Content is avaliable at SCOPUS

Meat Technology — Special Issue 66/3

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 664.9

ID: 179603977

https://doi.org/10.18485/meattech.2025.66.3.28

Original Scientific Paper

Organic trace minerals as an innovative nutritional solution for advancement of production performance and intestinal microbiota in piglets

Dejan Perić[1*], *Dragan Šefer*[1], *Roberto Barea*[2], *Stamen Radulović*[1], *Dragoljub Jovanović*[1], *Svetlana Grdović*[1] *and Radmila Marković*[1]

[1] University of Belgrade, Faculty of Veterinary Medicine, Bulevar Oslobodjenja 18, 11000 Belgrade, Serbia
[2] Novus Europe NV, Leuvensesteenweg 643, box 15, 1930 Zaventem, Belgium

ARTICLE INFO

Keywords:
Pigs
Animal nutrition
Organic trace minerals
Gut health
Intestinal microflora
Performance

ABSTRACT

After weaning, piglets are usually stressed by nutritional, psychological, environmental, physiological and social factors. During this period, piglets must rapidly adapt to a change in the type of feed they consume, from liquid sow's milk, which is easily digestible, tasty and evenly distributed throughout the day, to solid dry feed, which is less digestible and tasty. As a consequence, feed consumption usually decreases in the first days after weaning with bad influence on composition on intestinal microbiota. Nutrient additives belong to the basic group of feed additives, and the main aims in using them are to increase the overall nutritional value of feed and to ensure the safety of food of animal nutrition through feed. Due to the reduced usability, potential toxicity and negative impact on the environment of inorganic forms of trace elements, the attention of the scientific public in recent years has been focused on finding forms of minerals that will neutralize the potential harmful effects of use and at the same time ensure better mineral usability and economy in livestock production. The Department of Animal Nutrition and Botany, Faculty of Veterinary Medicine, University of Belgrade, organised a trial that aimed to test the effects of using organic trace elements in the diet on the health status, production performance measures and intestinal microbiota of the piglets. The use of new forms of trace elements showed a beneficial effect on piglet gut health, and consequently, on the yield of meat.

1. Introduction

The gastrointestinal tract has multiple functions, such as digesting and absorbing nutrients and electrolytes, maintaining the balance of body fluids, and secreting digestive enzymes, mucins, immunoglobulins and other components. In addition, it fulfils a barrier role for the host with the aim of protection from harmful pathogens and other antigens (*Gao et al*., 2019). After weaning, piglets are usually stressed by nutritional, psychological, environmental, physiological and social factors. During this period, piglets must rapidly adapt to a change in the type of feed they consume, from liquid sow's milk, which is easily digestible, tasty and evenly distributed throughout the day, to solid dry feed, which is less digestible and tasty. As a consequence, feed

***Corresponding author:** Dejan Perić, *dperic@vet.bg.ac.rs*

Paper received September 10th 2025. Paper accepted 15th 2025.
The paper was presented at the 63rd International Meat Industry Conference "Food for Thought: Innovations in Food and Nutrition" – Zlatibor, October 05th-08th 2025.
Published by Institute of Meat Hygiene and Technology – Belgrade, Serbia.

consumption usually decreases in the first days after weaning and the piglets become malnourished with a reduced growth rate, entering an energy and protein deficit. Piglets lose about 100-250 g of body weight on the first day after weaning and recover this loss of body weight in about four days (*Le Dividich and Seve*, 2000). Understanding the challenges and impact of low feed intake associated with weaning and the consequent impact on piglet performance can help nutritionists implement modern feeding strategies using various supplements that have been proven to directly or indirectly increase feed intake and digestibility.

2. The influence of the weaning process on the composition of the intestinal microbiota of piglets

Over recent decades, the interaction between nutrition and the gut microbiota, as well as its impact on health, has great attention. Initial studies have primarily focused on the classification of microorganisms that form the gut microbiota and the relationship of the composition of microorganisms to the health status of the host (*Hermann-Bank et al.*, 2013). The primary intestinal microbiota of piglets after birth is formed because of the milieu favouring the lactic acid bacteria in the sows' milk (*Zhao et al*., 2019). However, the transitional period of nutrition during the weaning period reduces the relative abundance of bacteria of the *Lactobacillus* and increases the abundance of *Clostridium* spp., *Prevotella* spp., *Proteobacteriaceae* and *Escherichia coli*, which increases microbial diversity in the gut (*Williams et al.*, 2005). The composition of the gut microbiota of piglets, in addition to the stressors that occur during the weaning process, is largely influenced by the levels and sources of protein and fibre in the feed mixtures during this period (*Hammer et al*., 2012). During the weaning period, piglets, despite the use of pre-starters, sharply reduce their feed intake due to their transition from a liquid diet to solid feed with a complex composition, and due to other stressors that were previously described in detail. Thus, the nutrients for the survival and reproduction of bacteria are also limited. Pathogenic bacteria are able to take advantage of special nutrients (e.g., ethanolamine, a compound that is a weak base and has an odour similar to ammonia), thereby enhancing the expression of their virulence factors. For example, both *Salmonella* and enterohemorrhagic *E. coli* can use ethanolamine as a source of carbon or nitrogen in order to gain a nutritional advantage in competition with other bacteria (*Ni Lochlainn et al.*, 2018). Enterohemorrhagic *E. coli* can also use the hexose deoxy sugar, fucose, for the purpose of activating the type III secretion system, which facilitates the adhesion of pathogenic bacteria to host enterocytes (*Bäumler and Sperandio*, 2016).

Considering the importance of the composition of the intestinal microbiota for piglets, the proper establishment of a complex and well-balanced ecosystem in the gut is currently recognized as a key point in any program to reduce the use of antibiotics in animal husbandry (*Liao and Nyachoti*, 2017). One of the main causes of low body weight of slaughtered piglets and variable production results of piglets during the suckling period is the high prevalence of digestive disorders associated with opportunistic pathogens. Among them, *E. coli* is the main pathogenic agent that causes neonatal diarrhoea and postinlet diarrhoea, and consequently is also responsible for the use of large amounts of antibiotics (*López-Colom et al.,* 2020). In recent years, various feeding programs, as well as the use of supplements, have been investigated as a way to combat this pathogen and reduce the incidence of diarrhoea in piglets.

3. Using innovative nutritional strategies in piglet nutrition

Due to the increasing demands of the global consumer lobby, and respecting the production priorities (better use of feed, longer sustainability, easier manipulation) with the ultimate aim of increasing production and improving the quality of food of animal origin, in addition to the basic nutrients, a large number of additives with different purposes are added to the feed mixtures (*Šefer et al.*, 2014). Nutrient additives belong to the basic group of feed additives, and their main aim is to increase the overall nutritional value of feed. Inorganic forms of trace elements in the form of salts have been used for years as additives in mixtures for piglets: oxides, carbonates, chlorides and sulphates. The bioavailability of minerals from these sources differs, with sulphates typically having higher relative bioavailability values than oxides. The bioavailability of inorganic forms of micronutrients is limited, and high doses are required to meet the requirements of animals, often leading to nutrient imbalances and potential toxicity problems (*Ma et al*., 2020).

Due to the low usability, potential toxicity and negative impact on the environment of inorganic forms of trace elements, the attention of the scientific public in recent years has been focused on finding forms of minerals that will neutralize the potential harmful effects of mineral use and, at the same time, ensure better usability and economy in livestock production. The formation of a complex molecule of trace elements with organic components can increase passive absorption in the intestine by reducing the interaction between minerals in the intestinal lumen and, thus, preventing the formation of insoluble complexes with substances, such as hydroxides, carbonates, phosphates, oxalates and phytates, which would make copper, manganese or zinc unavailable for absorption (*Peters and Mahan*, 2008). Mineral complexation with an organic component can also result in a more favourable water-lipid partition coefficient that favours absorption over a wide pH range. The strength of the bond between the organic ligand and the trace element during complex formation can prevent dissociation while passing through the gastrointestinal tract and improve the bioavailability of the mineral (*Byrne et al*., 2021). Organically bound trace elements are recognized as bioavailable sources of trace elements that are more usable than their traditional inorganic analogues, such as sulphates and oxides *(To et al*., 2021). The aim of the research was to investigate the justification and effects of using of organic forms of copper, manganese and zinc in the diet on the health status and production performance of piglets, as well as the on the microbiota composition in chosen segments of the piglet gastrointestinal tract.

4. Materials and methods

The experimental protocol was approved by the Veterinary Directorate of the Serbian Ministry of Agriculture, Forestry and Water Management and the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade (Resolution number: 23/2020).

During the formation of the trial, an individual clinical examination of the chosen piglets was carried out, so that all the selected individuals were healthy and vital. During the formation of experimental groups, all piglets were uniform in terms of origin, sex balance and body weight (± 10%). The health condition of the test subjects was monitored daily. The experiment was conducted on 48 weaned piglets of the same origin, with an equal sex ratio. Piglets aged 28±1 days were randomly assigned to two experimental groups (C and E-I). Each experimental group consisted of six pens (two male and two female individuals per pen). The study lasted 42 days and was divided into two phases. The first phase lasted from 0-21 days (starter phase) and the second phase from 21-42 days (grower phase). From the beginning (28-day-old piglets) to the end (70-day-old piglets) of the study, experimental groups of animals were fed with complete mixtures of standard chemical and raw material composition, and the mixtures were formulated to meet the recommended nutrient requirements according to the *NRC* (2012).

The composition of the premix at the starter stage for the control group was (in kg of complete mixture): 20,000 IU vitamin A, 2,000 IU vitamin D3, 80 mg vitamin E, 2.4 mg vitamin K3, 2.4 mg vitamin B1, 6 mg vitamin B2, 6 mg vitamin B6, 0.4 mg vitamin B12, 0.3 mg biotin, 32 mg niacin, 14 mg Ca-pantothenate, 5 mg folic acid, 3 mg I, 0.4 mg Se, 0.6 mg Co, 550 mg choline chloride, 240 mg Fe, 130 mg Cu, 120mg Mn, 100 mg Zn, 1000 mg phytase, 100 mg antioxidant BHT. In the first phase, in the complete mixture for feeding group E-I, trace elements Cu, Mn and Zn of inorganic origin were replaced by trace elements Cu, Mn and Zn of organic origin (130 ppm Cu, 60 ppm Mn and 60 ppm Zn), in organic form bound to methionine. The composition of the premix at the grower stage for the control group was (in kg of complete mixture): 20,000 IU vitamin A, 1,800 IU vitamin D3, 120 mg vitamin E, 2.8 mg vitamin K3, 4 mg vitamin B1, 8.8 mg vitamin B2, 6.8 mg vitamin B6, 0.04 mg vitamin B12, 0.28 mg biotin, 28 mg niacin, 16 mg Ca-pantothenate, 0.8 mg folic acid, 0.8 mg I, 0.4 mg Se, 0.6 mg Co, 500 mg choline chloride, 200 mg Fe, 80 mg Cu, 120mg Mn, 100 mg Zn, 1000 mg phytase, 40 mg antioxidant BHT.

In the second phase, in the complete mixture for feeding group E-I, trace elements Cu, Mn and Zn of inorganic origin were replaced by trace elements Cu, Mn and Zn of organic origin (80 ppm Cu, 60 ppm Mn and 60 ppm Zn), in organic form bound to methionine.

Production results (piglet body weight, body weight gain and feed conversion rate) were measured at the beginning if necessary and the end of the study, on day 42. Immediately after slaughter and evisceration of piglet organs, the contents of the jejunum and ileum were sampled (six samples of each intestinal segment from each group – one pig per subgroup) to determine the total number of aerobic bacteria (PCA), the total number of anaerobic bacteria (PCA an), and the numbers of *Enterococcus* (ECC), *E. coli*, and *Lactobacillus* (MRS). Samples for bacteriological tests were taken directly from the intestine with a sterile swab, 1 g of a sample of intestinal contents. Dilutions were formed in sterile saline solution for bacteria that were later incubated aerobically and in thioglycolate broth for bacteria that were incubated anaerobically.

5. Results and discussion

The health status of the individuals during the study was satisfactory, and no signs of disease were observed. The E-I group of piglets that consumed feed with added organic forms of copper, manganese and zinc achieved better production results, i.e., better growth and feed conversion rate, while consuming a smaller amount of feed per achieved unit of growth.

There were no differences in the two piglet groups from the point of view of statistical analysis at the beginning of the study. However, in percentage terms, the mean body weight of the E-I piglets was 6.06% greater compared to the control group at the end of study (Figure 1). Our results are in line with another study that proved that piglets fed with 50% copper, manganese, zinc and iron in organic form achieved better production results than piglets fed with similar concentrations of trace elements exclusively from inorganic sulphate forms (*Veum et al.*, 1995). The replacement of part of the inorganic trace elements with organic forms also improved the feed utilization in those piglets (*Veum et al.*, 1995). Additionally, piglets fed protein forms of copper and zinc had higher concentrations of these micronutrients in the liver than pigs fed sulphate forms (*Schiavon et al.*, 2000). This indicates a more efficient use of organic forms of copper and zinc compared to inorganic sources.

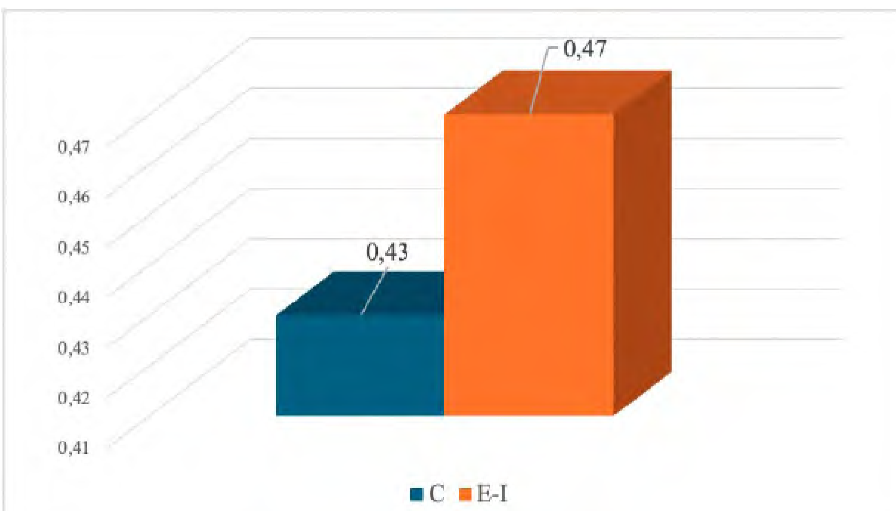

Figure 1. Mean body weight (kg) of control (C) (inorganic trace elements) and E-I (organic trace elements) groups of piglets (n = 24 per group) on days 1 (left side of figure) and 42 (right side of figure) of the study.

Analyzing the entire study period, the E-I group achieved a higher daily body weight gain compared to the control group (Figure 2). After calculating the feed conversion rate (Figure 3), and statistically analyzing the results (data not shown), significant differences were clearly shown between the examined nutritional treatments ($p<0.01$). *Mullan et al.* (2004) also described an improvement in the feed conversion rate following the inclusion of an organic source of trace elements in piglet feed mixtures. The authors of studies in which identical results were obtained generally attribute this effect to greater digestibility of nutrients, an increase in the height and width of the intestinal villi, modulation of the intestinal microbiota, but also to the antimicrobial properties of chelates that lead to an improvement in the immune function of the intestine, achieving to a greater extent the above effects (*Kong et al.*, 2010; *Han et al.*, 2014).

The numerical status ($\log_{10}$ CFU/g) of total aerobic bacteria, *E. coli*, *Enterococcus* spp., anaerobic bacteria and *Lactobacillus* spp. in the intestinal contents of the jejunum and ileum is shown in Figures 5 and 6, with the results indicating that the levels were within the physiological framework (*Dibner and*

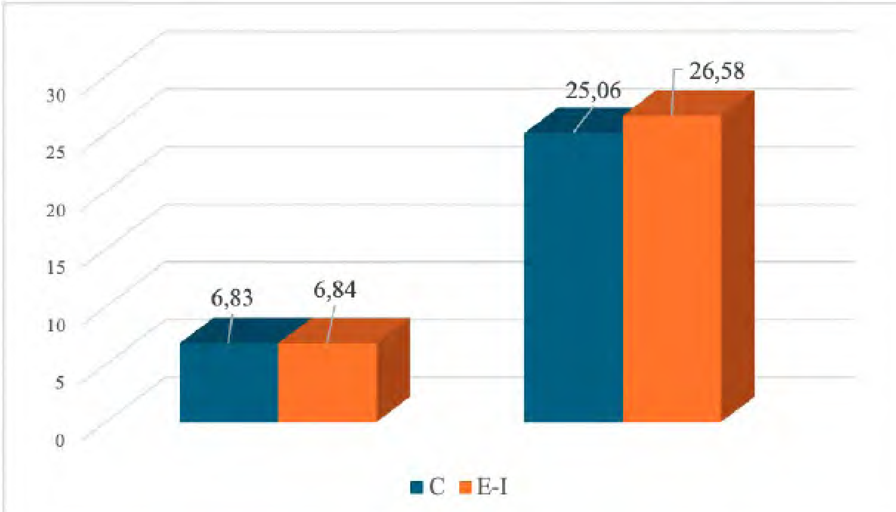

Figure 2. Mean daily body weight gain (kg) of control (C) (inorganic trace elements) and E-I (organic trace elements) groups of piglets (n = 24 per group) during the 42-day study

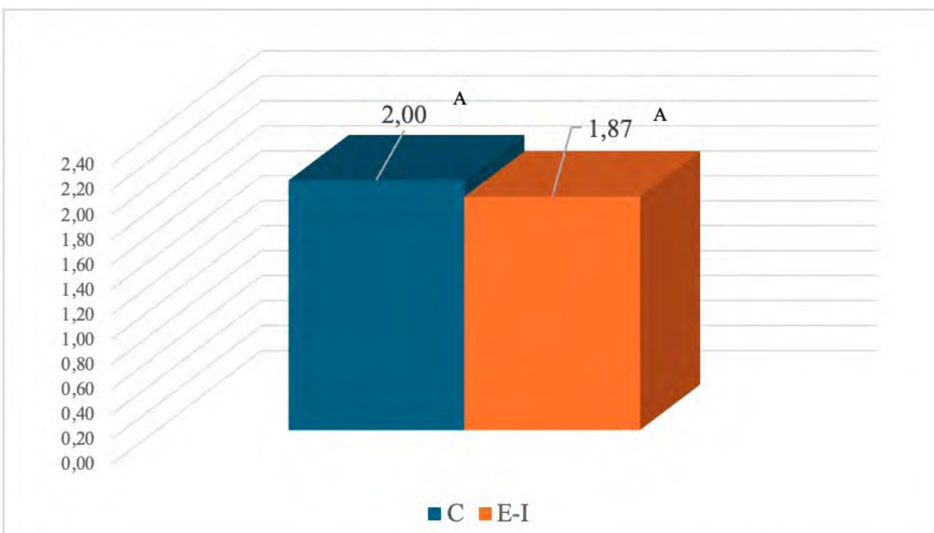

Figure 3. Mean feed conversion rate of control (C) (inorganic trace elements) and E-I (organic trace elements) groups of piglets (n = 24 per group) during the 42-day study

Buttin, 2002; *Ahmed et al*., 2014; *Diao et al.*, 2014). Analyzing the jejunum microbiota of the piglets, the highest mean (and statistically significant) total number of aerobic bacteria was found in the control group, compared to the E-I piglets ($p<0.01$). The effect in our study of adding organic forms of trace elements is consistent with the results of *Wang et al.* (2004). They conducted an experiment on piglets and demonstrated a reduction in the number of *E. coli*. However, the results of *Broom et al.* (2006) showed that the use of zinc oxide reduced the number of lactic acid bacteria, but had no effect on the number of *E. coli* in the intestines of piglets, probably due to an inorganic source of zinc in the mixtures. With the change to an organic source of zinc, a positive effect on *E. coli* numbers (lower in E-I piglets than in controls) was shown in our study. The *Enterococcus* spp. counts in our E-I group (i.e., no significant change with the use of organic trace elements compared with the control group; Figure 6) were not consistent with the results of *Broom et al.* (2006), which was expected because the oxide form of zinc was used in their study. However, the effect we measured on *Enterococcus* spp. counts was similar to that in a study by *Castillo et al.* (2008) with the use of the organic form of zinc in combination with mannan-oligosaccharides.

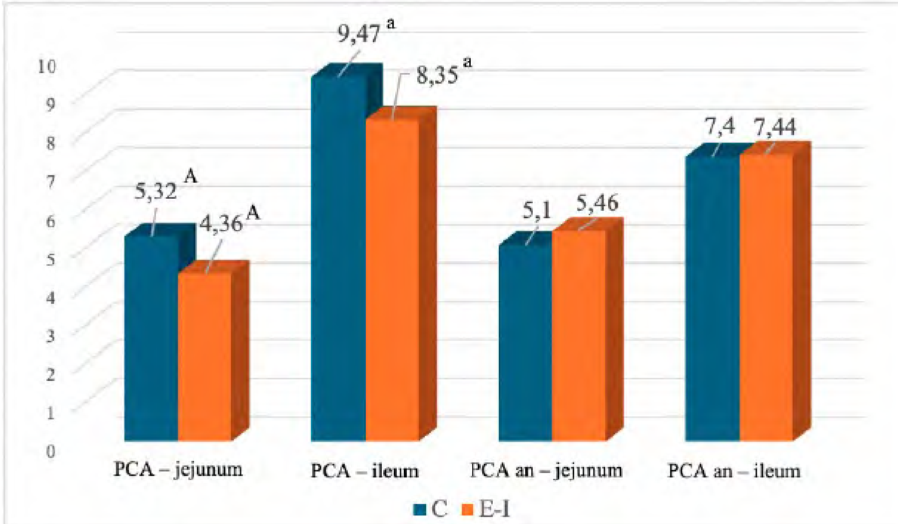

Figure 5. Total aerobic count (PCA) and total anaerobic count (PCA an) (log_{10} CFU/g) in jejunum and ileum of control (C) (inorganic trace elements) and E-I (organic trace elements) groups of piglets (n = 6)

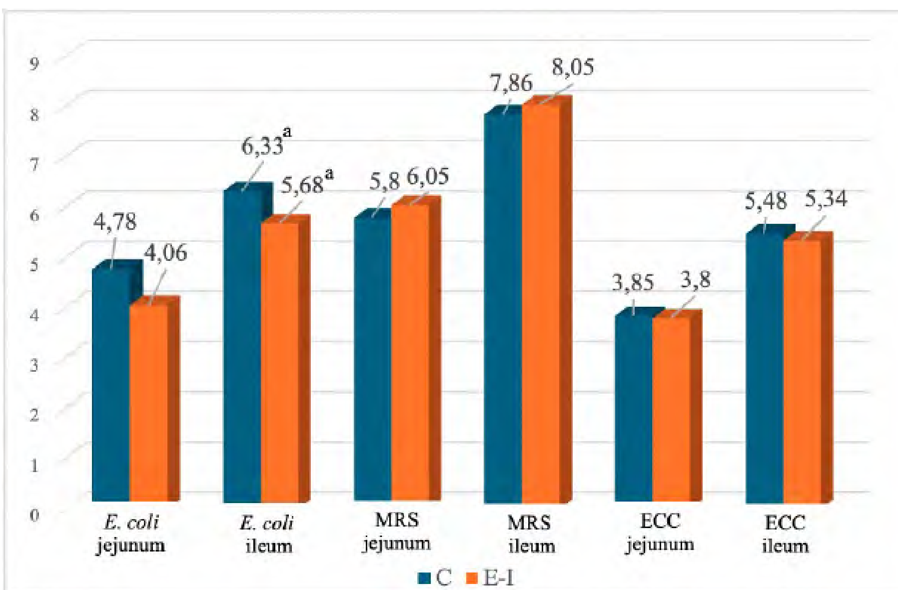

Figure 6. *Escherichia coli*, lactic acid bacteria (MRS) and *Enterococcus* (ECC) numbers (log_{10} CFU/g) in jejunum and ileum of control (C) (inorganic trace elements) and E-I (organic trace elements) groups of piglets (n = 6)

6. Conclusion

Based on the obtained results, we can conclude that the use of organic forms of copper, manganese and zinc in piglet nutrition has its own nutritional, medical and economic justification. Improving the ratios between intestinal microbiota led to better performance measures of piglets.

Disclosure Statement: No potential conflict of interest was reported by the authors.

Funding: The study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number 451-03-136/2025-03/200143).

References

Byrne, L., Hynes, J. M., Connolly, D. C. & Murphy, A. R. (2021). Influence of the Chelation Process on the Stability of Organic Trace Mineral Supplements Used in Animal Nutrition. *Animals*, 11, 1730.

Gao, J., Yin, J., Xu, K., Li, T. & Yin, Y. (2019). What Is the Impact of Diet on Nutritional Diarrhea Associated with Gut Microbiota in Weaning Piglets: A System Review. BioMed Research International,6916189.

LeDividich, J. & Sève, B. (2000). Effects of underfeeding during the weaning period on growth metabolism, and hormonal adjustments in the piglet. *Domestic Animal Endocrinology Journal*, 19, 63–74.

Schiavon, S., Bailoni, L., Ramanzin, M., Vincenzi, R., Simonetto, A. & Bittante, G. (2000). Effect of proteinate or sulfate mineral sources on trace elements in blood and liver of piglets. Animal Science, 71, 131–139.

Šefer, D., Grdović, S., Marković, R., Nedeljković-Trailović, J., Petrujkić, B. & Radulović, S. (2014). Primena biotehnologije u ishrani životinja, Zbornik radova i kratkih sadržaja 25.Savetovanja veterinara Srbije Zlatibor, 37–38.

To, V. P. T. H., Masagounder, K., & Loewen, M. E. (2021). Critical transporters of methionine and methionine hydroxy analogue supplements across the intestine: What we know so far and what can be learned to advance animal nutrition. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 255, 110908.

Veum, T. L., Bollinger, D. W. & Ellersieck, M. R. (1995). Proteinated trace mineral and condensed fish protein digestin weanling pig diets. Journal of Animal Science, 73, 308.

Hermann-Bank, M. L., Skovgaard, K., Stockmarr, A., Larsen, N., & Mølbak, L. (2013). The Gut Microbiotassay: a high-throughput qPCR approach combinable with next generation sequencing to study gut microbial diversity. *BMC Genomics*, 14, 1–14.

Zhao, J., Zhang, X., Liu, H., Brown, M. A., & Qiao, S. (2019). Dietary protein and gut microbiota composition and function. *Current Protein and Peptide Science*, 20(2), 145–154.

Williams, B. A., Bosch, M. W., Awati, A., Konstantinov, S. R., Smidt, H., Akkermans, A. D., ... & Tamminga, S. (2005). In vitro assessment of gastrointestinal tract (GIT) fermentation in pigs: Fermentable substrates and microbial activity. *Animal Research*, 54(3), 191–201.

Hamer, H. M., De Preter, V., Windey, K., & Verbeke, K. (2012). Functional analysis of colonic bacterial metabolism: relevant to health? *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 302(1), G1-G9.

Ni Lochlainn, M., Bowyer, R. C., & Steves, C. J. (2018). Dietary protein and muscle in aging people: the potential role of the gut microbiome. *Nutrients*, 10(7), 929.

Bäumler, A. J., & Sperandio, V. (2016). Interactions between the microbiota and pathogenic bacteria in the gut. *Nature*, 535(7610), 85–93.

Liao, S. F., & Nyachoti, M. (2017). Using probiotics to improve swine gut health and nutrient utilization. *Animal Nutrition*, 3(4), 331–343.

López-Colom, P., Castillejos, L., Rodríguez-Sorrento, A., Puyalto, M., Mallo, J. J., & Martín-Orúe, S. M. (2020). Impact of in-feed sodium butyrate or sodium heptanoate protected with medium-chain fatty acids on gut health in weaned piglets challenged with Escherichia coli F4+. *Archives of Animal Nutrition*, 74(4), 271–295.

Ma, L., He, J., Lu, X., Qiu, J., Hou, C., Liu, B., ... & Yu, D. (2020). Effects of low-dose organic trace minerals on performance, mineral status, and fecal mineral excretion of sows. *Asian-Australasian Journal of Animal Sciences*, 33(1), 132.

Peters, J. C., & Mahan, D. C. (2008). Effects of dietary organic and inorganic trace mineral levels on sow reproductive performances and daily mineral intakes over six parities. *Journal of animal science*, 86(9), 2247–2260.

NRC—National Research Council, (2012). Nutrient requirements of swine. Division on Earth, Life Studies, Board on Agriculture, & Committee on Nutrient Requirements of Swine. 11th Edition, National Academies Press 2012, Washington DC.

Castillo, M., Martin-Orue, S. M., Taylor-Pickard, J. A., Perez, J. F., & Gasa, J. (2008). Use of mannanoligosaccharides and zinc chelate as growth promoters and diarrhea preventative in weaning pigs: effects on microbiota and gut function. Journal of Animal Science, 86(1), 94–101.

Mullan, B. P., Hernandez, A., Pluske, J. R., Lyons, T. P., & Jacques, K. A. (2004). Influence of the form and rate of Cu and Zn supplementation on the performance of growing pigs. In Biotechnology in the Feed Industry, Proceedings of Alltech's 20th Annual Symposium (pp. 89-98). Nottingham University Press, UK.

Kong, M., Chen, X. G., Xing, K., & Park, H. J. (2010). Antimicrobial properties of chitosan and mode of action: a state of the art review. *International Journal of Food Microbiology*, 144(1), 51–63.

Han, X. Y., Ma, Y. F., Lv, M. Y., Wu, Z. P., & Qian, L. C. (2014). Chitosan-zinc chelate improves intestinal structure and mucosal function and decreases apoptosis in ileal mucosal epithelial cells in weaned pigs. *British Journal of Nutrition*, 111(8), 1405–1411.

Dibner, J. J., & Buttin, P. (2002). Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. Journal of applied poultry research, 11(4), 453–463.

Ahmed, S. T., Hwang, J. A., Hoon, J., Mun, H. S., & Yang, C. J. (2014). Comparison of single and blend acidifiers as alternative to antibiotics on growth performance, fecal microflora, and humoral immunity in weaned piglets. *Asian-Australasian Journal of Animal sciences*, 27(1), 93.

Diao, H., Zheng, P., Yu, B., He, J., Mao, X. B., Yu, J., & Chen, D. W. (2014). Effects of dietary supplementation with benzoic acid on intestinal morphological structure and microflora in weaned piglets. *Livestock Science*, 167, 249–256.

Broom, L. J., Miller, H. M., Kerr, K. G., & Knapp, J. S. (2006). Effects of zinc oxide and Enterococcus faecium SF68 dietary supplementation on the performance, intestinal microbiota and immune status of weaned piglets. *Research in Veterinary Science*, 80(1), 45–54.

Wang, X., Du, Y., & Liu, H. (2004). Preparation, characterization and antimicrobial activity of chitosan–Zn complex. *Carbohydrate Polymers*, 56(1), 21–26.

Authors info

Dejan Perić, https://orcid.org/0000-0002-4752-7489
Dragan Šefer, https://orcid.org/0000-0002-4394-6336
Roberto Barea /
Stamen Radulović, https://orcid.org/0000-0002-7250-537X
Dragoljub Jovanović, https://orcid.org/0000-0003-1665-7106
Svetlana Grdović, https://orcid.org/0000-0003-4445-2247
Radmila Marković, https://orcid.org/0000-0001-8467-0551