ZINC OXIDE IN PIGLET DIETS

1. What does the literature say about zinc homeostasis?

BY THE ANIMINE TECHNICAL TEAM. WWW.ANIMINE.EU

Welcome to the first in Animine's series of articles, which will review scientific literature on the effects of the pharmacological dosage of zinc oxide (ZnO) in weaned piglet diets. In the European Union, medicated ZnO will be totally banned from June 2022. It is interesting to note that abroad there is also a growing number of countries following this tendency, such as China, where the supplementation of pharmacological levels of ZnO is already restricted.

The pharmacological dosage of ZnO is well known for its effect on diarrhoea reduction and improved weight gain of weaned piglets. However, even after decades of use, there is still no consensus on its modes of action. This series of articles will review existing scientific literature with a particular focus on its key effects and mechanisms.

It will be also inspired from the latest outcomes of the ZincoSupp research network supervised by Animine. This ambitious scientific programme, involving several renowned universities, addresses the suppression of pharmacological ZnO, while studying the proper supplementation of the potentiated zinc source, HiZox.

Effect of dietary ZnO concentration on blood zinc levels

An exhaustive literature review was performed from the AniLib database. Out of 151 publications on the pharmacological use of ZnO in weaned piglets, 38 containing data on blood zinc concentration at the end of the study were selected for the purpose of this review.

As shown in Fig, 1, zinc supplementation below requirements (estimated to be at 100mg/kg, NRC 2012) can lead to low blood zinc levels. Although the majority of the observations indicated low zinc in the blood in this range, some trials observed a high concentration of zinc in the blood of piglets.

This could be due to a high zinc status at weaning due to the intake of zinc-rich creep feed before weaning.

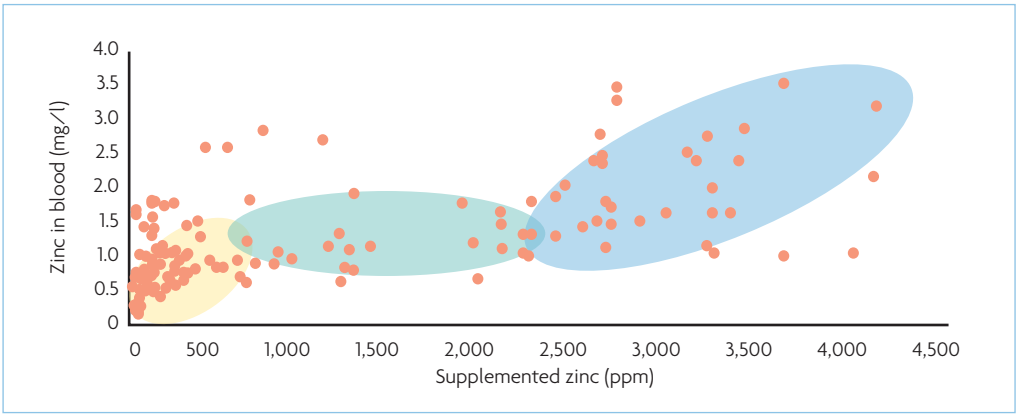
References of selected papers:

Acta Agriculturae Scandinavica	1995
Archives of Animal Nutrition	2011, 2013, 2014
Animal Feed Science and Technology	2017, 2018
Asian Australian JAS	2014, 2020
Asian Journal of Animal and Veterinary Advances	2012, 2013, 2018
British Journal of Nutrition	2014
Canadian JAS	2019
Journal of Animal Physiology and Animal Nutrition	2006
Journal of Animal Science	1970, 1993, 1996, 1999, 2001, 2002, 2009, 2013, 2019
Journal of Animal Science and Biotechnology	2020
Journal of Nutrition	1985, 2004
Journal of Trace Elements in Medicine and Biology	2018
Journal of Veterinary Diagnostic Investigation	2019
Journées de la Recherche Porcine	2003, 2005
KSU Swine Day	1998, 2008
PlOS one	2017
WUR report	2016

An increase in blood zinc levels, from 0 to 1.3mg/L, with higher ZnO supplementation dosages, from 200 to 500mg/kg, is also observed in dose-response behaviour. The supplementation of ZnO from 500 up to 2,000mg/kg zinc in the complete feed, seems to reach a plateau on blood levels, maintaining homeostatic regulation and buffering excess dietary zinc.

When the zinc supplementation exceeds 2,000mg/kg zinc, the homeostatic regulation capacity of piglets is bypassed increasing the blood zinc concentration. At such high levels, a higher variability of blood zinc concentrations is also observed. This might be related to weaning age and individual feed intakes. The higher the ZnO supplementation, the higher the feed consumption, resulting in a higher risk of piglet toxicity due to by-passed homeostatic regulation. ■

Fig. 1. Blood zinc concentration in piglets fed different dietary zinc content for two weeks post weaning.



ZINC OXIDE IN PIGLET DIETS

2. The role of blood Zn levels in growth improvement

BY THE ANIMINE TECHNICAL TEAM. WWW.ANIMINE.EU

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Relationship between blood Zn concentration and piglet growth performance

An exhaustive literature review was performed from the AniLib database. Out of 151 publications on pharmacological use of ZnO in weaned piglets, 19 papers were selected for the purpose of this review. The filtering criteria were: daily weight gain, blood zinc concentration at the end of 2-6 weeks studies (with focus on 2-3 weeks when possible) and ZnO supplementation levels at 2-3000mg Zn/kg complete diet. The stress of weaning creates anorexia in piglets from the first days after the suckling period. As a consequence of collapsed feed intake, the supply of nutrients becomes very limited at a critical time for the animal. The risk of transitory sub-clinical zinc deficiency appears, which can be compensated by very high dietary Zn concentrations. Pharmacological dosage of ZnO corrects sub-optimal blood zinc levels below 1-2mg/L. Growth improvement is observed with the repletion of Zn circulating pool.

Once minimum daily ingestion of zinc is achieved, blood Zn concentration is tightly regulated, and is stabilised by homoeostatic equilibrium.

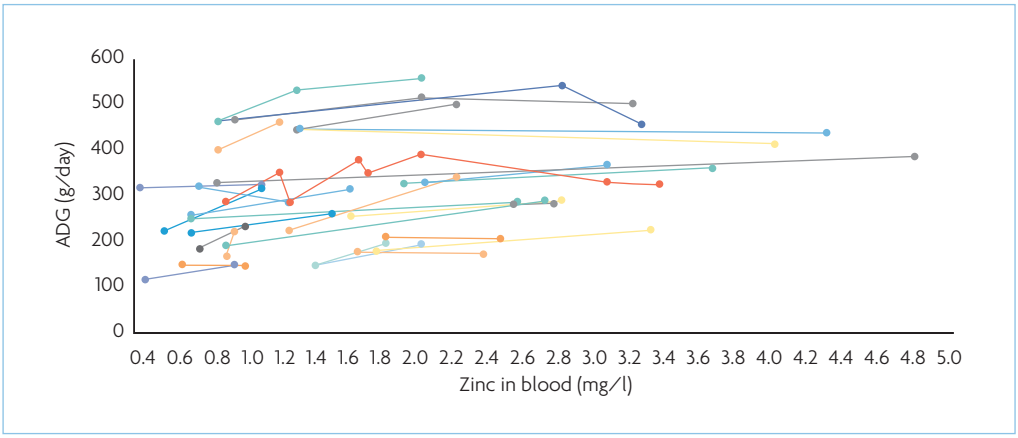
When blood Zn level is at the plateau, the effect of ZnO supplementation on body weight gain is less clear, but it is still observed.

Pharmacological dosage of ZnO may restore the Zn status of the weaned piglet, in combination with some non-nutritional functional effects, such as a local action of ZnO in the intestine, through a microbiota modulation. ■

References of selected papers:

Animal Feed Science and Technology	2018
Animal	2015
Animals	2021
Asian Australian JAS	2020
Asian Journal of Animal and Veterinary Advances	2012, 2013
British Journal of Nutrition	2014
Journal of Animal Science	1993, 1996, 1999, 2001, 2002, 2009
Journal of Animal Science and Technology	2014
Journal of Trace Elements in Medicine and Biology	2018
Murdoch University report	2010
KSU Swine Day	2008
Veterinarni Medicina	2015
WUR report	2016

Fig. 1. Correlation between blood Zn levels and average daily gain of weaned piglets fed pharmacological levels of ZnO. First point of each line is a negative control (without ZnO or low dietary Zn concentration) and the last point is pharmacological dosage.



ZINC OXIDE IN PIGLET DIETS

3. Does dietary Zn have an impact on inflammatory status?

BY THE ANIMINE TECHNICAL TEAM. WWW.ANIMINE.EU

Welcome to the third in Animine's series of articles, which reviews scientific literature on the effects of the pharmacological dosage of zinc oxide (ZnO) in weaned piglet diets. In the European Union, medicated ZnO will be totally banned from June 2022. It is interesting to note that abroad there is also a growing number of countries following this tendency, such as China, where the supplementation of pharmacological levels of ZnO is already restricted.

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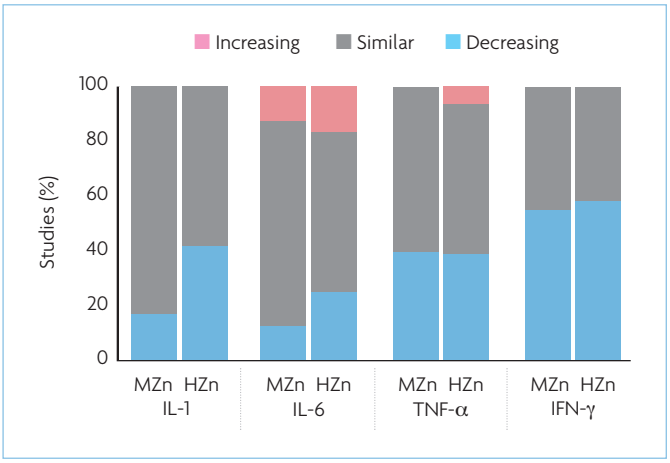
Relationship between dietary Zn and inflammation

A literature review was performed from AniLib database (Animine internal library). From a total of 152 publications on pharmacological use of ZnO in weaned piglets, 26 papers were selected for the purpose of this review. Filtering criteria were:

- ZnO supplementation at 2,000-3,000mg Zn/kg of complete diet compared to a negative control (supplementation <200ppm Zn).
- Analyses of gene expression in intestinal mucosa or quantification in blood of the following inflammatory biomarkers: interleukin 1 family (IL-1), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ).

Fig. 1. Effect of dietary Zn on inflammatory markers (significant effect or trend). MZn = medium Zn level (200-1000ppm Zn); HZn = high Zn level (>2,000ppm).



References of selected papers:

Animal Feed Science and Technology	2013, 2020
Animals	2015, 2021
Animal Nutrition	2017
Asian-Australasian JAS	2018
Animal Production Science Journal	2018
Animal Science Journal	2014
British Journal of Nutrition	2013, 2014, 2018
Biological Trace Element Research	2017, 2018
Canadian Journal of Animal Science	2006, 2014
Frontiers in Veterinary Science	2021
Innate Immunity	2014
Journal of Animal Science	2016
Journal of Nutritional Science and Vitaminology	2020
Journal of Trace Elements in Medicine and Biology	2018
Oncotarget	2017
PLOS One	2014, 2017
Scientific Reports	2019
WUR report	2016

These biomarkers are produced by cells of the immune system, including macrophages, and initiate inflammatory response.

Weaning is a stressful event which is associated with increased inflammation status. The gene expression of inflammatory cytokines in the gut is upregulated during this period. Some pro-inflammatory cytokines, like TNF-α and IFN-γ, increase the intestinal permeability through the disruption of tight junctions. Fig. 1 summarises what literature says about the effect of Zn on inflammatory cytokines. It represents the percentage of studies which demonstrated an increasing (in pink), decreasing (in blue), or no effect (grey) of medium (MZn) or high (HZn) zinc levels in comparison to a negative control. Only statistically significant differences were selected for increasing or decreasing effects.

High or medium levels of ZnO supplementation can reduce the overproduction of proinflammatory cytokines, according to this literature review (Fig. 1; blue colour). It was found that pharmacological levels of ZnO can significantly decrease the gene expression and/or the concentration of IL-1, IL-6, TNF-α, and IFN-γ, in 42%, 25%, 39%, and 60% of the selected studies, respectively, when compared to the control group. Medium zinc levels were often associated with alternative zinc oxide sources (ex: potentiated ZnO). ■

ZINC OXIDE IN PIGLET DIETS

4. Does dietary Zn have an impact on gut integrity?

BY THE ANIMINE TECHNICAL TEAM. WWW.ANIMINE.EU

Welcome to the fourth in Animine's series of articles, which reviews scientific literature on the effects of the pharmacological dosage of zinc oxide (ZnO) in weaned piglet diets. In the European Union, medicated ZnO has been totally banned since June 2022. It is interesting to note that abroad there is also a growing number of countries following this trend, such as China, where the supplementation of pharmacological levels of ZnO is already restricted.

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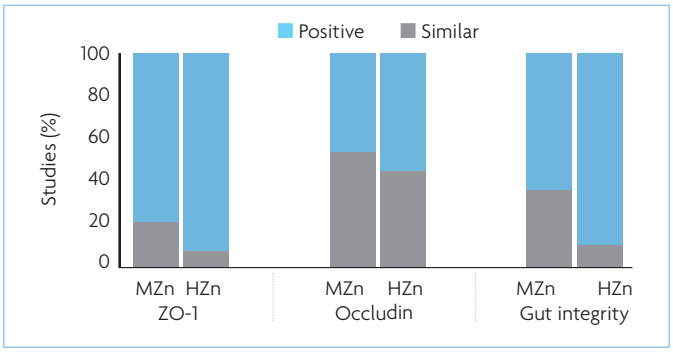
Relationship between dietary Zn and gut integrity

A literature review was performed from AniLib database (Animine internal library). From a total of 152 publications on pharmacological use of ZnO in weaned piglets, 20 papers were selected for this review. Filtering criteria were:
• ZnO supplementation at 2,000-3,000mg Zn/kg of complete diet compared to a negative control (supplementation <200ppm Zn).
• Criteria directly or indirectly related to gut permeability:
- Analyses of genes expression and/or activity of intestinal alkaline phosphatase (IAP), a metalloenzyme that plays various roles in the gastrointestinal tract such as regulation of intestinal pH at the surface of enterocytes, detoxification of bacterial lipopolysaccharides, modulation of intestinal inflammation.
- Analyses of gene expression involved in tight junctions' proteins (zona-occludens ZO-1 and occludin), which form the continuous intercellular barrier between epithelial cells.
- Markers of gut integrity: transepithelial electrical resistance (TEER), FD4-flux, serum level of diamine oxidase (DAO).

Fig. 1. Effect of dietary Zn on gut integrity parameters (significant effect or trend) MZn = medium Zn level (200-1,000ppm Zn); HZn = high Zn level (>2,000ppm).



References of selected papers:

Animal Feed Science and Technology	2012, 2013
Animals	2015
Animal Physiology and Animal Nutrition	2014, 2018
Animal Science Journal	2019
Archives of Animal Nutrition	2013
Asian-Australasian JAS	2018
Asian Journal of Animal and Veterinary Advances	2014
British Journal of Nutrition	2009, 2013, 2014
Biological Trace Element Research	2016, 2018
Innate Immunity	2014
Journal of Animal Science	2015
Journal of Applied Animal Research	2019
Oncotarget	2017
PLOS One	2017
Scientific Reports	2019
PLOS One	2014, 2017
Scientific Reports	2019

At weaning, piglets face an important dietary change from a liquid milk-based diet to a solid cereal-based feed. Alterations in gut morphology are usually observed and often related to gut permeability increase, both can be a predisposing factor for diarrhoea. Despite IAP being a promising biomarker for gut health in piglets, only two papers measured this particular parameter. In these given studies, the gene expression and/or the activity of IAP increased with Zn supplementation, which can be related to a better control of the inflammation status and a better gut integrity.

Fig. 1 summarises literature findings about the effects of Zn on markers of gut integrity. It represents the percentage of studies which demonstrated a positive effect (blue) or no effect (grey) of medium zinc levels (MZn – from 200-2,000mg Zn) or high zinc levels (HZn – from 2,000-3,000mg Zn) in comparison to a negative control (<200mg Zn). The effect of Zn is considered positive when the expression for tight junction's proteins increased. For gut integrity, the permeability decreases when TEER increases, or when FD4-flux and DOA increase. In all the selected studies, Zn supplementation showed a neutral or a positive effect. For the expression of ZO-1 protein involved in tight junction, effect is mainly positive: 92% with pharmacological dosage in 12 studies, 78% with intermediary dosage in nine studies. Increasing the gut integrity reduces passive transcellular absorption and can prevent uncontrolled transport of potentially harmful agents (ex: endotoxins). In summary, it is clearly observed that zinc and its increasing levels are directly involved in gut integrity. This aspect is crucial during post-weaning phases and can contribute to a better nutrient absorption for optimised performance. ■

ZINC OXIDE IN PIGLET DIETS

5. Gut microbiota and performance: the paradox

BY THE ANIMINE TECHNICAL TEAM. WWW.ANIMINE.EU

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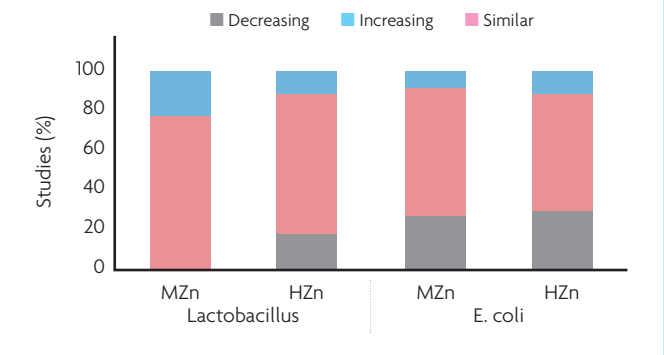
Relationship between dietary Zn and gut microbiota

A literature review was performed from AniLib database (Animine internal library). From a total of 152 publications on pharmacological use of ZnO in weaned piglets, 25 papers were selected for the purpose of this review. Filtering criteria were:

- ZnO supplementation at 2,000-3,000mg Zn/kg of complete diet compared to a negative control (supplementation <200ppm Zn), with or without intermediary level (200-1,000ppm).
- Quantification of common gut bacteria, in faecal samples or in intestinal digesta: focus on E. coli and lactobacillus as common denominators.

The gastrointestinal tract of piglets is colonised by thousands of different bacterial species that vary depending on host genotype and phenotype. The current understanding of this microflora is still very limited but some of the bacterial species are commonly accepted to be associated with gut health and weight gain. As an example, enteric colibacillosis is clearly due to an excess of E. coli and leads to diarrhoea and economic losses.

Fig. 1. Effect of ZnO on microflora modulation (significant effect or tendency) MZn = medium Zn level (200-1,000ppm Zn); HZn = high Zn level (>2,000ppm).



In contrast, lactobacillus are generally considered as beneficial bacteria. Nevertheless, recent findings revealed that excess lactobacillus can also negatively affect fat digestibility by deconjugating the host's bile salts, to obtain a source of energy. This stresses the fact that interpretation of the changes in microflora is a complex exercise that cannot be restricted to one flora analysis, but should consider the total balance and resilience of the population as well as the role of this flora and their produced metabolites.

What about the effects of pharmacological ZnO?

For decades, ZnO has been well appreciated for its antibacterial properties, especially at the pharmacological level. Considering its important effect on performance, common sense would tell us that ZnO positively affects beneficial bacteria (for example, lactobacillus) and decreases negative bacteria (for example, pathogenic E. coli).

However, the results in literature are not fully consistent with this statement. Indeed, and according to this review, high levels of ZnO tend to decrease lactobacillus abundance (reduction in 18% of the studies vs. increasing in 12% of the studies) in line with Starke et al., 2013. This reduction of lactobacillus is still associated with better performance.

More recent papers suggest that the effect of ZnO is more connected to the global change in bacterial diversity, rather than in the direct regulation of beneficial or pathogenic floras. It is also important to point out that its positive impact on gut integrity could also play an important role in the cross-talks between the host and its microflora, an area still very unknown.

ZnO a complex mode of action requiring more understanding

The mechanism by which high levels of zinc oxide improves performance is a complex one. Besides gut barrier integrity, zinc requirements, intestinal secretions and appetite regulation, the microflora modulation is an important one, but NOT the only one to be considered. The effect of ZnO on E. coli seems more obvious than its effect on lactobacillus and further analysis is required on its final impact on piglets' microbiota resilience. Pharmacological levels of ZnO seems to decrease lactobacillus abundance, but this effect was not observed with medium ZnO levels (200-1,000ppm). For E. coli abundance, both pharmacological and medium levels of ZnO seems to have the same effect. A nutritional dose of potentiated zinc oxide acts as a multifunctional alternative to pharmacological zinc oxide. ■

References for 14 selected papers are available on demand

6. Correlation between dietary ZnO and oxidative status

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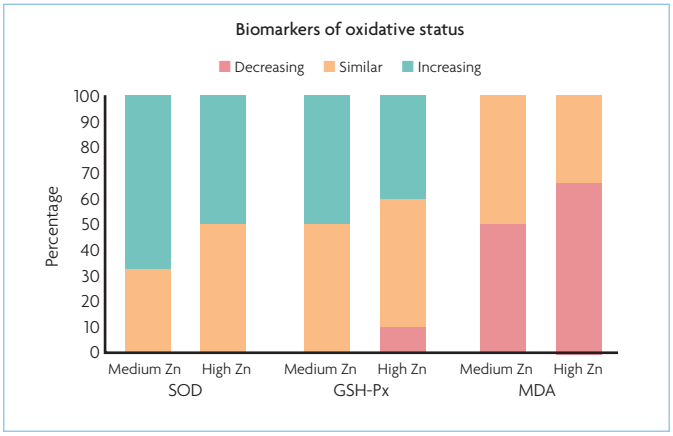
Relationship between dietary Zn and oxidative status

A literature review was performed from AniLib database (Animine internal library). From a total of 153 publications on pharmacological use of ZnO in weaned piglets, 14 papers were selected for the purpose of this review. Filtering criteria were:

- ZnO supplementation at 2,000-3,000mg Zn/kg of complete diet compared to a negative control with 'nutritional' zinc levels (<200ppm Zn).
- Parameters evaluated: markers of oxidative status in blood or in tissues (intestinal mucosa, liver, kidney):

• Superoxide dismutase SOD (enzyme quantification or activity, mRNA quantification), which transforms the superoxide radical into dioxygen and limit oxidative stress.

Fig. 1. Effect of dietary Zn on gut integrity parameters (significant effect or tendency). MZn = medium Zn level (200-1,000ppm Zn); HZn = high Zn level (>2,000ppm).



References of selected papers:

Amino Acids	2009
Animal Feed Science and Technology	2019
Animal Nutrition	2017
Biological Trace Element Research	2016
Canadian Journal of Animal Science	2010
Journal of Animal Science	2019
Journal of Animal Science and Biotechnology	2020
Journal of Animal Science and Technology	2022
Journal of Animal and Veterinary Advances	2012
Journal of Applied Animal Research	2019
Journal of Trace Elements in Medicine and Biology	2016
Livestock Science	2021
Oncotarget	2017
PlOS one	2017

• Glutathione peroxidase GSH-Px (enzyme quantification or activity), which protects the cells from oxidative damage.
• Malondialdehyde (MDA) quantification, a product of the lipid peroxidation.

After weaning, signs of stress are systematically recorded in piglets. Signs include oxidative stress with accumulation of reactive oxygen species (ROS), lipid peroxidation and generation of MDA. The ROS can cause cell damages and consequently decrease the integrity of the intestinal mucosa and other tissues. On the other hand, zinc is an important cofactor of numerous metalloenzymes, including cytosolic superoxide dismutase SOD. As a matter of fact, zinc displays indirect antioxidant properties.

High or medium levels of ZnO supplementation increases the activity and/or the concentration of antioxidant enzymes (SOD and GSH-Px), while it decreases MDA concentration. These results demonstrate that ZnO can mitigate oxidative stress following weaning and protect the tissues of the animal.

Nevertheless, these positive impacts should also be connected to other positive effects of ZnO on animal health: control of inflammation, regulation of intestinal microbiota, and direct effect on intestinal integrity through the synthesis of the intestinal tight junction proteins.

This article is highlighting an additional positive effect of supplementation of zinc oxide in piglets. The antioxidative properties can only be achieved with proper bioavailability. Despite zinc oxide standards sources used as pharmacological levels are poorly bioavailable, their application at high levels are somewhere compensating this weakness by a dose effect. Any strategy of replacing pharmacological applications requires the selection of a proper zinc source with optimal bio-accessibility, combined with an adequate antimicrobial effect. ■