
CURRENT APPROACHES IN MAINTENANCE GUT HEALTH IN FARMING ANIMALS. PART 3. THE PROSPECTS OF ORGANIC ACIDS SUPPLEMENTATION IN POULTRY FEED ADDITIVES

Masiuk Dmytro, Nedzvetsky Victor, Kokariev Andrii
DOI <https://doi.org/10.30525/978-9934-26-714-7-10>

INTRODUCTION

Poultry farming is the most widespread form of livestock production in the world, due to its relatively low cost and short production cycle. At the current stage of poultry development, the best possible conditions have been created to fulfil the genetic potential of animals. According to N. Haulisah et al. (2021)¹, one of the key methods of increasing broiler productivity since the 1950s has been the use of antibiotics as growth promoters (GPs). However, the use of these substances has brought the issue of antibiotic resistance to a new level that threatens humanity. The most widely used antibiotics, as GPs, have been used to improve feed conversion and health status of poultry². The abandonment of antibiotics has initiated an increase in the number of studies on effective alternative methods of monitoring and correcting animal health, welfare and productivity, including in poultry farming.

A wide range of products have been proposed to replace antibiotics in feed, among which organic acids (OAs) show promising results³. The pool of carboxylic acids that are most common in living organisms is represented by free fatty acids and those that are part of the lipids of living organisms. According to D. Venegas et al. (2019)⁴, according to the chain length, fatty acids

¹ High levels of antibiotic resistance in isolates from diseased livestock / N. A. Haulisah et al. *Frontiers in Veterinary Science*. 2021. Vol. 8. Art. 652351.

² Salah A. S., Ahmed-Farid O. A., El-Tarabany M. S. Carcass yields, muscle amino acid and fatty acid profiles, and antioxidant indices of broilers supplemented with synbiotic and/or organic acids / A. S. Salah, O. A. Ahmed-Farid, M. S. El-Tarabany. *Journal of Animal Physiology and Animal Nutrition*. 2019. Vol. 103, No. 1. P. 41–52.

³ In vitro investigation of the antibacterial activity of nine commercial water disinfectants, acidifiers, and glyceride blends against the most important poultry zoonotic bacteria / T. Mantzios et al. *Pathogens*. 2023. Vol. 12, No. 3. Art. 381. DOI: <https://doi.org/10.3390/pathogens12030381>.

⁴ Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases / D. Parada Venegas et al. *Frontiers in Immunology*. 2019. Vol. 10. Art. 277.

are divided into short-chain fatty acids (SCFAs) – containing up to 6 carbon atoms, medium-chain fatty acids (MCFAs) – containing 6–12 carbon atoms, and long-chain fatty acids (LCFAs) – containing 13–21 carbon atoms. There is also (very long) elongated chain fatty acids (VLCFAs), which have over 22 carbon atoms. All SCFAs have varying degrees of water solubility, which distinguishes them from LCFAs, which are insoluble. In the poultry industry, SCFAs are used more frequently and somewhat less frequently than MCFAs as an alternative to antibacterial preparations and growth stimulants. G. Galli et al. (2021)⁵ note that the effectiveness of SCFAs and MCFAs is influenced by a considerable number of factors, among which the key ones are the chemical structure, form of additive, its amount and method of supplementation, buffer capacity of feed, and its nutritional value.

Organic acids are widely used as additives in drinking water or as feed additives. They are often used in the salt forms (sodium, potassium, or calcium and/or partially esterified). According to M. Aljumaah et al. (2020)⁶, the advantage of using salts is their odourlessness, solid form (the purest organic salts are volatile), lower corrosivity, and better solubility. The efficiency of an organic acid depends on its molecular weight, pKa value and form (undissociated or dissociated), which together determine the difference in antimicrobial activity and bioavailability. Several OAs have a specific bioavailability. The bioavailability of OAs can be improved by choosing the correct form of additive that promotes OA adsorption by cells. F. Mannelli et al. (2019)⁷, N. Qi et al. (2023)⁸ pointed out that the use of fatty acid salts is the simplest solution, as these salts are solid, which facilitates the technology of feed production and improves their organoleptic properties. Microencapsulation is considered a promising way to deliver organic acids to the digestive tract. Therewith, the composition of the coating that envelops the OA ensures a more efficient release of these substances in the right place in the digestive tract.

The purpose of this study was to summarise the current comprehension and prospects for the use of short- and medium-chain organic acids in poultry farming as safe substitutes for antimicrobial agents and/or growth promoters based on the analysis of available literature.

⁵ Growth performance and meat quality of broilers fed with microencapsulated organic acids / G. M. Galli et al. *Animal Feed Science and Technology*. 2021. Vol. 271. Art. 114706.

⁶ Organic acid blend supplementation increases butyrate and acetate production in *Salmonella enterica* serovar Typhimurium challenged broilers / M. R. Aljumaah et al. *PLoS ONE*. 2020. Vol. 15, No. 6. Art. e0232831.

⁷ Effect of chestnut tannins and short chain fatty acids as anti-microbials and as feeding supplements in broilers rearing and meat quality / F. Mannelli et al. *Animals*. 2019. Vol. 9, No. 9. Art. 659.

⁸ Study of microencapsulated fatty acid antimicrobial activity in vitro and its prevention ability of *Clostridium perfringens* induced necrotic enteritis in broiler chicken / N. Qi et al. *Gut Pathogens*. 2023. Vol. 15. Art. 1.

1. Application of SCFAs in poultry farming

OAs are organic compounds with acidic properties, the most common of which are carboxylic acids, whose acidity is related to their carboxylic group – COOH. These acids are weak, partially dissociated and have a pKa (pH at which the acid is half dissociated) of 3 to 5. SCFAs used in poultry production include oil, vinegar, propionic, dairy, formic, apple, wine, fumaric, and sorbic⁹.

SCFAs (acetate, propionate, and butyrate) are produced by bacterial fermentation in the intestine and have certain effects on metabolism and the immune system. Apart from being important cross-nutrition products, microbial metabolites also have a positive effect on the mucous membrane of the digestive tract. SCFAs play an important role in the metabolic and immune homeostasis of the digestive tract, as well as in the integrity of the intestinal barrier, which opens great opportunities for therapeutic development¹⁰.

The intestinal microbiota releases SCFAs (mainly acetate, propionate, and butyrate) in the colon during fermentation, from where they are absorbed by the colon epithelium through passive and active transport. The pathways of formation of various SCFAs and the corresponding microbial producers were identified. The products of microbial fermentation of SCFAs are the main source of energy for colonocytes¹¹. At the same time, all commensal gut taxa ferment pyruvate to produce butyrate, while pathogenic bacteria, such as *Fusobacterium*, use different pathways, such as glutamate (4-aminobutyrate) and lysine, which are associated with the release of harmful by-products, such as ammonia.

The key factors that limit the production of SCFAs in the digestive tract include pH, growth factors, and gas levels. Carbohydrates, which lead to high production of SCFAs, lower the pH in the colon, which affects the microbial composition and production of SCFAs. The availability of growth factors such as iron is crucial for the production of SCFAs. Significantly lower concentrations of butyrate and propionate were found in the faeces of iron-deficient rats. Iron deficiency induces an increase in the number of *Lactobacilli* and *Enterobacteriaceae* species and, conversely, a decrease in *Roseburia* and *Eubacterium rectale* species, which are the main producers of butyrate. Oxygen and Hydrogen levels affect the fermentation process and the production of SCFAs¹². Overall, only 4 SCFAs are currently widely used in poultry production (Table 1).

⁹ Ricke S. C., Dittoe D. K., Richardson K. E. Formic acid as an antimicrobial for poultry production: A review. Richardson. *Frontiers in Veterinary Science*. 2020. Vol. 7. Art. 533419.

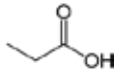
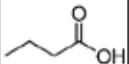
¹⁰ Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases / D. Parada Venegas et al. *Frontiers in Immunology*. 2019. Vol. 10. Art. 277.

¹¹ Short chain fatty acids and its producing organisms: An overlooked therapy for IBD? / S. Deleu et al. *EBioMedicine*. 2021. Vol. 66. Art. 103293.

¹² Louis P., Flint H. J. Formation of propionate and butyrate by the human colonic microbiota / P. Louis, H. J. Flint. *Environmental Microbiology*. 2017. Vol. 19, No. 1. P. 29–41.

Table 1

Short-chain fatty acids used in poultry production

Name		Formula		Mass (g/mol)	Diagram
Common	Systematic	Molecular	Structural		
Formic acid	Methanoic acid	CH_2O_2	COOH	46.0	
Acetic acid	Ethanoic acid	$\text{C}_2\text{H}_4\text{O}_2$	CH_3COOH	60.1	
Propionic acid	Propanoic acid	$\text{C}_3\text{H}_6\text{O}_2$	$\text{CH}_3\text{CH}_2\text{COOH}$	74.1	
Butyric acid	Butanoic acid	$\text{C}_4\text{H}_8\text{O}_2$	$\text{CH}_3(\text{CH}_2)_2\text{COOH}$	88.1	

Source: compiled by the authors of this study based on the literature data

Acetic, butyric, and propionic acids are the most abundant SCFAs in the colon, where they have a positive effect on energy status by providing a carbon source for the intestinal microbiota through the activation of glyoxylate pathway enzymes. In commercial poultry production, SCFAs are mainly associated with antimicrobial activity and increased productivity¹³. The antimicrobial effect of propionic and butyric acids is conditioned by the acid dissociation constant ($\text{pK}_a=3-5$). Once in the intestine, they lower the pH, which inhibits potentially pathogenic bacteria, and increase the level of calcium, phosphorus, and magnesium in the blood serum due to improved absorption¹⁴.

Lactic acid is used in poultry production both in pure form and in the form of butyrates, coated/encapsulated butyrate salts¹⁵ and butyrate glycerols (butyrine). Each product formulation has its own advantages and limitations in terms of bioavailability, cost, biosafety, stability, processing temperature/pressure, and digestive release. The use of an encapsulated form of lactic acid in laying hens showed better results than butyrate.

¹³ Polyphenols and organic acids as alternatives to antimicrobials in poultry rearing: A Review / F. Scicutella et al. *Antibiotics*. 2021. Vol. 10, No. 8. Art. 1010.

¹⁴ Effect of supplementation of salts of organic acids on serum and haematological parameters of broilers / V. Us et al. *International Journal of Current Microbiology and Applied Sciences*. 2017. Vol. 6, No. 11. P. 4211–4218.

¹⁵ Evaluation of the blend of organic acids and medium-chain fatty acids in matrix coating as antibiotic growth promoter alternative on growth performance, nutrient digestibility, blood profiles, excreta microflora, and carcass quality in broilers / D. H. Nguyen et al. *Poultry Science*. 2018. Vol. 97, No. 12. P. 4351–4358.

Butyric acid is one of the main acids that are successfully used in industrial poultry farming. This acid modulates the state of symbiotic microbiota and improves immunological homeostasis in the intestine. Butyrates (calcium or sodium salts) are easily converted to butyric acid in the digestive tract and are considered safe. Compared to other MCFAs, butyrate has a slight antibacterial effect, but is not expensive. The use of butyrate reduces the concentration of total circulating triglycerides and cholesterol in broilers¹⁶. Sodium butyrate is the most common form of butyric acid. There (are a series) is a number of studies showing that the addition of butyric acid, in various doses, from 0.2 g/kg to 0.6 g/kg, to broiler chickens' diets improves performance, digestibility, and nutrient absorption, and reduces the incidence of disease. The mechanism of butyrate effect is that when sodium butyrate enters the stomach, it releases the Na ion¹⁷. Due to the low pH, butyrate is rapidly converted to the undissociated form of butyric acid¹⁸.

Butyric acid is formed by microbial fermentation in the colon and is the main source of energy for colonocytes, affects their proliferation, differentiation, gene expression, and protein synthesis, and improves its absorption. Butyric acid reduces bacterial colonisation of the intestinal wall by lowering the pH, which reduces the tendency to diarrhoea. The addition of butyric acid or butyric acid glycerides to turkey diets was shown to have a beneficial effect on feed conversion, intestinal morphology, and bird health by reducing pathogen concentrations in faeces¹⁹. The use of feed additives based on butyric acid inhibits the spread of Salmonella infection and contamination of bedding with pathogens.

The addition of 0.5% acetic, lactic, or formic acid to drinking water limits the growth of *S. Typhimurium* in chickens²⁰. However, sublethal concentrations of undissociated acetic acid may not always stimulate the acid resistance of *Salmonella enterica* sub. *enterica* serovar Enteritidis Phage²¹. A relatively new

¹⁶ Effects of dietary short-and medium-chain fatty acids on performance, carcass traits, jejunum morphology, and serum parameters of broiler chickens / A. Khatibjoo et al. *Journal of Applied Animal Research*. 2018. Vol. 46, No. 1. P. 492–498.

¹⁷ Dietary sodium butyrate and/or vitamin D3 supplementation alters growth performance, meat quality, chemical composition, and oxidative stability in broilers / H. Gao et al. *Food Chemistry*. 2022. Vol. 390. Art. 133138.

¹⁸ Effect of sodium butyrate on intestinal health of poultry – a review / S. S. Elnesr et al. *Annals of Animal Science*. 2020. Vol. 20, No. 1. P. 29–41.

¹⁹ Makowski Z., Lipiński K., Mazur-Kuśnerek M. The effects of different forms of butyric acid on the performance of turkeys, carcass quality, incidence of footpad dermatitis and economic efficiency / Z. Makowski, K. Lipiński, M. Mazur-Kuśnerek. *Animals*. 2022. Vol. 12, No. 11. Art. 1458.

²⁰ The control of poultry salmonellosis using organic agents: An updated overview / M. T. El-Saadony et al. *Poultry Science*. 2022. Vol. 101, No. 4. Art. 101716.

²¹ Gavriil A., Thanasoulia A., Skandamis P. N. Sublethal concentrations of undissociated acetic acid may not always stimulate acid resistance in *Salmonella enterica* sub. *enterica* serovar Enteritidis Phage Type 4: Implications of challenge substrate associated factors / A. Gavriil, A. Thanasoulia, P. N. Skandamis. *PLoS ONE*. 2020. Vol. 15, No. 7. Art. e0234999.

area is the use of dietary fermented fibres to produce bioactive fatty acids in the intestines of animals²². The addition of 1% wheat bran with a particle size of 280 µm to the feed leads to its rapid fermentation in the digestive tract with the formation of butyric acid. These feed additives improve the performance of broiler chickens. Glycerol monolaurate is successfully used in poultry feeding, which not only improves meat quality, but also has antibacterial properties at a dose of 300 mg/kg against *Escherichia coli* and *Eimeria* spp.²³. The expressed antimicrobial effect of LCFAs formed as a result of fermentation of cranberry processing waste was shown. The beneficial effect of high doses of α -linolenic acid (21.0%) and linoleic acid (39.7%) obtained from fermentation products was aimed at preventing encephalomalacia, improving the immune response against infectious bursal disease virus (IBDV) and Newcastle disease²⁴.

2. Effect of SCFA on metabolism and immune defence

SCFAs are transported through the basolateral mucosa of the colon to the portal bloodstream, probably with the participation of SCFA-specific receptors. SCFA receptors, which are activated by acetate and propionate, were found on intestinal endocrine L cells. The GPR 109A receptor, which is expressed by colon epithelial cells, is activated by butyrate and provides IL10 production and an anti-inflammatory response. The OLFR 78 receptor is expressed in renal blood vessels and is involved in blood pressure regulation²⁵. Despite a considerable amount of information on the effect of SCFAs on metabolism and energy homeostasis, the mechanisms of transport and biological activity of SCFAs are still understudied.

Along with the studied protective effects, there is an evidence of the damaging effect of butyrate on the intestinal barrier in the inflammation presence²⁶. Comparable results were obtained regarding the protection of colon stem cells from microbial metabolites. Some studies have shown conflicting data on the positive effect of butyrate on the intestinal barrier. For instance,

²² Microbial short-chain fatty acids: A bridge between dietary fibers and poultry gut health – A review / Q. Ali et al. *Animal Bioscience*. 2022. Vol. 35, No. 10. P. 1461–1478.

²³ Glycerol monolaurate in the diet of broiler chickens replacing conventional antimicrobials: Impact on health, performance and meat quality / B. F. Fortuoso et al. *Microbial Pathogenesis*. 2019. Vol. 129. P. 161–167.

²⁴ Dietary organic cranberry pomace influences multiple blood biochemical parameters and cecal microbiota in pasture-raised broiler chickens / M. R. Islam et al. *Journal of Functional Foods*. 2020. Vol. 72. Art. 104053.

²⁵ The olfactory G protein-coupled receptor (Olfir-78/OR51E2) modulates the intestinal response to colitis / K. Kotlo et al. *American Journal of Physiology-Cell Physiology*. 2020. Vol. 318, No. 3. P. 502–513.

²⁶ Butyrate does not protect against inflammation-induced loss of epithelial barrier function and cytokine production in primary cell monolayers from patients with ulcerative colitis / M. Vancamelbeke et al. *Journal of Crohn's & Colitis*. 2019. Vol. 13, No. 10. P. 1351–1361.

butyrate promotes the growth of SCFA-producing bacteria in patients with digestive disorders²⁷. Oral administration of butyrate to mice aggravated colitis, while its intraperitoneal administration improved the condition of the animal health. On the other hand, inhibition of butyrate-producing microorganisms caused an exacerbation of colitis in mice. Acetate supplementation in mice plays a crucial role in the intestinal response to tissue damage and repair in colitis²⁸. Undoubtedly, further study (more research) is needed on both microbiome-derived and dietary-supplemented SCFAs pool to apply SCFAs in digestive tract diseases. Despite the aforementioned data, the use of SCFAs for therapeutic purposes, their optimal therapeutic doses and indications for the use of SCFA-producing bacteria or SCFAs are still unresolved.

In vitro studies have provided evidence of the beneficial effects of butyrate on the intestinal barrier. Butyrate contributes to the Butyrate contributes to the intestinal barrier integrity by activating genes whose products maintain transepithelial electrical resistance (TEER). Butyrate has been shown to stimulate the energy metabolism of intestinal epithelial cells, modulate the production of hypoxia inducible factor 1 (HIF-1) and transcription factors, which together support the intestinal barrier function. Particularly important are the results of SCFAs' impact on the intestinal barrier and the immune system. SCFAs can modulate the adaptive immune system by stimulating macrophages and dendritic cells. SCFAs can initiate the transformation of naïve T cells into regulatory T cells²⁹. Apart from polarising regulatory T cells, SCFAs also affect the polarisation and activation of Th1, Th2, and Th17 cells by inhibiting histone deacetylase (HDAC). Acetate, propionate, and butyrate also promote Th1 and Th17 differentiation in vitro. HDAC inhibition by butyrate and propionate promotes IFN- γ expression, which enhances antiviral immunity³⁰.

The effect of SCFAs on certain types of immune cells (neutrophils, monocytes, and macrophages) is also achieved through a decrease in histone deacetylase (HDAC) levels³¹. The effects of SCFA on cytokine transcription are conditioned by the activation of the nuclear factor kappa B cell (NF- κ B). Thus, SCFAs are involved in the regulation of cellular response genes expression

²⁷ Microbiota changes induced by microencapsulated sodium butyrate in patients with inflammatory bowel disease / S. Facchin et al. *Neurogastroenterology & Motility*. 2020. Vol. 32, No. 10. Art. e13914.

²⁸ A high-sugar diet rapidly enhances susceptibility to colitis via depletion of luminal short-chain fatty acids in mice / M. Laffin et al. *Scientific Reports*. 2019. Vol. 9. Art. 12294.

²⁹ Oliveira L. M., Teixeira F. M. E., Sato M. N. Impact of retinoic acid on immune cells and inflammatory diseases / L. M. Oliveira, F. M. E. Teixeira, M. N. Sato. *Mediators of Inflammation*. 2018. Vol. 2018. Art. 3067126.

³⁰ Regulation of the effector function of CD8⁺ T cells by gut microbiota-derived metabolite butyrate / M. Luu et al. *Scientific Reports*. 2018. Vol. 8. Art. 14430.

³¹ Immunomodulatory potential of gut microbiome-derived short-chain fatty acids (SCFAs) / W. Ratajczak et al. *Acta Biochimica Polonica*. 2019. Vol. 66, No. 1. P. 1–12.

to damage, including infectious agents. Stimulation of cytokine production is a universal mechanism that ensures an adequate cellular response. In this response, it is critical to ensure a balance between pro- and anti-inflammatory factors. The ability of SCFAs to regulate anti-inflammatory cytokines and pro-inflammatory cytokines including IL-1 β , IL-6, IL-8, and IFN- γ was found in broilers and piglets³². Furthermore, SCFAs affect neutrophil chemotaxis induced by inflammatory mediators. SCFAs can affect the differentiation and function of dendritic cells, specifically, *in vitro* studies showed butyrate inhibiting their maturation during incubation with various inflammatory inducers.

In addition to their regulatory effects, SCFAs are a complete source of energy for enterocytes. The content of SCFAs in the chicken caecum varies according to the content of the dominant microbiota³³. Microbiota modification with antibiotics in mice showed a strong association between SCFAs content and the number of *Bacteroides* in the caecum. Elevated concentrations of SCFAs are considered beneficial for gut health by lowering pH and inhibiting pathogens. However, too high a SCFAs content can inhibit beneficial taxa together with pathogens. This undesirable effect of organic acids supplementation to the diet reduces feed intake and inhibits weight gain in broiler chickens.

SCFAs inhibit cholesterol synthesis by inhibiting the activity of the enzymes 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR). Thus, SCFAs are involved in the indirect regulation of energy metabolism by modulating cholesterol-dependent hormone synthesis and lipid metabolism. Another way in which SCFAs are involved in metabolic regulation is by reducing plasma glucose levels through the activation of the fatty acid receptors Ffar2 and Ffar3. The lack of butyrate-producing bacteria in the microbiome induced a decrease in FFAR2/3 receptor signalling, suppressed mucin formation, and increased intestinal permeability³⁴. Considering all the above-mentioned data, the use of SCFAs in poultry production requires a detailed (understanding) comprehension of the protective mechanisms in the intestinal system cells, as well as the possible risks of inhibiting intestinal function.

3. Use of medium-chain fatty acids

Medium-chain fatty acids (MCFAs) are considered a promising alternative to antibiotics due to their beneficial effects on digestion. MCFAs exhibit antibacterial activity, activate absorption, inhibit lipase production by bacteria

³² Dietary sodium butyrate improves intestinal development and function by modulating the microbial community in broilers / W. Wu et al. *PLoS ONE*. 2018. Vol. 13, No. 5. Art. e0197762.

³³ 16S rRNA sequencing analysis of the gut microbiota in broiler chickens prophylactically administered with antimicrobial agents / M. Cuccato et al. *Antibiotics*. 2021. Vol. 10, No. 2. Art. 146.

³⁴ Abnormalities in microbiota/butyrate/FFAR3 signaling in aging gut impair brain function / S. P. Mishra et al. *JCI Insight*. 2024. Vol. 9, No. 3. Art. e168443.

and have a lower ability to dissociate³⁵. The mechanisms of action of MCFA are still unclear. MCFAs can act as non-ionic surfactants, incorporating into the bacterial double layer of lipids to form pores, leading to a critical increase in permeability and cell destruction. MCFAs are rapidly absorbed in the small intestine, transported to the liver as free fatty acids, and enter the mitochondria independently of fatty acyl-CoA carnitine transferases³⁶. The administration of MCFAs reduces lipogenesis, lipid uptake, fatty acid biosynthesis, and increases their oxidation.

It is assumed that the antibacterial efficacy of MCFAs is comparable to that of SCFAs, but their mechanism of impact is different in respect to that of SCFAs. MCFAs have a pKa value of about 4.9 and their efficiency decreases with molecular weight magnification. An important feature of MCFAs is their ability to easily penetrate dense peptidoglycan (Gram+ bacteria) and/or phospholipid (Gram- bacteria) membranes in undissociated form³⁷. The MCFAs adsorbed by the cells dissociate in the protoplasm, lowering the pH, which initiates critical damage to microbial cells. This property of MCFAs makes them promising substitutes for antibiotics, especially against gram-positive cocci and *Escherichia coli*. *Campylobacter* spp. infections cause an estimated 250,000 cases of gastroenteritis in the EU and a cost of over EUR 2.4 billion annually (The European Union One Health 2018; Zoonoses Report, 2019)³⁸. Despite the widespread occurrence of *Campylobacter* in warm-blooded animals, the main source of infection is birds, specifically broiler chickens³⁹. Addition of MCFAs to the feed for 3 days reduces *Campylobacter* colonisation in 27-day-old broilers experimentally infected with *C. jejuni* at 15 days of age. Feed additives with MCFA significantly reduce *Campylobacter* carriage in broiler chickens.

MCFAs, namely caproic, caprylic, and capric acids, are digested and absorbed faster than long-chain fatty acids, improve digestion, absorption, and

³⁵ The combined use of triacylglycerols containing medium-chain fatty acids (MCFAs) and exogenous lipolytic enzymes as an alternative for nutritional antibiotics in piglet nutrition: I. In vitro screening of the release of MCFAs from selected fat sources by selected exogenous lipolytic enzymes under simulated pig gastric conditions and their effects on the gut flora of piglets / N. A. Dierick et al. *Livestock Production Science*. 2002. Vol. 75, No. 2. P. 129–142.

³⁶ Roopashree P. G., Shetty S. S., Suchetha Kumari N. Effect of medium chain fatty acid in human health and disease / P. G. Roopashree, S. S. Shetty, N. Suchetha Kumari. *Journal of Functional Foods*. 2021. Vol. 87. Art. 104724.

³⁷ Intestinal mucus protects *Campylobacter jejuni* in the ceca of colonized broiler chickens against the bactericidal effects of medium-chain fatty acids / D. Hermans et al. *Poultry Science*. 2010. Vol. 89, No. 6. P. 1144–1155.

³⁸ The European Union One Health 2018 Zoonoses Report. *EFSA Journal*. 2019. Vol. 17, No. 12. Art. e05926.

³⁹ Peh E., Kittler S., Reich F., Kehrenberg C. Antimicrobial activity of organic acids against *Campylobacter* spp. and development of combinations – A synergistic effect? / E. Peh et al. *PLoS ONE*. 2020. Vol. 15, No. 9. Art. e0239312.

lipid transport. Furthermore, MCFAs prevent the adsorption of bacteria to the intestinal wall and reduce invasion by inhibiting the production of bacterial lipases. The beneficial effect of MCFAs (C6-C12) alone and their mixture with SCFAs (C2-C6) was shown in (relation) respect to performance, carcass characteristics, haematological and biochemical parameters of broiler chickens' serum. Monoglycerides synthesised using MCFAs are also considered as a promising alternative. Recently, the results of their beneficial effects on performance, intestinal histomorphology, amino acid digestibility, and blood chemistry of broiler chickens have been presented. A metagenomic analysis of organic acid use has shown a stimulating effect on the diversity of beneficial microorganisms, nutrient digestion, and muscle growth⁴⁰.

4. Application of organic acid mixtures in poultry farming

Numerous studies on feed additives applying have shown that mixtures of organic acids (two or more) have significantly better efficacy than any one acid alone⁴¹. Specifically, SCFAs enhances the antimicrobial effect of MCFAs, which supports the gut microbiota of piglets and feed conversion in laying hens. The polymodal effects of a mixture of fumaric, citric, malic acids with capric and caprylic acids on productivity, Lactobacillus content, IgG concentration and inhibition of E. coli growth were shown. The mixture of phosphoric acid (0.2 g/kg) and lactic acid (0.3 g/kg) increases the pH of the pectoralis major and thigh muscle within 24 hours. The use of a mixture of microencapsulated organic acids with MCFAs has a positive effect on egg production, egg strength, calcium concentration, and the content of Lactobacillus and E. coli in the faeces of laying hens.

There is a fairly considerable number of results showing the beneficial effect of organic acid mixtures on the intestinal function and health of productive poultry. Specifically, the use of mixtures of butyric, fumaric, and lactic acids in different proportions had a positive effect on poultry body weight gain, feed conversion rate, and increase in the height of villi in the small intestine. A positive effect of sodium butyrate supplementation with MCFAs salts on the intestinal health of broiler chickens has been shown⁴². The addition of SCFAs and MCFAs to the broiler diet reduces serum cholesterol levels, abdominal fat, and thigh fat percentage and improves meat quality. Combined use of MCFA

⁴⁰ A comparison study of the caecum microbial profiles, productivity and production quality of broiler chickens fed supplements based on medium chain fatty and organic acids / A. Dauksiene et al. *Animals*. 2021. Vol. 11, No. 3. Art. 610.

⁴¹ Effects of short- and medium-chain fatty acids on production, meat quality, and microbial attributes – A Review / R. T. Szabó et al. *Molecules*. 2023. Vol. 28, No. 13. Art. 4956.

⁴² Impact of dietary supplementation with sodium butyrate protected by medium-chain fatty acid salts on gut health of broiler chickens / M. Sadurní et al. *Animals*. 2022. Vol. 12, No. 19. Art. 2496.

and organic acids increases duodenal villi height and crypt depth in broiler chickens. In addition to blends of single organic acids, blends of essential oils with organic acids have recently been proposed. Testing of such mixtures has shown their effectiveness against pathogen contamination of poultry feed⁴³. However, the scheme of feed additives use is of fundamental significance. For instance, the use of a mixture of organic acids and essential oils throughout the production cycle (35 days) with the addition of MCFAs for 5 days immediately before slaughter negatively affected broiler performance⁴⁴. The authors suggest that this result may be due to changes in the microbiota of the small intestine caused by prolonged exposure to acid. Recent results of a study in Ukraine on the effects of the original SCFAs blend have shown the vital role of adhesion proteins and extracellular matrix in the barrier function of the small intestine⁴⁵. Therewith, mixtures of SCFAs with MCFAs have been shown to be effective in treating poultry infected with *Clostridium perfringens*, *Eimeria* spp., and *Salmonella typhimurium*. At the same time, mixtures of formic acid, propionic acid, and sodium formate, varying in ratio, showed no differences in effectiveness against *Salmonella*.

CONCLUSIONS

According to the current regulatory framework, specifically EU regulations, the use of antibiotics in poultry farming is critically limited. Currently, OAs are successfully used as a prophylactic alternative to antibiotics. Research has shown that OAs are a cost-effective means of ensuring productivity, antimicrobial protection, and maintaining poultry health. Recent studies have shown that certain OAs improve digestion, immune response, and suppress pathogenic microflora. On the other hand, mixtures of OAs may have more powerful protective and stimulating effects depending on the composition and relative content of each component.

SCFAs have a wide range of effects on metabolism and immune defence in poultry, but despite the available data respectively their effects on metabolism and energy homeostasis, the mechanisms of transport and biological activity is still understudied. Butyrate has been shown to have a positive effect on the intestinal barrier and stimulates the metabolism of enterocytes. SCFAs can modulate the adaptive immune system by stimulating macrophages and dendritic

⁴³ Synergistic effects of essential oils and organic acids against *Aspergillus flavus* contamination in poultry feed / T. Satterlee et al. *Toxins*. 2023. Vol. 15, No. 11. Art. 635.

⁴⁴ An investigation of the effect of water additives on broiler growth and the caecal microbiota at harvest / G. Greene et al. *Pathogens*. 2022. Vol. 11, No. 8. Art. 932.

⁴⁵ Fibronectin measurement as a potential molecular marker for barrier function assessment of piglet intestine / D. M. Masiuk et al. *Theoretical and Applied Veterinary Medicine*. 2023. Vol. 11, No. 2. P. 3–8.

cells. SCFAs are involved in the regulation of gene expression in the cellular response to damage, including infectious agents. Therewith, there is an evidence of the damaging effect of butyrate on the intestinal barrier during inflammation. Considering the above data, the use of SCFAs in poultry production requires a detailed understanding of the protective mechanisms of action on the cells of the intestinal system, as well as the possible risks of inhibiting intestinal function. The total use of SCFAs requires further studies of their effect on the efficiency of the intestinal barrier function and the expression of molecular markers of epithelial cell intercellular adhesion. Along with determining the expression, a significant criterion for assessing the beneficial effect of SCFAs is the production of pro- and anti-inflammatory cytokines by cells of the intestinal system, fatty acid receptors, energy metabolism, and proliferation of intestinal epithelial cells.

Recently, MCFAs have been increasingly considered as an alternative to antibiotics due to their high antibacterial activity, stimulation of absorption, inhibition of lipase production by bacteria and lower dissociation ability compared to other surfactants. However, the mechanisms of MCFAs effect are still unclear. A significant feature of MCFAs is their ability to easily penetrate microbial membranes in undissociated form, which is effective against Gram-positive cocci. The study of the antimicrobial effects of MCFAs mixtures is a crucial component of creating a modern antibiotic-free strategy.

Mixtures of OAs are more effective than their individual use. Mixtures of SCFAs with MCFAs have the most significant beneficial effect since SCFAs act better as growth stimulants and MCFAs have higher antibacterial properties. At the same time, the optimal content of individual OAs in the mixture, doses, and exposure times stays a compromise issue. The use of specific molecular markers to evaluate the effectiveness of OA mixtures will allow the development of criteria for the formation of antimicrobial feed additives with unique properties and a focus that meets the challenges in poultry production. Further research into the mechanisms of action of the OAs will allow the development of their mixtures with optimised properties in terms of antimicrobial protection, intestinal functions, and metabolic stimulation. In addition, OA mixtures can be useful for the prevention of chronic diseases, such as bacterial chondronecrosis and osteomyelitis in broiler chickens.

SUMMARY

The use of antibiotics in poultry farming is critically limited, which leads to the search for and research of alternative compounds to replace antibiotics. Organic acids are considered one such alternative, but the antimicrobial and metabolic effects of fatty acid blends are still controversial and understudied.

All this necessitates a systematic analysis of the current data on effective antibiotic replacement strategies. The purpose of this study was to analyze and summarize current ideas on the use of organic acid mixtures as an alternative strategy for sustainable poultry production. An analysis of current literature showed that one of the most promising alternatives to the use of antibiotics in poultry farming is mixtures of organic acids and their derivatives, which have antibacterial effects, lower pH, are involved in energy metabolism, and all this together contributes to intestinal function. Organic acids have a positive effect on physiological functions, namely, digestion and the immune system, are the main source of energy for colonocytes, and reduce the pathogenic bacterial load on the digestive tract. Mixtures of organic acids were shown to be more effective than their individual use, specifically, mixtures of short- and medium-chain fatty acids were shown to be highly effective in supporting the intestinal barrier, microbiome, and immunity, with the former acting better as growth promoters and the latter having higher antibacterial properties. The efficiency of organic acids alone or in mixtures depends on many factors, depending on the type of molecule, form, and dose. The expediency of further studies of the effects of organic acids was substantiated, which will be useful for the development of antibiotic-free strategies using the synergistic effects of their mixtures and multidirectional cytoprotective effect. The findings of this study will be useful for scientists and veterinarians to learn about the prospects of using organic acid compositions as antibiotic alternatives, specifically for the development of technological approaches to minimize their applying.

Bibliography

1. Microbial short-chain fatty acids: A bridge between dietary fibers and poultry gut health – A review / Q. Ali et al. *Animal Bioscience*. 2022. Vol. 35, No. 10. P. 1461–1478. DOI: <https://doi.org/10.5713/ab.21.0562>.
2. Organic acid blend supplementation increases butyrate and acetate production in *Salmonella enterica* serovar Typhimurium challenged broilers / M. R. Aljumaah et al. *PLoS ONE*. 2020. Vol. 15, No. 6. Art. e0232831. DOI: <https://doi.org/10.1371/journal.pone.0232831>.
3. 16S rRNA sequencing analysis of the gut microbiota in broiler chickens prophylactically administered with antimicrobial agents / M. Cuccato et al. *Antibiotics*. 2021. Vol. 10, No. 2. Art. 146. DOI: <https://doi.org/10.3390/antibiotics10020146>.
4. A comparison study of the caecum microbial profiles, productivity and production quality of broiler chickens fed supplements based on medium chain fatty and organic acids / A. Dauksiene et al. *Animals*. 2021. Vol. 11, No. 3. Art. 610. DOI: <https://doi.org/10.3390/ani11030610>.

5. Short chain fatty acids and its producing organisms: An overlooked therapy for IBD? / S. Deleu et al. *EBioMedicine*. 2021. Vol. 66. Art. 103293. DOI: <https://doi.org/10.1016/j.ebiom.2021.103293>.
6. The combined use of triacylglycerols containing medium-chain fatty acids (MCFAs) and exogenous lipolytic enzymes as an alternative for nutritional antibiotics in piglet nutrition: I. In vitro screening of the release of MCFAs from selected fat sources by selected exogenous lipolytic enzymes under simulated pig gastric conditions and their effects on the gut flora of piglets / N. A. Dierick et al. *Livestock Production Science*. 2002. Vol. 75, No. 2. P. 129–142. DOI: [https://doi.org/10.1016/S0301-6226\(01\)00303-7](https://doi.org/10.1016/S0301-6226(01)00303-7).
7. Effect of sodium butyrate on intestinal health of poultry – a review / S. S. Elnesr et al. *Annals of Animal Science*. 2020. Vol. 20, No. 1. P. 29–41. DOI: <https://doi.org/10.2478/aoas-2019-0077>.
8. The control of poultry salmonellosis using organic agents: An updated overview / M. T. El-Saadony et al. *Poultry Science*. 2022. Vol. 101, No. 4. Art. 101716. DOI: <https://doi.org/10.1016/j.psj.2022.101716>.
9. Microbiota changes induced by microencapsulated sodium butyrate in patients with inflammatory bowel disease / S. Facchin et al. *Neurogastroenterology & Motility*. 2020. Vol. 32, No. 10. Art. e13914. DOI: <https://doi.org/10.1111/nmo.13914>.
10. Glycerol monolaurate in the diet of broiler chickens replacing conventional antimicrobials: Impact on health, performance and meat quality / B. F. Fortuoso et al. *Microbial Pathogenesis*. 2019. Vol. 129. P. 161–167. DOI: <https://doi.org/10.1016/j.micpath.2019.02.005>.
11. Growth performance and meat quality of broilers fed with microencapsulated organic acids / G. M. Galli et al. *Animal Feed Science and Technology*. 2021. Vol. 271. Art. 114706. DOI: <https://doi.org/10.1016/j.anifeedsci.2020.114706>.
12. Dietary sodium butyrate and/or vitamin D3 supplementation alters growth performance, meat quality, chemical composition, and oxidative stability in broilers / H. Gao et al. *Food Chemistry*. 2022. Vol. 390. Art. 133138. DOI: <https://doi.org/10.1016/j.foodchem.2022.133138>.
13. Gavriil A., Thanasoulia A., Skandamis P. N. Sublethal concentrations of undissociated acetic acid may not always stimulate acid resistance in *Salmonella enterica* sub. *enterica* serovar Enteritidis Phage Type 4: Implications of challenge substrate associated factors / A. Gavriil, A. Thanasoulia, P. N. Skandamis. *PLoS ONE*. 2020. Vol. 15, No. 7. Art. e0234999. DOI: <https://doi.org/10.1371/journal.pone.0234999>.
14. An investigation of the effect of water additives on broiler growth and the caecal microbiota at harvest / G. Greene et al. *Pathogens*. 2022. Vol. 11, No. 8. Art. 932. DOI: <https://doi.org/10.3390/pathogens11080932>.

15. High levels of antibiotic resistance in isolates from diseased livestock / N. A. Haulisah et al. *Frontiers in Veterinary Science*. 2021. Vol. 8. Art. 652351. DOI: <https://doi.org/10.3389/fvets.2021.652351>.
16. Intestinal mucus protects *Campylobacter jejuni* in the ceca of colonized broiler chickens against the bactericidal effects of medium-chain fatty acids / D. Hermans et al. *Poultry Science*. 2010. Vol. 89, No. 6. P. 1144–1155. DOI: <https://doi.org/10.3382/ps.2010-00717>.
17. Dietary organic cranberry pomace influences multiple blood biochemical parameters and cecal microbiota in pasture-raised broiler chickens / M. R. Islam et al. *Journal of Functional Foods*. 2020. Vol. 72. Art. 104053. DOI: <https://doi.org/10.1016/j.jff.2020.104053>.
18. Effects of dietary short-and medium-chain fatty acids on performance, carcass traits, jejunum morphology, and serum parameters of broiler chickens / A. Khatibjoo et al. *Journal of Applied Animal Research*. 2018. Vol. 46, No. 1. P. 492–498. DOI: <https://doi.org/10.1080/09712119.2017.1345741>.
19. The olfactory G protein-coupled receptor (Olf-78/OR51E2) modulates the intestinal response to colitis / K. Kotlo et al. *American Journal of Physiology-Cell Physiology*. 2020. Vol. 318, No. 3. P. 502–513. DOI: <https://doi.org/10.1152/ajpcell.00454.2019>.
20. A high-sugar diet rapidly enhances susceptibility to colitis via depletion of luminal short-chain fatty acids in mice / M. Laffin et al. *Scientific Reports*. 2019. Vol. 9. Art. 12294. DOI: <https://doi.org/10.1038/s41598-019-48749-2>.
21. Louis P, Flint H. J. Formation of propionate and butyrate by the human colonic microbiota / P. Louis, H. J. Flint. *Environmental Microbiology*. 2017. Vol. 19, No. 1. P. 29–41. DOI: <https://doi.org/10.1111/1462-2920.13589>.
22. Regulation of the effector function of CD8+ T cells by gut microbiota-derived metabolite butyrate / M. Luu et al. *Scientific Reports*. 2018. Vol. 8. Art. 14430. DOI: <https://doi.org/10.1038/s41598-018-32860-x>.
23. Makowski Z., Lipiński K., Mazur-Kuśnerek M. The effects of different forms of butyric acid on the performance of turkeys, carcass quality, incidence of footpad dermatitis and economic efficiency / Z. Makowski, K. Lipiński, M. Mazur-Kuśnerek. *Animals*. 2022. Vol. 12, No. 11. Art. 1458. DOI: <https://doi.org/10.3390/ani12111458>.
24. Effect of chestnut tannins and short chain fatty acids as anti-microbials and as feeding supplements in broilers rearing and meat quality / F. Mannelli et al. *Animals*. 2019. Vol. 9, No. 9. Art. 659. DOI: <https://doi.org/10.3390/ani9090659>.
25. In vitro investigation of the antibacterial activity of nine commercial water disinfectants, acidifiers, and glyceride blends against the most important poultry zoonotic bacteria / T. Mantzios et al. *Pathogens*. 2023. Vol. 12, No. 3. Art. 381. DOI: <https://doi.org/10.3390/pathogens12030381>.

26. Fibronectin measurement as a potential molecular marker for barrier function assessment of piglet intestine / D. M. Masiuk et al. *Theoretical and Applied Veterinary Medicine*. 2023. Vol. 11, No. 2. P. 3–8. DOI: <https://doi.org/10.32819/2023.11006>.

27. Abnormalities in microbiota/butyrate/FFAR3 signaling in aging gut impair brain function / S. P. Mishra et al. *JCI Insight*. 2024. Vol. 9, No. 3. Art. e168443. DOI: <https://doi.org/10.1172/jci.insight.168443>.

28. Evaluation of the blend of organic acids and medium-chain fatty acids in matrix coating as antibiotic growth promoter alternative on growth performance, nutrient digestibility, blood profiles, excreta microflora, and carcass quality in broilers / D. H. Nguyen et al. *Poultry Science*. 2018. Vol. 97, No. 12. P. 4351–4358. DOI: <https://doi.org/10.3382/ps/pey339>.

29. Oliveira L. M., Teixeira F. M. E., Sato M. N. Impact of retinoic acid on immune cells and inflammatory diseases. *Mediators of Inflammation*. 2018. Vol. 2018. Art. 3067126. DOI: <https://doi.org/10.1155/2018/3067126>.

30. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases / D. Parada Venegas et al. *Frontiers in Immunology*. 2019. Vol. 10. Art. 277. DOI: <https://doi.org/10.3389/fimmu.2019.00277>.

31. Peh E., Kittler S., Reich F., Kehrenberg C. Antimicrobial activity of organic acids against *Campylobacter* spp. and development of combinations – A synergistic effect? / E. Peh et al. *PLoS ONE*. 2020. Vol. 15, No. 9. Art. e0239312. DOI: <https://doi.org/10.1371/journal.pone.0239312>.

32. Study of microencapsulated fatty acid antimicrobial activity in vitro and its prevention ability of *Clostridium perfringens* induced necrotic enteritis in broiler chicken / N. Qi et al. *Gut Pathogens*. 2023. Vol. 15. Art. 1. DOI: <https://doi.org/10.1186/s13099-022-00526-9>.

33. Immunomodulatory potential of gut microbiome-derived short-chain fatty acids (SCFAs) / W. Ratajczak et al. *Acta Biochimica Polonica*. 2019. Vol. 66, No. 1. P. 1–12. DOI: https://doi.org/10.18388/abp.2018_2648.

34. Ricke S. C., Dittoe D. K., Richardson K. E. Formic acid as an antimicrobial for poultry production: A review. *Frontiers in Veterinary Science*. 2020. Vol. 7. Art. 533419. DOI: <https://doi.org/10.3389/fvets.2020.00563>.

35. Roopashree P. G., Shetty S. S., Suchetha Kumari N. Effect of medium chain fatty acid in human health and disease / P. G. Roopashree, S. S. Shetty, N. Suchetha Kumari. *Journal of Functional Foods*. 2021. Vol. 87. Art. 104724. DOI: <https://doi.org/10.1016/j.jff.2021.104724>.

36. Impact of dietary supplementation with sodium butyrate protected by medium-chain fatty acid salts on gut health of broiler chickens / M. Sadurní

et al. *Animals*. 2022. Vol. 12, No. 19. Art. 2496. DOI: <https://doi.org/10.3390/ani12192496>.

37. Salah A. S., Ahmed-Farid O. A., El-Tarabany M. S. Carcass yields, muscle amino acid and fatty acid profiles, and antioxidant indices of broilers supplemented with synbiotic and/or organic acids / A. S. Salah, O. A. Ahmed-Farid, M. S. El-Tarabany. *Journal of Animal Physiology and Animal Nutrition*. 2019. Vol. 103, No. 1. P. 41–52. DOI: <https://doi.org/10.1111/jpn.12994>.

38. Synergistic effects of essential oils and organic acids against *Aspergillus flavus* contamination in poultry feed / T. Satterlee et al. *Toxins*. 2023. Vol. 15, No. 11. Art. 635. DOI: <https://doi.org/10.3390/toxins15110635>.

39. Polyphenols and organic acids as alternatives to antimicrobials in poultry rearing: A Review / F. Scicutella et al. *Antibiotics*. 2021. Vol. 10, No. 8. Art. 1010. DOI: <https://doi.org/10.3390/antibiotics10081010>.

40. Effects of short- and medium-chain fatty acids on production, meat quality, and microbial attributes – A Review / R. T. Szabó et al. *Molecules*. 2023. Vol. 28, No. 13. Art. 4956. DOI: <https://doi.org/10.3390/molecules28134956>.

41. The European Union One Health 2018 Zoonoses Report. *EFSA Journal*. 2019. Vol. 17, No. 12. Art. e05926. DOI: <https://doi.org/10.2903/j.efsa.2019.5926>.

42. Effect of supplementation of salts of organic acids on serum and haematological parameters of broilers / V. Us et al. *International Journal of Current Microbiology and Applied Sciences*. 2017. Vol. 6, No. 11. P. 4211–4218. DOI: <https://doi.org/10.20546/ijcmas.2017.611.493>.

43. Butyrate does not protect against inflammation-induced loss of epithelial barrier function and cytokine production in primary cell monolayers from patients with ulcerative colitis / M. Vancamelbeke et al. *Journal of Crohn's & Colitis*. 2019. Vol. 13, No. 10. P. 1351–1361. DOI: <https://doi.org/10.1093/ecco-jcc/jjz064>.

44. Dietary sodium butyrate improves intestinal development and function by modulating the microbial community in broilers / W. Wu et al. *PLoS ONE*. 2018. Vol. 13, No. 5. Art. e0197762. DOI: <https://doi.org/10.1371/journal.pone.0197762>.

Information about the authors:

Masiuk Dmytro,

Doctor of Veterinary Sciences, Professor,

Head of the Department of Physiology, Animal Biochemistry
and Laboratory Diagnostics

Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

Nedzvetsky Victor,

Doctor of Biological Sciences, Professor,
Vice-director of Scientific Research Center of Biosafety and Environmental
Control Agro-Industrial Complex
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine

Kokariiev Andrii,

Candidate of Veterinary Sciences, Associate Professor,
Head of the Department of Immunochemical and Molecular Genetic
Analysis of Scientific Research Center of Biosafety and Environmental
Control Agro-Industrial Complex
Dnipro State Agrarian and Economic University
25, Serhiya Yefremova Street, Dnipro, Ukraine