Review

Effects of short and medium-chain fatty acids on production, meat quality and gut hygiene of broiler chickens – A review

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Abstract: The non-therapeutic use of antimicrobials in poultry production contribute to the spread of drug-resistant pathogens in both birds and humans. Antibiotics are known to enhance feed efficiency and promote growth and weight gain of poultry. New regulatory requirements and consumer preferences have led to a reduced use of antibiotics in poultry production and discover natural alternatives to the antibiotic growth promoters. This interest is not just focused on the direct removal or inhibition of the causative microorganisms but also the prevention diseases caused by enteric pathogens using a range of feed additives. A group of promising feed additives is short- and medium-chain fatty acids and their derivatives. MCFAs possess antibacterial, anticoccidial and antiviral effects. Also, it has been proven that these acids act synergistically if they are used together with organic acids, essential oils, or probiotics. These fatty acids also benefit intestinal health integrity and homeostasis in broilers. Other effects have also been documented, including increases in intestinal angiogenesis and gene expression of tight junctions. The aim of this review is to give an overview of SCFA, MCFA as alternatives of antibiotic growth promoters and by summarizing the current finding in the literature, to show their possible benefits on production, meat quality and gut health in poultry.

Keywords: SCFA, MCFA, broilers, poultry, gut health, antimicrobial effect

1. Introduction

Organic acids as feed additives are common subjects of research in poultry nutrition due to food safety aspects, particularly on lowering the incidence of foodborne pathogens in poultry. Research most often pay attention to particular lipid groups (saturated, unsaturated, polyunsaturated fats or fats grouped by the length of their fatty acid chains into short, medium or long chain fatty acids). It has been proven that short and medium chain fatty acids (SCFA and MCFA, respectively) have positive effects on health, production, feed digestibility and lower body and muscle fats in broilers [1].

Non-therapeutic use of antibiotics increases the rate of weight gain and/or the efficiency of feed utilization in food animals [2]. Since the 1980s antibiotic growth promoters (AGPs) have been utilized widely in poultry diets to improve performance and feed conversion [3]. They have also been utilized to protect animals from the adverse effects of enteric microorganisms [4] as well as to modulate inflammation [5].

The trend of banning the use of AGPs in poultry nutrition has developed from the early 2000s' because consumers are becoming increasingly concerned about the nutritional and health aspects of their food, as these compounds contribute to the spread of antibiotic resistance in microbes, as well as to the presence of antibiotic residues in poultry products [6, 7, 8]. Removing antibiotics from compound feeds, after they were banned by the European Union in 2006, has put great pressure on poultry producers to find alternatives to antibiotics to improve gut health and growth performance [9, 10, 11, 12]. As alternatives for AGPs, MCFAs are widely used as feed additives for broiler diets as natural sources to inhibit bacterial growth as well as enteric pathogen colonization [13, 14]. There are several hypotheses presented in the literature on the antimicrobial activity of these fatty acids. One is based on the basic principle that undissociated organic acids (non-ionized, more lipophilic) can penetrate the bacterial cell wall triggering an ionization of fatty acids, which results in disruption of the normal physiology of certain types of bacteria [15, 16, 17] including an increase in fluidity, solubility, permeability, and instability of the bacterial membrane [18, 19]. There is another theory as well, which roots in modifying gene expression in pathogens as MCFAs are proven to be involved in the down-regulation of certain genes (i.e., hil A, hil D etc.) responsible for virulence [20].

MCFAs are obtained from edible fats such as coconut oil and milk fat by lipid fraction separation [21]. They are also present in palm oil (8%) and cuphea oil [22, 23, 24, 25]. This group of fatty acids comprises of caproic (C6), caprylic (C8), capric (C10) and lauric (C12) acids [26]. SCFAs are saturated aliphatic organic acids that consist of one to six carbon atoms. The most abundant are acetate (C2), propionate (C3) and butyrate (C4) and these acids are produced in the gastrointestinal tract of humans and animals [27, 28]. They have different important roles in the development of intestinal epithelial cells, they modulate some processes in the gastrointestinal tract such as electrolyte and water absorption or regulate several leukocyte functions including production of cytokines [29]. SCFA are known for their antimicrobial characteristics due to their ability to cross bacterial membranes in their undissociated form. There they dissociate which results in anion concentration and bactericidal effect [30].

In this review, current findings and prospects for further research are summarized on the poultry microbiome and feeds supplemented with SCFA and MCFA.

2. Natural sources of SCFA and MCFA

The fatty acids most commonly used in diet supplementation for poultry to control microorganisms are formic acid, benzoic acid, citric acid, carboxylic acids (all SCFA) and their salts as well as some MCFA including Caproic (C6:0), Caprylic (C8:0), Capric (C10:0) and Lauric acid (C12:0). Coconut oil is a highly saturated oil (about 90%), and 60% of its total fatty acid composition are MCFA with a chain length of 6 to 12 carbon atoms [22], which are absorbed directly into the portal circulation without re-esterification in intestinal cells [31]. The MCFA are partly independent of the carnitine transport mechanism into the mitochondria of the liver and are rapidly and exclusively oxidized for the production of energy [32]. Medium chain fatty acids (MCFA) are naturally found in many plants oil (palm, red palm, palm kernel, babassu, coconut, murumuru) or dairy products [33, 35, 35]. Palm and red palm oils contains palmitic (42.9%, 41.96%) and oleic (40.28%, 40.6%) fatty acids in the largest proportion. Lauric and oleic acids are the top components of coconut (41.31%, 11.72%) and babassu (43.98%, 13.62%) oils [33]. However, oil composition of the same lipid sources might show differences as the extraction method might alter the amount of different fatty acids [34]. MCFAs and myristic acid (C14) can influence the immune function, so it plays an important role in stimulating cell-mediated immunity. The hydrophilic and hydrophobic characteristics of MCFAs have a key role in its antimicrobial activity, it can create the lysis of the cell [35, 36]. MCFAs have also an indirect role in the availability of nutrients for the birds [37].

SCFAs are formed from polysaccharide, oligosaccharide, protein, peptide and glycoprotein precursors by anaerobic micro-organisms. Carbohydrates are the most important SCFA progenitors [38]. SCFAs are carboxylic acids and have the presence of an aliphatic tail of two to six carbons. SCFAs can be produced naturally through host metabolic pathways (fermented by symbiotic microbiota) and by SCFAs-producing probiotics [39, 40, 41]. The beneficial microbiota of broiler produces SCFAs as acetic, propionic or butyric acids [41]. SCFAs are potential mediators of microbiota-gut-brain axis, thus they might have a role in neurological processes [42, 40]. Furthermore, they have anti-inflammatory, antitumorigenic, and antimicrobial effects [39]. In broiler feed, the most frequently studied SCFAs are the acetate, propionate and butyrate with two, three and four carbon molecules in their chemical structure [41]. These three fatty acids accounted for more than 95% of the total SCFA group [43].

New products have been developed through the formation of calcium and/or sodium salt with the fatty acids or esterification of these acids prior to addition to feed. Esterification has an important advantage as the esterified SCFA and MCFA escape gastric digestion thus reaching the small intestine where they can exert their effect [44, 45]. When these acids, in salt or esterified form are fed to animals, positive effects on growth performance, intestinal microbial growth and health status of the animals are seen [46]. The potential effect of SCFAs and MCFAs without any protection would be limited because of prompt absorption and metabolism or both in the gastric area of the intestinal tract.

3. Production parameters

Rapid growth and high meat production efficiency are essential for successful broiler chicken production. Due to genetic development the present days' hybrids have extremely high growth potential, which must be realised to meet the increasing demand for chicken meat worldwide. Improving production parameters is an important objective for producers. Several results have been reported the effect of coconut and/or palm oils on the production parameters of broilers. The studies focus on growth performance, body weight, weight gain, feed efficiency, mortality, feed intake, feed conversion, relative liver, heart and breast weight.

The slaughter weight of broilers is commonly 2-2.5 kg, modern meat-producing broilers reach this market ready weight within 35-45 days [47]. The Cobb 500 hybrid attains a slaughter weight of 2.5 kg at 37-38 days of age with a feed conversion of 1.5 kg/kg [48]. The study of Wang et al. [24] indicated that feeding MCFAs in the form of coconut oil do not improve growth performance but reduces fat deposition and favorably affect lipid profiles without impairing performance in broilers.

Coconut diet (1.5% coconut oil) significantly increased the body weight gain of Cobb chickens in the 1-21-day period (590 g) compared to other oil diets (fish, canola and mixed fish + canola + coconut); however, this benefit disappeared by 42 days (2038 g) [49]. Similar result was found by Khosravinia [50] with 2 g/kg MCFA in the same period of life in daily weight gain. 0.25% lauric acid and 0.25% capric acid alone or in combination represented significantly improved live body weight and cumulative weekly gain [12]. Zimborán et al. [51] observed no differences between control and supplemented groups (5% coconut oil, 5% palm oil, 2.5% coconut + 2.5% palm oil) in terms of body weight from day 1 to day 28 of age. On day 42 live body weight was 2007.3 g in the coconut group, while it was 2013.1 g in the control. Several authors reported that palm oil supplementation does not affect body weight and weight gain [52, 53, 54]. However, the study of Rahman et al. [55] revealed negative effect of adding 4% palm oil in the diet on the growth parameters from the second and fourth week of the experiment. Zimborán et al. [51] described 2005.9 g body weight on day 42 with 5% palm oil in feed, the mix group (2.5% coconut and 2.5% palm oil supplementation) gained not significantly lower (1952.34 g) body weight. This result is in agreement with the data of Khatun et al. [56], where Cobb-500 hybrids achieved the body weight of 2126.8 g was by day 42 with 6% palm oil supplementation. 5% palm oil supplementation generated the same slaughter weight of Hubbard Classic broiler than of control group on day 42 [55]. Khatun et al. [56] measured a gain of 1454.8 g with 6% palm oil supplementation between days 21 and 42, while 5% palm oil in feed resulted 1430.4 g body weight gain [51]. In the similar period, from day 21 to day 42, Attia et al. [49] obtained 2038 g gain with 1.5% coconut oil to the diet. This result exceeded the weight gain of 1446.3 g measured with 5% coconut oil supplementation [51]. The 2.5-2.5% mixed coconut and palm mixed oil group showed the lowest body weight gain (1369.5 g) during the same growing period compared to pure coconut or palm oil addition [51]. Comparing the effect of short and medium fatty acids, no significant differences were found in the body weight and weight gain parameters by [28] using pure MCFA- and SCFA supplements alone and in combination.

Some studies reported beneficial effects of oil supplements on the feed efficiency of broiler chickens [57, 58], but there are controversial results too [59]. Khosravinia [50] represented reduced

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mortality of broilers by the high dose (2 g/kg) of MCFAs. Furthermore, MCFAs were also beneficial even in the case of high flock density (16, 18 birds/m^2), resulted low frequency of foot morbidities.

Wang et al. [24] and Attia et al. [49] measured the feed intake and feed conversion between 21 and 42 days of life by Arbor Acres and Cobb hybrids. The coconut oil group (1.5% supplementation) had lower total daily feed intake result [24], than Attia et al. [49] with the same supplementation, however, these values were more or less the same as the control values in the two experiments (Table 1.). Similarly, Khosravinia [50] did not found significant differences between control and experimental groups (1.5 and 2 g/kg MCFAs) in feed intake. Rebolé et al. [60] found the same non-significant effect of palm oil (90 g/kg diet) supplementation. Birds with 5% coconut oil supplementation consumed a total of 2510 g of feed, 119.5 g per day between day 21 and 42 [51]. Feed intake of Hubbard Classic [55] and Cobb 500 hybrids [56] supplemented with 5% and 6% palm oil, respectively, did not differ from the control. Birds supplemented with 5% palm oil, the mixed group (2.5-2.5% coconut and palm oil) and the control group consumed similar amounts of feed [51]. Feed conversion was not affected by MFCA supplementation, while body weight gain was 49 g higher in broilers fed with supplemented feed [59].

Table 1. Feed intake and feed conversion changes by fatty acid supplementation

Supplementation	Feed intake (g)[1]	Work of
1.5% coconut oil	2914.8^T	[24]
1.5% coconut oil	3064T	[49]
1.5 and 2 g/kg MCFA	82.06; 83.09^D	[50]
5% coconut oil	2510^T	[51]
2.5+2.5% coconut and palm oil	2760^T	[51]
5% palm oil	2860^T	[51]
5% palm oil	3064^T	[55]
6% palm oil	2530.7^T	[56]
Supplementation	**Feed conversion (g/g)**	**Work of**
1.5% coconut oil	1.82	[24]
1.5% coconut oil	1.52	[49]
2.5+2.5% coconut and palm oil	1.79	[51]
6% palm oil	2.05	[56]
1.5 and 2 g/kg MCFA	1,9; 1.79	[50]

[1] T: total feed intake, D: daily feed intake

Considering feed conversion results, Wang et al. [24] and Attia et al. [49] showed more pronounced differences among groups supplemented with variable oil sources compared to the feed intake data. Wang et al. [24] observed higher feed efficiency than Attia et al. [49] with the same amount of coconut oil supplementation (Table 1.). The results of Zimborán et al. [51] are consistent with those of Wang et al. [24], with coconut and palm oil supplementation. Khatun et al. [56] observed that palm oil supplementation decreased feed conversion lower than 2.05 g/g [51]. 1.5 g/kg MCFAs supplementation generated higher feed conversion ratio than 2 g/kg MCFAs [50], while the cumulative weekly feed consumption and the cumulative feed conversion ratio were improved in broilers fed with high MCFA diet [12].

The relative liver weight was 2.31% in the 1.5% supplemented coconut oil group of Attia et al. [49] compared to the results (1.5%) found by Zimborán et al. [51]. In the case of relative heart weight, the tendency was similar. Attia et al. [49] described 0.55% relative heart weight in the coconut oil group, while Zimborán et al. [51] observed lower values (0.38%) in the coconut and coconut + palm oils supplemented groups, respectively. The relative liver weight was not affected by the palm oil (90 g/kg diet) treatment in the work of Rebolé et al. [60]. Khatibjoo et al. [28] also failed to find differences in spleen, bursa and thymus weight due to the supplementation with MCFAs, SCFAs and the combination of those. MCFA or SCFA supplementation affected neither the breast meat, the thigh meat yield and carcass weight, nor the protein and lipid percentage of breast and thigh [28]. European

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performance efficiency factor (EPEF) was better in the case of 0.25% of lauric acid supplementation (324.6) and in its combination with capric acid, respectively (323.8) [12]. EPEF was also improved by alpha-monolaurin (0.25 g/kg) [61] and 2% MCFA supplementation [50] in broilers, respectively.

The parallel use of MCFA and coccidiostats positively influenced production results in broilers (weight at the end of the production cycle, feed intake, growth and conversion rates) [62]. Fat, protein and crude fiber digestibility was improved if MCFA was used with selected organic acids (propionic, fumaric) [63]. Zeits et al. [64] used a different ratio of C12 and C14 in broiler nutrition and did not find any differences in microbiota, intestine morphology, or fat or cholesterol content in meat or liver, but adding MCFA had a positive effect on feed conversion and pectoral weight of broilers.

4. Meat quality parameters

Maintaining high meat quality is essential for quality food and its longer shelf life. For meat quality testing, it is necessary to measure parameters such as pH, drip loss, colour, kitchen losses (cooling and cooking) and tenderness [65, 66, 67, 68].

In a recent experiment, 5% coconut oil and mixed oil supplementation (2.5-2.5% coconut + palm oil) resulted the same pH 24 hours after slaughter at 42 days of age, while it lower when adding 5% palm oil in the diet [51] (Table 2). These results are in line with those of Souza et al. [69] and Khatun et al. [56] and correspond to the normal pH of broiler meat [70]. According to Jiang et al. [71] pH24, varied between 6.01 and 6.06, which is consistent with the average final pH of broiler breast meat (6.03) [72]. 2% coconut oil in feed resulted in breast [73] not similar data with the results of Zhang et al. [74] and Gao et al. [75] with sodium butyrate. Ogunwole et al. [76] measured the effect of 2% coconut oil and 2% palm oil in feed separately. The coconut group had higher pH value than the palm oil group at hour 24. According to these data palm oil might result in lower pH than coconut oil, which hypothetically might be related to the different fatty acid composition of the two oils and the consequent difference in the fatty acid composition of the meat itself.

Table 2. Changes of pH after 24 hours by different fatty acid supplementation

Supplementation	pH 24h	Work of
5% coconut oil	5.98	[51]
2.5+2.5% coconut and palm oil	5.98	[51]
5% palm oil	5.89	[51]
6% palm oil	5.80	[56]
4%+2% palm and sunflower oil	5.79	[56]
2%+4% palm and sunflower oil	5.78	[56]
2% coconut oil	6.25	[73]
0.4 g/kg sodium butyrate	5.88	[74]
1 g/kg sodium butyrate	5.50	[75]
2% coconut oil	5.91	[76]
2% palm oil	5.35	[76]

According to literature data different types of fat sources often cause changes in the meat colour as well. Abdullah et al. [77] found that the L* value for broilers slaughtered on day 42 was 53.35. Prayitno et al. [73] did not represent differences in colour in groups with different percentages (0-2%) of coconut oil. The other colour parameters were not affected by the supplementation. In the study of Ogunwole et al. [76] the L*, the a* and the b* were measured to detect the colour of the meat when 2% coconut oil supplementation was present in the diet. L* value and b* were higher, the other parameter was lower in the group fed with same amount palm oil supplementation. The higher (6%) amount of palm oil in feed resulted colour changes, represented in Table 3 [56]. A 5% coconut oil or 5% palm oil supplementation resulted in similar colour results. The same trend was observed in the mixed group (2.5-2.5% coconut and palm oil supplement) [51]. Sn1-monoglycerides as a source of saturated short medium chain fatty acids (SMCFA) represented significantly increased L* and b* values compare to the control group. Based on the ΔE results (ΔE > 5) referring to the visibility of colour differences [78], Sn1 monoglycerides strongly modified meat coloration [66].

Table 3. Changes of color parameters by different fatty acid supplementation

Supplementation	L*	a*	b*	Work of
5% coconut oil	63.04	11.18	9.38	[51]
2.5+2.5% coconut and palm oil	62.87	10.98	9.39	[51]
5% palm oil	63.58	11.58	8.80	[51]
6% palm oil	50.31	3.93	14.21	[56]
4%+2% palm and sunflower oil	49.36	3.94	14.22	[56]
2%+4% palm and sunflower oil	48.20	4.71	14.24	[56]
3.0 g/kg Sn1-monoglycerides	63.80	5.60	8.99	[78]
2% coconut oil	45.35	8.31	5.45	[76]
2% palm oil	52.31	6.02	5.45	[76]

Literature data on water holding capacity/drip loss of meat are highly variable. In the study of Prayitno et al. [73] with different amount coconut oil supplementation, the water holding capacity of broiler meat was diverse (Table 4). A similar finding was obtained where the water holding capacity of broiler chickens fed with 2% coconut oil was higher than that of 2% palm oil [76]. Zimborán et al. [51] observed similar drip loss data in 5% coconut and 5% palm oil groups. 2.5-2.5% coconut and palm oil in the feed resulted higher drip loss values. However, Khatun et al. [56] found that drip loss was only 3.06% in the group of 6% palm oil supplementation. The variability of these results is at least partially due to the differences in the analytical methods. However, irrespectively of the different methods it seems, that the different types of oils do not affect the water holding capacity of meat [79].

Measuring kitchen losses is an important part of meat quality assessment and it comprises for loss in the mass of meat due to freezing and thawing as well as during cooking and consequent cooling. Similarly to the drip loss, it is important not only for the costumers, but also for the processing companies. Most commonly cooking loss is monitored in the research and this parameter shows high variability according to the literature data. Thus Soeparno [80] found that cooking losses of broiler chickens at 6 and 7 weeks of age were 24.89% and 34.57%, respectively, and high individual variance Somewhat lower values were reported by Prayitno et al. [73] about the cooking loss without added oil in the diet, while it was lower than 2% coconut oil supplementation (Table 4). It was also revealed that 2% palm oil inclusion in the diet caused higher cooking loss than the same rate (2%) of coconut oil [76]. However, when palm oil was added in higher inclusion rate (6%) lower cooking loss has occurred compared to the 2% inclusion rate value [56]. In another experiment the overall kitchen losses were presented for different oil supplementation treatments. The 5% coconut oil inclusion in the diet resulted higher kitchen loss than 5% palm oil in the feed, while mixed 2.5-2.5% coconut and palm oil supplementation group has shown lowest value [51]. Based on these results, coconut oil supplementation has negative effect on kitchen losses compared to palm oil.

Table 4. Drip and cooking loss values from different fatty acid supplementation experiments.

Supplementation	Tenderness	Work of
5% coconut oil	10.33	[51]
2.5+2.5% coconut and palm oil	11.15	[51]
5% palm oil	10.44	[51]
6% palm oil	3.06	[56]

4%+2% palm and sunflower oil	3.08	[56]
2%+4% palm and sunflower oil	3.09	[56]
0.5 % coconut oil	35.84	[73]
1 % coconut oil	35.85	[73]
1.5 % coconut oil	40.99	[73]
2% coconut oil	42.21	[73]
2% coconut oil	10.21	[76]
2% palm oil	39.87	[76]
Supplementation	**Cooking loss %**	**Work of**
0.5 % coconut oil	25.32	[73]
1 % coconut oil	25.54	[73]
1.5 % coconut oil	19.14	[73]
2% coconut oil	18.87	[73]
2% coconut oil	17.98	[76]
2% palm oil	29.32	[76]
6% palm oil	24.83	[56]
4%+2% palm and sunflower oil	24.85	[56]
2%+4% palm and sunflower oil	24.86	[56]
5% coconut oil	19.32	[51]
2.5+2.5% coconut and palm oil	15.47	[51]
5% palm oil	18.74	[51]

Tenderness shows how though the meat is. It can be characterised with shear force and in chicken the normal values vary around 1.5-2.0 kg/cm2. Whether the diet was based on corn, or milo or wheat, the tenderness of the breast meat ranged between 1.82 kg/cm^2 and 2.19 kg/cm^2 [81]. However, in several scientific work focused on oil supplementation, much higher shear force data were reported. Like the values measured by Prayitno et al. [73] which were the lowest for 2% coconut oil supplementation and the highest for 0.5% coconut oil (Table 5). Khatun et al. [56] recorded lower shear force in Cobb 500 broiler chicken breast meat when 6% palm oil was added in the diet. However, 2% palm oil or 2% coconut oil supplementation resulted in an increased value in this parameter, which means the meat became firmer due to additional oil [76]. The results of Zimborán et al. [51] are closest to those of Lyon et al. [81]. In this research shear force value of the group fed 5% coconut oil or palm oil was lower than 2 kg/cm^2, while it was higher in the samples of mixed 2.5-2.5% coconut and palm oil treatment.

Table 5. Tenderness results from different fatty acid supplementation experiments.

Supplementation	**Tenderness kg/cm^2**	**Work of**
5% coconut oil	1.93	[51]
2.5+2.5% coconut and palm oil	2.10	[51]
5% palm oil	1.80	[51]
6% palm oil	1.20	[56]
4%+2% palm and sunflower oil	1.19	[56]
2%+4% palm and sunflower oil	1.19	[56]
0.5 % coconut oil	5.56	[73]
1 % coconut oil	5.28	[73]

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1.5 % coconut oil	4.34	[73]
2% coconut oil	3.38	[73]
2% coconut oil	6.63	[76]
2% palm oil	5.67	[76]

Based on the literature, short-chain fatty acids have a positive effect on meat quality parameters, which ensure the enjoyment value of meat and provide the basis for quality production. The performance of organoleptic tests may in the future provide greater insight into consumer feedback.

5. Molecular and biochemical parameters

Short and long chain fatty acids might cause changes in several biochemical processes and parameters in poultry. They are thought to be involved in energy metabolism signalling. Lipid parameters are highly variable in poultry, therefore absolute values available in the literature from different research groups cannot be easily compared. However, Khatibjoo et al. [28] showed that MCFAs, SCFAs and the combination of these can decrease serum cholesterol concentration of broilers. Similar results were found by Khatun et al. [56], when the effects of 6% pure palm oil supplementation was compared with mixed oil supplementation. The mechanism in the background is not totally clear, but SCFAs for instance are able to reduce the activity of certain enzymes, like 3-hydroxy-3-methylgltaryl-CoA synthase and 3-hydorxy-3-methylglutaryl-CoA reductase. As these are key enzymes for cholesterol synthesis in the liver, this effect is likely to lower the blood cholesterol concentration.

Khatun et al. [56] used also 6% palm oil supplementation, 0.68 mmol/L of blood plasma TG (triglycerides) and 3.26 mmol/L cholesterol were detected. Lower amount of palm oil (4% palm oil + 2% sunflower oil, 2% palm oil + 4% sunflower oil) resulted decrease of TG (0.51, 0.55 mmol/L) and cholesterol (2.67, 2.64 mmol/L). However, in the study by Attia et al. [49], 1.5% coconut oil supplementation did not affect the plasma lipid profile of 42-day-old broiler chickens. Same results were published by Wang et al. [24] with 1.5% coconut oil supplementation. In the experiment of Zimborán et al. [51] significant changes were found in plasma TG and cholesterol concentration due to 5% coconut, or palm or mixed (coconut+palm) oil inclusion in the diet with the exeption of TG concentration in the coconut oil treated group. This latter value was significantly higher than the control, which is contradictory to the other research finding.

As fatty acids are good electron donors in the mitochondrial electron transfer, reactive oxygen species might be released due to their ◎ -oxidation. Excessive accumulation of MCFAs might result in increased ROS production and consequently leads to lipid peroxidation. In certain conditions these fatty acids might induce apoptosis and parallel the modification of glutathione level and generate reactive oxygen species. Protective activity of certain MCFAs against oxidative stress. When 2, 4 and 6% palm oil were added to the feed in the work of Long et al. [82], the blood plasma malondialdehyde (MDA) concentration, an oxidative stress indicator was 7.93 nmol/ml, 6.44 nmol/ml and 6.44 nmol/ml respectively on the day 42, while the activity of an antioxidant enzyme, the glutathione peroxidase (GSHPx) in the blood plasma was 765.3 U/ml, 789.3 U/ml, 880.5 U/ml. In another experiment with 5% coconut oil, or 5% palm oil or their combination also some reduction was found in the MDA concentration, while the differences in the GSHPx activities were not consistent. Similar results were reported by Attia et al. [49] with 1.5% coconut oil supplementation, where the MDA concentration was markedly reduced. So, in general the higher the SCFA and MCFA in the diet, the better the oxidative status in the different tissues.

Sodium butyrate could lower the MDA level while increases the serum superoxide dismutase enzyme activity in chickens in normal conditions [83]. 1 g sodium butyrate/ kg (which is at the highest end of regular inclusion rate range) diet did not influence the MDA amount [75] and did not cause any change in the oxidative status of chicken meat [78]. However, in the work of Saleh et al. [61] with a potential MCFA additive, alpha-monolaurin (0.25 and 1g/kg diet suppl.) the MDA levels were significantly reduced in birds.

Altogether, there is no clear conclusion for antioxidant defence parameters regarding supplementation of short and long chain fatty acids. However, as the use of fatty acids does not have any proven negative effect on lipid peroxidation processes, their administration is preferable due to their other beneficial effects.

6. Gut health of chicken

Enteric diseases are major concerns in the poultry industry due to production losses, increased mortality, reduced bird welfare and increased risk of contamination of poultry food products. A reduction of pathogenic microorganisms in the digestive tract decreases the contamination of poultry products Salmonella and Campylobacter are considered major public health burdens worldwide, and epidemiological studies demonstrate that poultry are known to be one of the main reservoirs for these zoonotic pathogens, therefore, poultry products are frequent sources of these pathogenic agents.

According to literature data, MCFA inhibit bacterial toxin production and expression of other virulence factors by interfering with signal transduction [84, 85]. The effect of these acids is both bactericidal (killing) and bacteriostatic (growth-inhibiting) depending on the concentration, synergism among them, and target bacterial strain [44,86].

The details of the mechanism of the antimicrobial effects of SCFA and MCFA or the synergistic activities between these acids are not perfectly revealed [1, 87, 88]. It is assumed that due to their lipophilic character, in undissociated form they can penetrate the microbial cell wall and can deteriorate the cell membrane causing increased permeability and cell leakage, causing the death of bacteria [17]. It is also assumed the within the cell these acids might reduce the pH which can alter the activity of the cytosolic enzymes [1].

Mathis et al. [89] proved that the combination of organic acids and MCFA significantly reduced clinical symptoms of diseases in artificial necrotic enteritis of broilers. They showed in broilers infected with viral malabsorption syndrome (MAS) that adding SCFA and MCFA together in feed increased broiler growth and resulted higher broiler weight at the end of the production cycle. How this directly impacts viruses is not known, but it is considered that SCFA and MCFA together have a synergistic effect on bacteria, whereby MCFA damages microorganisms' cell walls, thus allowing SCFA access into the bacterial cytoplasm to produce an antibacterial effect. Butyrate appears to bactericidal and can stimulate villi growth

Salmonellosis

Salmonella typhimurium and *enteritidis* are often detected in poultry and foods, being associated with several human foodborne outbreaks [90]. Formic acid has strong antimicrobial effects in poultry as reviewed by Ricke et al. [27]. Feeding formic acid (4 kg/ton) for 6 weeks resulted in no recovery of *Salmonella* from ceca in broilers compared to the control prevalence of 17% (Bourassa et al.., 2018). Deschepper et al. [91] showed that MCFA at 0.8 and 1.2 g/kg feed reduced the invasion of Salmonella enteritidis in the intestine of broilers. Van Immerseel et al. [92] found that MCFA reduces the colonization and invasion of Salmonella enterica shortly after infection in chickens, and similar result together with increased transcription of antimicrobial peptide was revealed. Evans et al. [93] reported that addition of MCFA in turkey poults diet could lower the colonization of Salmonella in early period of their life.

Campylobacteriosis

Broiler chicken is a natural host for Campylobacter spp., and contaminated poultry meat products are major sources for transmitting pathogenic Campylobacter strains to humans [94, 95]. MCFA shows marked anti-Campylobacter activity in vitro. In vivo studies indicated that the supplementation of MFCAs may be a promising tool either for the prevention or reduction of Campylobacter jejuni colonization in commercial broiler flocks [60]. Solis de los Santos et al. [96] evaluated the effect of caprylic acid on the cecal Campylobacter jejuni colonization at experimental infection in broilers and the dose of 0.35% in a regular starter diet was more efficient compared with the spectrum of other five higher supplementations up to 1.4%.

Molatová et al. [97] examined the effects of supplementation of feed with a coated or non-coated mixture of fatty acids (caprylic and capric acid) in broiler chickens experimentally infected with Campylobacter jejuni. Their results indicated that MCFA, and especially their coated form, may help to reduce the level of colonization of chicken intestine by C. jejuni. In the study of van Gerwe et al. [59] supplementing the feed with a mixture of MCFAs (C8–C12) decreased the susceptibility of broilers for Campylobacter colonization.

Drinking water is a prominent source of horizontal Campylobacter transfer [98]. Results on the application of MCFA in drinking water are contradictory. Metcalf et al. [99] reported that treatment with caprylic acid in water had an inconsistent effect on the intestinal Campylobacter counts, whereas drinking water application of MCFA was effective in combating Campylobacter colonization in poultry [100]. Although Campylobacter colonization and transmission was not reduced by adding an emulsion of a mixture of caproic, caprylic, capric, and lauric acids to the drinking water of broiler chicks, they were found to be less susceptible to colonization of Campylobacter jejuni, which seems to be the result of reduced survival of the bacteria in the drinking water. Considering these results, the benefit of water applications of MCFA is thought to be the reduced probability of Campylobacter invasion in the digestive tract of a bird and then the transmission of the pathogen throughout a flock.

Clostridiosis

Clostridium perfringens is within the five most frequent pathogens causing foodborn disease in humans. This microbe is a normal member of chickens' intestinal microbiome as it is acquired from the environment like water, food, litter etc. [92]. As certain strains might produce toxins, adhesins, special proteolytic and collagenolytic enzymes which can alter the healthy structure of the intestinal mucosa, altering the physiological status of the gastrointestinal tract and resulting in necrotic enteritis. Supplementation with MCFA-containing fats and oils in feed, using coconut and palm oil, was found to be effective against *Clostridium perfringens*, mainly as a preventative treatment [25]. However, coconut oil and palm oil did not affect the production parameters of broilers during *Clostridium perfringens* challenge [37]. Hovorková et al. [33] found in vitro inhibitory effect of palm kernel oil against clostridia. Similarly, the data of Shilling et al. [101] indicated that lauric acid (C12) can significantly inhibit the growth of Clostridium difficile, while Capric (C10) and caprylic acid (C8) were less effective. However, in an in vivo experiment Clostridium perfringens was not inhibited by lauric acid in the work of Yang et al. [102], while Abdelli et al. [103] have found improved histomorphology of the intestinal mucosa and reduced clostridia excretion in the excreta. Sodium lauryl lactylate was also found to prevent and inhibit colonisation of Clostridium perfringens. Qi et al. [36] showed that microencapsulated C8–C12–C14, C12–C14 or C10–C12 combinations displayed efficient antibacterial effects on the wild isolated avilamycin-resistant strains (CP-MZ1, C. perfringens type G strains, P-C8-1, C. perfringens type A strains), suggesting that microencapsulated mix of different MCFAs together with myristic acid have the potential for clinical treatment of necrotic enteritis.

E. Coli

E. coli is a normal inhabitant of chicken gut microbiome. Though many of the E. coli are not pathogenic, some have acquired virulence factors and can cause colibacillosis, which manifests in inflammatory process in different organs of the birds. As such, this is the most common and economically detrimental bacterial disease in avian species worldwide.

Short and medium chain fatty acids are thought to be beneficial to reduce the effects of coli pathogeneicity due to their antimicrobial effects. However, results are quite contradictory. No difference was found in the number of coliforms (TCC) in the digesta of broilers treated with coconut and/or palm oil in feed [25]. Similar results were reported by Jadhav et al.. [12] with lauric and/or capric acid supplementation, as the TCC was slightly, but not significantly reduced. However, Skřivan et al. [104], revealed that supplementation of caprylic acid reduced coliform count in broilers.

TCC was also significantly lower due to an MCFA mix supplementation compared to the control within the initial 28 days of fattening period [105]. According to Ripon et al. [106] the antimicrobial activity of fatty acids need time to develop, as in their broiler experiment TCC count was not affected by a mixture of medium chain fatty acids in the first 21 days of growth, while by day 42 it was reduced significantly compared to the control values.

Coccidiosis

Avian coccidiosis is an infectious enteric disease caused by protozoan parasites of the genus Eimeria with major impact in poultry production. Although coccidiosis may be subclinical, mucosal intestinal lesions can compromise performance of broilers [41]. Coccidia are host-specific in poultry and the different species are likely to infect different locations of the intestine, i.e., E. acervulina and E. maxima the two most prevalent species in chicken cause infection in the small intestine, while E. tenella is localized in the ceca. There are several studies outlined the positive effect of SCFAs and MCFAs in coccidiosis challenge. According to these results modifying microbiome composition, anti-inflammatory effects and improving gut barrier are the probable mechanisms behind the veneer. Thus, higher number of goblet cells, helping to create intestinal barrier in the mucosa, and reduced villus : crypt ratio, as well as increased lactic acid bacteria count were found by Sadurni et al. [107] in the intestine of infected birds in infected birds when a mixture of fatty acid salts distilled from coconut oil was added in their feed. It is proven by Place et al. [108] that butyrate inhibits nuclear factor kappa B activation, and consequently it results in reduced expression of proinflammatory cytokines. This effect of tributyrin (a glycerol ester of the butyrate) was confirmed by Hansen et al. [109] as the gene expression of certain enzymes and proteins (junctional adhesion molecule, liver enriched antimicrobial protein 2, mucin 2) related to gut immunity has been changed due to butyrate supplementation especially in birds with coccidiosis challenge. It is also important, that though there are several types of butyrate feed additives, the most efficient forms are the glycerol esters, like tributiryn, because they cannot be absorbed before reaching the small intestine.

7. Implications for future research

According to the results of several decades' research with short and medium chain fatty acids in broiler chicken diet, it is clear by now that these acids may improve performance of chickens directly and indirectly, as well. However, the results are not consistent when we consider the form of supplementation. When single fatty acids, like lauric or miristic acid are used it is obviously easier to evaluate their effects [61]. However, a blend of MCFAs and SCFAs are commonly used in form of natural vegetable fat sources, like the coconut oil or palm oil composed by numerous different fatty acids. Thus, in general, more complex the composition of a fat source is, the more complicated to evaluate its effects. It is also reasonable to mention that when a blend of fatty acids are used, they might have some interaction (synergism or antagonism) as well, which makes even more difficult to identify the mechanism behind the effect [88]. However, from practical point of view, it is more reasonable to use natural vegetable oils in broiler nutrition, but in this case we have to consider not only the possible biological activity of the individual fatty acids included, but also their energy value, which makes even more complicated to evaluate research results.

As it is highlighted several times in the different sections of the article, research results even with the same fatty acid supplementation may vary. Therefore, need for further research with pure fatty acids supplements and natural vegetable oils in this field is inevitable. Probably antimicrobial or bacteriostatic effects of MCFAs and SCFAs are the most prosperous side of the story. In this field there are still so many questions we cannot answer up to date. As microbiome is a key factor in nutrient utilisation and immune status of the birds it is essential to clarify how the different members of the microflora can be supported or inhibited by these fatty acids and their blends and thus how these supplements can help to stabilise the microbiome composition and maintain eubiosis.

8. Conclusions

Nutritional strategies to improve gut health are under extensive research to reduce antibiotic use in poultry production. Previous and current literature proved that as alternatives for antibiotics, the use of SCFA and MCFA, has important health and performance benefits in broilers both at experimental and commercial levels. When these acids, in salt or esterified form are fed to animals, positive effects on growth performance, intestinal microbial growth and health status of the animals are seen. Although the full mechanism of action of SCFA and MCFA is not well-known, broad-spectrum activity has been demonstrated against gram positive and gram-negative pathogenous bacteria such as Salmonella, E. coli, and Clostridium spp, making them a viable solution to reduce the use of antibiotics. They also have synergistic effects when used together and can thus reduce the magnitude and duration of treatments. However, using natural oil sources rich in SCFAs and/or MSCFAs the results are often contradictory, as the fatty acid composition of these feed additives might be variable. In these blends only the proportion of the different fatty acids are measured, while we do not really know what is their absolute concentration in the oil and therefore in the diet. Thus, research results from the experiments working with single or blended fatty acids are not easily comparable. Also the potential effect of SCFA and MCFA without any protection would be limited because of the prompt absorption and metabolism or both in the stomach or in the small intestine, while their bacteriostatic activity can be realized in the ceca and in the lower gut.

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