

An answer to the problem of swine weaning without the use of zinc oxide

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In the pig industry, the use of high levels of zinc oxide (ZnO) is used for prevention and treatment of diarrhoea in weaning piglets, but the mechanism of action of Zinc has not yet been known. Some studies tested some hypothesis as WANG ET AL that demonstrated the ZnO alters the expression of intestinal proteins that are related to the regulation of oxidative stress, cell proliferation and apoptosis in weaning piglets¹. In light of these studies, this work intends to propose a new approach to use a feed additive without ZnO, based on the mechanism of immune tolerance to food antigens and the role of the oxidative stress in the intestinal system. The idea is that the ZnO modulate the inflammatory process towards foods antigen and infectious agents. For this reason, the hypothesis is that is important to limit the proinflammatory cytokines and activate the anti-inflammatory cytokines. In this paper, to demonstrate this hypothesis, the KK-0-18 feed supplement produced by Farmavet srl company was tested². Briefly the KK-0-18 contains only raw materials as Calcium Salt of Butyric Acid, Common Wheat Flour, Inulin from Chicory Root, Calcium Propionate (E282) 80.000 mg, Calcium Acetate (E263) 17.000 mg., Betaine Hydrochloride (3a925) 23.000 mg., Saccharomyces Cerevisiae (MUCL 39885-4b1710) 450x109 UFC., Endo-1-4-beta glucanase EC 3.2.1.4 (4a1602i) 3.840 u. , Endo-1-3(4)-beta glucanase EC 3.2.1.6 (4a1602i) 3.360 u., Endo-1-4-beta xylanase EC 3.2.1.8 (4a1602i) 12.960 u. ². This feed supplement is used as a 4% in complete feed for weaning of pigs. Normally, is integrated into corn, barley, soya 48%. The trial was performed in the "Eredi Antognozzi" breeding with 80 piglets with 25 days. The 80 piglets were divided into two groups, control and test. In the "control group" was used the medicated feed presented on the market with 3000 ppm of ZnO and 400 ppm of amoxicillin for a kilo. In the "test group" was used the feed (with the same analytical values of the feed described for the first group) but with the feed supplement KK-0-18. For each group, were chosen 8 piglets randomly. For these piglets, two blood samples were taken, one on the second day and one on the seventh day of the weaning period. The samples collected were centrifuged to separate the plasma from the blood at the "Zooprofilattico Institute of Perugia Tolentino". After that, the plasma samples were analysed by Millipex method to find the cytokines by Aarhus University³. In detail, the following 12 cytokines were identified: IFNG, IL-1a, IL-1B, IL-1RA, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, TNF-a. The table indicates the results of the analysis as a percentage based on the mean of the blood samples for the 8 piglets for the control and test group, following this equation:

$$\frac{(\text{Mean of the blood samples of the 7th day} - \text{Mean of the blood samples of the 2nd day})}{\text{Mean of the blood samples of the 2nd day}}$$

| Cytokines | IFN gamma | IL 1a | IL 1b | IL 1ra | IL 2 | IL 4 |
|-----------|-----------|--------|--------|---------|---------|---------|
| Test | 1,19% | 19,35% | 6,97% | 126,41% | 27,80% | 30,69% |
| Control | -11,61% | 2,50% | -3,42% | 6,81% | 5,10% | 7,87% |
| Cytokines | IL 6 | IL 8 | IL 10 | IL 12 | IL 18 | TNF a |
| Test | -37,65% | 12,12% | 9,79% | -15,80% | -7,76% | 4,76% |
| Control | 3,08% | -5,88% | -4,03% | -9,22% | -13,43% | -13,46% |

Analysis of data shows that the cytokines values between the test feed and the control feed with zinc oxide 3000 ppm kg tend to be homogeneous. Following are summarized several considerations:

INF gamma, IL 1a, IL 1b have higher values in the test feed, most likely because their presence is stimulated by bacteria or their toxins, while in the case of the control feed the presence of 400 ppm kg of amoxicillin determines an inhibition of pathogens. The **IL1ra** has an extremely high value as a reaction to the presence of **IL1a** and **IL1b** as it plays a strong inhibitory action thus protecting the intestine from the unbalanced inflammatory process. **IL 2** shows an increase, it is the cytokine that activates ILC (Cellular innate lymphocytes) which represent hubs of the immune system and among the numerous functions, one is the protection of the intestinal barrier from pathogens⁴. **IL 6, IL 12, IL 18**: In the test, they have a much lower value than the control and among all the cytokines they are those that have a marked inflammatory action, so their regulation allows optimal intestinal homeostasis. **IL 8** is slightly up by 12.12%, its action is chemotactile towards granulocytes and macrophages. **IL 10** shows a significant increase in the 9.79% differential compared to the control. This cytokinin is certainly the most important in immunotolerance processes since it regulates the regression of the inflammatory process. **TNF a** has a low-test value 0.82 pg/ml both in the test and in the control 0.73 pg/ml. It is the cytokine that maintains a high level even on the eighth-day post-weaning at colon level and is responsible for the enteric forms. In this study, we presented and evaluated a new complementary feed the KK 0-18, the results presented shown that may completely substitute the zinc oxide in swine weaning feeds.

¹ X. O. D. Y. J. W. G. & W. J. Wang, «Proteomic analysis reveals altered expression of proteins related to glutathione metabolism and apoptosis in the small intestine of zinc oxide-supplemented piglets.», *Amino acids* 37.1, pp. 209-218, 2009.

² S. Calamanti, 2019. [Online]. Available: <http://www.farmavet.it/en/supplement-and-feed/pigs/kk-premix-0-18/>.

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⁴ C. WILHELM, S. KHARABI MASOULEH e A. KAZAKOV, «Metabolic Regulation of Innate Lymphoid Cell-Mediated Tissue Protection—Linking the Nutritional State to Barrier Immunity,» *Frontiers in immunology*, n. 8: 1742., 2017