg)	1.980	2.030	2.043	2.076	0.0326	0.47	0.58	0.91
FC [3]	2.603	2.413	2.686	2.580	0.0723	0.44	0.36	0.79
Weight to 90 d (kg)	107.83	112.23	104.29	109.29	1.9853	0.46	0.29	0.94
Gaining of weight to 50-90 d (kg)	38.35	40.83	36.92	37.26	0.8972	0.20	0.46	0.57
DWG [1] (kg)	0.910	0.972	0.879	0.888	0.0213	0.21	0.43	0.55
DFC [2] (kg)	3.030	3.016	2.850	2.863	0.0568	0.20	1.00	0.91
FC [3]	3.326	3.106	3.270	3.226	0.0634	0.82	0.36	0.53
Gaining of weight in the whole period (kg)	75.700	82.267	74.133	77.400	1.9356	0.46	0.29	0.94
DWG [1] (kg)	0.841	0.914	0.826	0.859	0.0211	0.45	0.26	0.66
DFC[2] (kg)	2.493	2.513	2.443	2.466	0.0334	0.54	0.78	0.98
FC [3]	2.963	2.760	2.976	2.876	0.0497	0.53	0.16	0.61
Dead	0.67	0.33	0	0	0.1306	0.06	0.50	0.50

Treatments: Control = unsupplemented dams-unsupplemented pigs, ZnDF = unsupplemented dams-supplemented pigs; ZnGL = supplemented dams-unsupplemented pigs; ZnGL + ZnDF = supplemented dams-supplemented pigs. Supplement of 100 mg of Zn / kg of feed, provided from zinc methionine (Zinpro 100; Zinpro, Eden Praire, MN). Two Factors: supplementation method = ZnGL and ZnCF, Zn addition level (0 and 100 mg / kg food); [1]Standard error of the mean; [2]Daily body weight gain; [3] Daily food intake and [4] Diet conversion

Epithelial morphology of the duodenum. The analysis indicates an interaction (p <0.01) of the climate with zinc, observing that in cool climate the pigs have a better response to the additional consumption of 100 mg of Zn/kg of feed, with a greater height of the villi with respect to the pigs supplemented during hot weather. In the case of the depth of the crypts, the pigs that consumed feed added with zinc methionine, both in the warm season and in the cool season, had a lower depth (p <0.02); while the V:C relationship was influenced (p <0.01) by the climate; being older during the cool season.

Jejunum epithelial morphology. The climate was the factor that modified (p <0.01) the height of the villi, observing a higher height of these in the pigs treated during the cool season. However, the depth of the crypts was lower (p <0.05) in the pigs that consumed feed added with zinc methionine, both in the warm and cool seasons, as well as in those that did not consume additional zinc during the cool season. (p <0.05). Similarly, the highest (p <0.03) V: C relationship was d in the animals and times of the year observe previously referred.

Ileum epithelial morphology. In the ileum region, the height of the villi was also higher (p <0.01) in the pigs treated during the cool season. However, the depth of the crypt was lower (p <0.03) in the pigs that consumed feed added with zinc methionine, both in the warm season and in the cool one, observing an interaction (p <0.03) between the factors climate and zinc an interaction (p <0.04) between climate and zinc was found in the V: C ratio.

Table 7. Influence of climate and zinc supplementation on the morphology of the different regions of the small intestine, in fattening pigs

Variables	Heat Season				Cool Season				Main factors		Interaction
	Pig - Zn		Pig + Zn		Pig - Zn		Pig + Zn				
	Mean	± EE	Mean	± EE	Mean	± EE	Mean	± EE	Climate	Zinc	C*Z
Duodenum											
Pigs, n	14		13		10		15				
High V, µm	391	15.90 b	327	15.97c	423	17.86ab	451	14.58b	< 0.01	0.26	<0 .01
Deep C, µm	153	7.88a	115	8.18b	121	9.33ab	116	7.62b	0.07	0.02	0.06
V:C Ratio	2.70	0.190c	2.98	0.197bc	3.69	0.215ab	3.90	0.190a	< 0.01	0.24	0.86
Jejunum											
Pigs, n	15		13		10		15				
High V, µm	383	12.60b	408	13.54ab	458	15.44a	452	12.60a	<0.01	0.51	0.25
Deep C, µm	136	6.24a	111	6.71ab	111	7.64ab	108	6.24b	0.05	0.05	0.13
V:C Ratio	2.97	0.190b	3.80	0.204 a	4.18	0.222 a	4.25	0.197 a	< 0.01	0.03	0.07
Ileum											
Pigs, n	13		14		9		15				
High V, µm	363	14.03b	351	13.52b	423	16.86^{a}	435	13.06a	<0 .01	0.98	0.43
Deep C, µm	140	6.31a	110	6.38b	112	7.58b	113	5.87b	0.07	0.03	0.03
V:C Ratio*	2.66	0.16b	3.30	0.15a	3.95	0.187a	3.86	0.15a	< 0.01	0.11	0.04

*V:C ratio indicates the product of the division of the villus height by crypt depth. $^{a\ b,\ c,}$ indicates statistical difference (Tuckey, P<0.05)

When doing the pooled analysis (see Table 8) of the epithelial morphology of the three segments of the small intestine, it was observed that the climate has a greater effect (p<0.01) on the height of the villi, and that there is an interaction (p<0.02) between climate and zinc supplementation in reducing crypt depth and increasing V: C ratio.

Table 8. Influence of the climate and the addition of zinc methionine to the diet on the intestinal morphology of fattening pigs (combined values of the three regions: Duodenum, jejunum and ileum)

Variables	Heat Season				Cool Season				Main factors		Interaction
	Pigs - Zn		Pigs + Zn		Pigs - Zn		Pigs + Zn				
	Mean	± EE	Mean	± EE	Mean	± EE	Mean	± EE	Clima	Zinc	C*Z
Small intestine											
Pigs, n	15		14		10		15				
Samples, n	42		40		29		45				
High V, µm	378	8.213b	362	8.414b	436	9.467a	446	8.210a	< 0.01	0.68	0.13
Deep C, µm	143	3.897a	112	3.992b	113	4.463b	113	3.895b	< 0.01	< 0.01	< 0.01
V:C Ratio *	2.77	0.105c	3.36	0.107b	3.94	0.119a	4.00	0.105a	< 0.01	< 0.01	0.02

* Ratio V: C, indicates the product of the division of the height of villi by depth of crypt. a b, c, indicates statistical difference (Tuckey, P <0.01)

The results of the present study indicate that supplemental zinc at the level of 100 mg / kg of feed, from zinc methionine, improves the intestinal morphology of fattening pigs reared under a hot environment; but when it is supplemented to pigs raised under cool environments the response is improved.

It has been suggested that a low V: C ratio may indicate villus atrophy, associated with an increased rate of cell loss from the villus apex, concurrent with increased cell production in the crypts, and thus therefore, a greater depth of these. A higher V: C ratio suggests a healthier state of the intestine (Tang *et al*., 1999). Al *et al*. (2015) indicated that the height of the villi and the depth of the crypt show remarkably interdependent developmental changes. Therefore, its morphometric measurement cannot be considered individually; instead the V: C ratio should be evaluated. It suggests that the higher V: C ratio observed in the present study (3.36 *vs*. 2.77; p <0.01) in pigs that received supplementation with 100 mg of Zn (ZnMet) kg^{-1} during the hot season, regarding of those who did not receive additional zinc. It is due to the beneficial action of organic Zn on the maintenance of intestinal integrity and morphology. These results are congruent with those obtained by Bouwhuis *et al*. (2016), who reported that the inclusion of zinc methionine increased the height of the villi in the jejunum, but did not increase the weight gain of pigs. In addition, studies that are more recent demonstrated that zinc supplementation (450 mg kg^{-1} of nano-ZnO and 3,000 mg kg^{-1} of ZnO) protects the morphology of the small intestine, by increasing the height of the villi (Long *et al*., 2017; Pei *et al*., 2018). For their part, Li *et al*. (2001) observed that the pigs that received supplemental ZnO had greater thickness of the mucosa and height of the villi, in the proximal and medial sites of the small intestine, with respect to the pigs that received the control diet.

Heat stress is known to affect gastrointestinal health and function (Eshel *et al*., 2001), since during heat stress blood flow is diverted from the splenic system to the skin, in an attempt to dissipate excess heat (Lambert, 2009). A reduced blood flow and hyperthermia lead to hypoxia, oxidative and nitrosating stress in the enterocyte (Pearce *et al*., 2013a, b, c), damaging cell and mucous membranes; as well as tight junctions, increasing intestinal permeability (Lambert *et al*., 2002). In this regard, Pearce *et al*. (2015) observed that heat stress increases the passage of high molecular weight substances and circulating endotoxins, in addition to an increase in the autolysis of the intestinal epithelium.

Zinc oxide supplementation has been shown to reduce intestinal permeability in piglets at weaning, while increasing the amount and expression of tightly bound proteins (Zhang and Guo, 2009). Also increasing levels of the zinc complex AA (220 and 320 mg/kg) increased the integrity of the gastrointestinal tract, compared to control pigs that received 120 mg/kg of $ZnSO_4$ (Sanz-Fernandez *et al*., 2014). In addition, this mineral protects cells against oxidative damage by stabilizing membranes, inhibiting the pro-oxidant enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH-Oxidase), and inducing metallothionein synthesis (Marreiro *et al*., 2017). In this regard, it has been reported that zinc supplementation induces the expression of metallothioneins in Caco-2 cells (Wang *et al*., 2013), which could act as antioxidants, due to their ability to sequester reactive oxygen species and nitrogen intermediates (Waeytens *et al*., 2009). Furthermore, zinc increases the expression and concentration of antimicrobial substances such as *β*-defensins in IPEC-J2 cells (Mao *et al*., 2013).

On the other hand, Zn is a structural component of the enzyme superoxide dismutase (SOD), present in the cytoplasm of cells. SOD promotes the conversion of two superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), reducing the toxicity of reactive oxygen species (ROS); because it converts a highly reactive species into a less harmful one (Cruz and Soares, 2011; Marreiro *et al*., 2017). Studies have also highlighted its role in the regulation of glutathione peroxidase and in the expression of glutamate-cysteine ligase, an enzyme involved in the synthesis of glutathione that acts directly on the neutralization of free radicals (Marreiro *et al*., 2017). Consequently, there appear to be a variety of mechanisms by which dietary zinc can maintain or restore the integrity and morphology of the intestinal epithelium, under conditions of oxidative stress.

CONCLUSION

Supplementation with 100 mg of zinc/kg of feed, from zinc methionine, improves the integrity of the intestinal epithelium in fattening pigs, reared under conditions of high caloric load, and supplementation during the gestation-lactation period. It can be used as a strategy to reduce mortality during the fattening stage.

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