Literature Reviews

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Dietary organic acids for broiler chickens: a review¤

Ácidos orgánicos en la dieta de pollos de engorde: revisión de literatura

Dietas ácidos orgânicos sobre frangos de corte: revisão de literatura

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Summary

The objective was to summarize and describe the possible mode of action of dietary organic acids and their effects on growth performance of broiler chickens. Previous experiments have suggested that dietary organic acids decrease pH in diets and subsequently reduce pH in the proximal and distal intestine, increase nutrient utilization, and inhibit pathogenic bacterial growth in the gastrointestinal tract (GIT). The degree of pH reduction is usually greater in the upper part of the GIT (crop, proventriculus, and gizzard) than in the lower part of the GIT (duodenum, jejunum, ileum, and cecum). Bactericidal effects of dietary organic acids have been observed for pathogenic bacteria and even for beneficial bacteria to some extent. However, few significant results regarding bacterial modulation in the GIT have been reported. Dietary organic acids can improve dry matter and protein utilization in some experiments, but the extent of improvement in nutrient utilization is smaller than has been anticipated. Growth performance is likely improved, but results have been inconsistent due to variations in sources and inclusion levels of dietary organic acids. Differences in other dietary components and experimental environments among previous experiments likely contribute to the variable results. This review suggests that the effects of dietary organic acids on broiler chickens are not fully understood. Further experiments are required to reliably demonstrate the mode of action of dietary organic acids and their growth-promoting effects on broiler chickens.

Keywords: *acidifiers, gastrointestinal pH, growth performance, microbial population, nutrient utilization.*

Resumen

El objetivo fue resumir y describir el posible modo de acción de los ácidos orgánicos en la dieta y sus efectos sobre el crecimiento de los pollos de engorde. Experimentos previos sugieren que los ácidos orgánicos dietarios disminuyen el pH de la dieta y posteriormente reducen el pH en el intestino proximal y distal, aumentan la utilización de los nutrientes, e inhiben el crecimiento de bacterias patógenas en el tracto gastrointestinal (GIT).

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El grado de reducción del pH es generalmente mayor en la parte superior (buche, proventrículo y molleja) que en la parte inferior del GIT (duodeno, yeyuno, íleon y ciego). Se han observado efectos bactericidas de los ácidos orgánicos sobre bacterias patógenas e incluso sobre bacterias beneficiosas en cierta medida. Sin embargo, se han reportado algunos resultados significativos con respecto a la modulación bacteriana en el GIT. Los ácidos orgánicos en la dieta pueden mejorar la utilización de la materia seca y la proteína en algunos experimentos, pero el grado de mejora en la utilización de los nutrientes es más bajo que lo esperado. El crecimiento probablemente mejora, pero los resultados han sido inconsistentes debido a las variaciones en las fuentes y a los niveles de inclusión de los ácidos orgánicos en la dieta. Las diferencias en otros componentes de la dieta y entornos experimentales entre los ensayos anteriores probablemente contribuyen a la variacion en los resultados. Esta revisión sugiere que los efectos de los ácidos orgánicos en la dieta de pollos de engorde no son totalmente comprendidos. Se requieren más experimentos para demostrar de manera fiable el modo de acción de los ácidos orgánicos dietarios y sus efectos sobre la promocion del crecimiento en pollos de engorde.

Palabras clave: *acidificantes, desempeño del crecimiento, pH gastrointestinal, población microbiana, utilización de nutrientes.*

Resumo

Este estudo se fez para resumir e descrever o possível modo de ação dos ácidos orgânicos na dieta e seus efeitos sobre o desempenho do crescimento de frangos de corte. Pesquisas feitas nesta área tem descrito que os ácidos orgânicos nas dietas diminuem o pH da dieta e subsequentemente diminuem o pH no intestino proximal e distal, aumentam a utilização de nutrientes e inibem o crescimento de bactérias patogénicas no trato gastrointestinal (GIT). O grau de redução do pH é normalmente maior na parte superior do GIT (colheita, proventrículos e moela) do que na parte inferior do GIT (duodeno, jejuno, íleo e ceco). Com a inclusão de ácidos orgânicos nas dietas tem-se observado efeitos bactericidas tanto sobre as bactérias patogénicas quanto para as bactérias benéficas, em certa medida. Porém, tem-se reportado alguns resultados significativos enquanto à modulação bacteriana no GIT. A adição de ácidos orgânicos na dieta pode melhorar o aproveitamento de matéria seca e proteína em alguns testes, mas o grau de melhora na utilização dos nutrientes é menor do que o esperado. Provavelmente melhora o crescimento, mas os resultados têm sido inconsistentes, devido as variações nas fontes e aos níveis de inclusão de ácidos orgânicos na dieta. As diferenças em outros componentes da dieta e os lugares onde se fazem os testes contribuem para a variação dos resultados. Esta análise sugere que os efeitos dos ácidos orgânicos nas dietas de frangos de corte não são totalmente compreendidos. Precisam-se mais pesquisas para demonstrar uma maneira fiável do modo de ação dos ácidos orgânicos incluídos na dieta e seus efeitos sobre a promoção do crescimento em frangos de corte.

Palavras chave: *acidificantes, desempenho produtivo, pH gastrointestinal, população microbiana, utilização de nutriente.*

Introduction

Antibiotic growth promoters (AGPs) have been widely used in poultry diets for years. The use of AGPs, however, has been either regulated or banned because of public concerns over possible antibiotic residual problems and the development of antibiotic-resistant bacteria (Leeson, 2007). Consequently, many researchers have searched for potential alternatives to AGPs. Organic acids, organic minerals, bacteriophages, probiotics, and prebiotics have been suggested as a useful dietary means for compensating the loss in productive performance when AGPs are removed from poultry diets (Jackson *et al.*, 2004; Yan *et al.*, 2012). Among these alternatives, dietary organic acids have gained great attention because of their antimicrobial activity against pathogenic bacteria and the fact that these compounds can induce a pH reduction in the gastrointestinal tract (GIT), which can improve nutrient utilization in poultry diets (Eidelsburger *et al.*, 1992; Boling *et al.*, 2000; Partanen, 2001; Kil *et al.*, 2011a).

Dietary acids for poultry diets are classified as inorganic and organic acids. However, organic acids have been more often used for poultry diets. Organic acids can be defined as carboxylic acids including fatty acids, which have the chemical structure of R-COOH with acidic properties. However, not all organic acids have been used as feed additives in poultry diets. Short chain fatty acids such as formic (C1), acetic (C2), propionic (C3), and butyric acid (C4), and other carboxylic acids such as lactic, malic, tartaric, fumaric, and citric acid have been most commonly used in the poultry industry because their chemical and physical properties are applicable to poultry diets (Dibner and Buttin, 2002). Previously, several reviews have discussed the effects of dietary organic acids on broiler chickens (Dibner and Buttin, 2002; Ricke, 2003; Anjum and Chaudhry, 2010; Islam, 2012). However, previous reviews have not provided a complete evaluation of the potential mechanisms, and have not compiled the effects of dietary organic acids on broiler performance with the recent data. The objective of this review, therefore, was to summarize and describe the possible mode of action of dietary organic acids for broiler chickens and the effects of dietary organic acids on the growth performance of broiler chickens.

Potential mode of action of dietary organic acids

The mode of action of organic acids in animal diets has not been clearly elucidated; this incomplete understanding has limited the application of organic acids in broiler diets. However, several possible mechanisms have been proposed and most of them have been associated with: (1) decreased pH in diets and subsequent reduction of the pH in the GIT, (2) improved nutrient utilization in diets by increasing nutrient retention, and (3) inhibition of pathogenic bacterial growth (Afsharmanesh and Pourreza, 2005; Mroz, 2005). Further research has been performed to elucidate the mode of action of dietary organic acids in various animal species, but the results remain controversial.

Effects on the pH of the gastrointestinal tract (GIT)

The degree of pH reduction in diets and digesta by dietary organic acids is likely dependent of both the pK_a values of the respective organic acids and the pH conditions of the GIT (Kim *et al.*, 2005). As expected, the pH of broiler diets was clearly decreased with increasing inclusion levels of dietary organic acids in a dose-dependent manner (Table 1), as was also observed in pig diets (Kil *et al.*, 2011a). Subsequently, the addition of organic acids to broiler diets resulted in the pH reduction of digesta in various parts of the GIT. In general, the degree of pH reduction was usually greater in the upper part of GIT (crop, proventriculus, and gizzard) as compared to the lower part of the GIT (duodenum, jejunum, ileum, and cecum). In seven previous experiments, 11 of 13 organic acid-supplemented groups showed decreased crop pH compared with the control groups, with 7 of 11 observations being significant. Three experiments reported that the pH reductions in the crop were dose-dependent. The average pH reduction in the crop was 0.37 (standard error [SE] = 0.10) and it is likely that, of all locations in the GIT, the crop showed the greatest pH reduction. This observation may be related to the short transit of the acids to the crop in addition to the less acidic conditions of the crop (Thompson and Hinton, 1997).

In four previous experiments (Paul *et al.,* 2007; Samanta *et al.*, 2008; Panda *et al.*, 2009a; Salgado-Tránsito *et al.*, 2011), 6 out of 10 organic acid-supplemented groups showed pH reduction in the proventriculus compared with the control groups. However, the reduction achieved statistical significance in only one experiment (Panda *et al.*, 2009a). The average pH reduction in the proventriculus was 0.12 (SE = 0.07), which was less than the pH reduction as observed in the crop. In the lower part of the GIT, the effects of dietary organic acids on digesta pH were more variable than in the upper part of the GIT. Samanta *et al.* (2008), Panda *et al.* (2009a), and Nourmohammadi *et al.* (2011) reported a significant pH reduction in the duodenum, whereas other studies found no significant pH reductions in the duodenum. Similar tendencies for pH reduction were observed in the jejunum, ileum, and cecum. It has been reported that only small amounts of added organic acids in diets may reach the lower part of the GIT because organic acids are very readily absorbed in the upper part of the GIT (Hume *et al.*, 1993). This may explain the lack of pH reduction in the lower part of the GIT as a result of dietary organic acids. Taken together, the data indicate that the effects of dietary organic acids on the pH of the GIT may be limited to the upper part of the GIT in broiler chickens.

Effects on nutrient utilization

Reduced pH in the upper part of the GIT may increase nutrient digestibility, and therefore, nutrient utilization in diets. In the stomach, a reduction in gastric pH activates pepsinogen and other zymogens by adjusting gastric acidity closer

Table 1. Effects of dietary organic acids on diet pH and the digesta pH in the gastrointestinal tract of broiler chickens[1].

Organic acid	Inclusion g/kg	Change in pH[2], unit								References
		Diet pH	Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Cecum	
Citric acid	20		-0.15*		0.02	0.26	-0.11	0.13	-0.13	Esmaeilipour *et al.*, 2011
	40		-0.31*		0.12	0.28	0.19	-0.15	-0.05	
Citric acid	6.25	-0.60*		0.10	-0.20	-0.10	-0.10	0.10		Salgado-Tránsito *et al.*, 2011
	12.5	-1.10*		0.00	0.00	-0.20	0.00	0.00		
	25	-1.70*		-0.10	0.30	-0.20	-0.10	-0.10		
	50	-2.30*		-0.10	-0.10	0.10	0.00	0.10		
Citric acid	10	-0.64*	-0.30		-0.40			-0.06	-0.20	Atapattu and Nelligaswatta, 2005
	20	-1.14*	-1.10		0.20			-0.26	-0.23	
Citric acid	30		-0.17*		-0.02	-0.01	-0.14*	-0.01		Nourmohammadi *et al.*, 2011
	60		-0.28*		-0.12*	-0.09*	-0.40*	-0.06*		
Citric acid	30							-0.08		Aydin *et al.*, 2010
Butyric acid	2		-0.57*	-0.30*	-0.24*	-0.12*	-0.13	-0.18		Panda *et al.*, 2009a
	4		-0.82*	-0.44*	-0.40*	-0.24*	-0.22	-0.22		
	6		-0.83*	-0.46*	-0.42*	-0.27*	-0.18	-0.08		
Blend[3]	6		-0.28				0.12	0.24	0.46	Smulikowska *et al.*, 2010
Blend[4]	1		0.15	-0.08	-0.64*	-0.32*		-0.03		Samanta *et al.*, 2008
Ammonium formate	3		0.00	0.00	-0.21	0.30	-0.05	0.00		Paul *et al.*, 2007
Calcium propionate	3		-0.10	0.20	-0.15	0.10	0.00	0.00		
Mean		-1.25	-0.37	-0.12	-0.14	-0.04	-0.08	-0.04	-0.03	
SE[5]		0.27	0.10	0.07	0.06	0.06	0.04	0.03	0.13	

[1]An asterisk mark (*) represents significant difference compared with the control group ($p<0.05$).

[2]Changes in pH (unit) = pH measured in broiler chickens fed diets containing organic acids minus the pH measured in broiler chickens fed diets containing no organic acids.

[3]Blend = lactic acid + formic acid + citric acid and their salts.

[4]Blend = formic acid + propionic acid + calcium propionate + ortho phosphoric acid.

[5]Standard error.

to that required for optimal activity (Jongbloed *et al.*, 2000); this increased enzyme activity can improve the digestion of proteins and possibly other nutrients. Furthermore, acidic digesta may decrease gastric emptying, and therefore provide more time for nutrient digestion in the GIT (Kidder and Manners, 1978; Mayer, 1994). Several researchers have demonstrated that dietary supplementation of organic acids can improve the retention of protein and other nutrients. The data from the five previous experiments indicated that broiler chickens fed diets containing various inclusion levels of dietary organic acids generally had greater retention of dry matter (DM) and protein than those fed control diets (Table 2). Average improvements in the retention of DM and protein were 1.0% (SE = 0.60) and 1.7% (SE = 0.88), respectively. However, we excluded the data from Nezhad *et al.* (2011) in the calculations of the average improvements in the retention of DM and protein because of the unexpectedly high improvement in the retention of protein. Among 14 organic acid-supplemented groups, 7 and 9 groups showed a numerical increase in the retention of DM and protein, respectively; however, no significant improvements were verified. In addition, it is unlikely that there were dose-dependent responses of organic acids to nutrient retention. When we considered the ratio of the number of positive responses to the number of negative responses by dietary organic acid supplementation, however, broiler chickens fed diets containing organic acids may have improved nutrient retention. However, it appears that the extent of the improvements in nutrient retention may be smaller than anticipated. Surprisingly, there have been few data pertaining to the effects of dietary organic acids on amino acid digestibility in diets fed to broiler chickens compared with other animal species. Further experiments investigating standardized ileal digestibility and true ileal digestibility of amino acid are required to verify the effects of dietary organic acids on nutrient utilization especially for amino acids in diets fed to broiler chickens.

Table 2. Effects of dietary organic acids on dry matter (DM) and protein retention in broiler chickens.

Organic acid	Inclusion, g/kg	Changes in retention[1], %		References
		DM	Protein	
Citric acid	15	-0.3	-1.4	Ao *et al.*, 2009
	20	1.3	2.1	
Citric acid	20	0.9	-0.1	Esmaeilipour *et al.*, 2011
	40	4.4	2.9	
Citric acid	25	7.2	27.9	Nezhad *et al.*, 2011
	50	-2.2	8.1	
Formic acid	5	1.4	-1.6	Hernández *et al.*, 2006
	10	-1.4	-4.7	
Formic acid	2	0.2	1.6	Panda *et al.*, 2009b
	4	-0.7	1.3	
	6	-1.7	5.9	
	8	-0.2	5.7	
	10	0.0	5.7	
Fumaric acid	5	2.7	2.2	Pirgozliev *et al.*, 2008
	10	6.4	5.5	
	15	0.8	-1.5	
Mean[2]		1.0	1.7	
SE[3]		0.60	0.88	

[1]Changes in retention (%): the percentage increase or decrease in the retention of DM and protein measured in the organic acid-supplemented groups relative to the control groups.

[2]Values for the mean were calculated with the exclusion of the data from Nezhad *et al.* (2011), in order to prevent the biased overestimation.

[3]Standard error.

Effects on pathogenic bacteria

An increased population of pathogenic bacteria in the GIT often results in reduced growth performance of broiler chickens. Therefore, the prevention of pathogenic bacterial over-growth in the GIT may be one of the most important strategies for enhancing growth performance when supplemental AGPs are not used in animal diets. Organic acids can easily penetrate the bacteria cell wall and disrupt normal cellular functions, including replication and protein synthesis of bacteria (Denyer and Stewart, 1998; Davidson, 2001). The proposed sequential mechanisms of bactericidal action are followed as (Mani-Lopez *et al.*, 2012): (1) acid form of organic acids (protonated form) can penetrate across the bacteria cell wall, (2) penetrated organic acids within bacterial cells dissociate into the conjugated base form (non-protonated form) with a concomitant reduction in cellular pH, and (3) decreased pH creates a stressful environment leading to cellular dysfunctions, and thus prevents bacterial growth. Such reactions are likely to occur mainly with pH-sensitive bacteria species, which include the wide range of pathogenic bacteria. Akyurek *et al.* (2011) reported that broiler chickens fed diets containing organic acid blends had less pathogenic bacterial loads such as coliforms and *Clostridia* but greater beneficial bacteria such as *Lactobacilli* in the ileum compared with those fed diets containing AGPs. It is also likely that the decreased pH in the GIT induced by dietary organic acids may play a role in preventing bacterial transfer from the diet or environment. However, most of the previous experiments regarding the effects of dietary organic acids on microbial populations in the GIT reported few significant benefits on microbial populations in the GIT (Table 3). In our summary, moreover, the birds fed diets containing organic acids had slightly lower lactic acid-producing bacteria or *Lactobacilli* counts in the ileum (0.44 $\log_{10}$ ± 0.20 colony forming units [CFU]) and the cecum (0.37 ± 0.07 $\log_{10}$ CFU) than those fed control diets although these species are generally considered as beneficial bacteria. This observation was inconsistent with the findings of Akyurek *et al.* (2011). There have been only few experiments showing a significant reduction in coliform bacteria or *Escherichia coli* counts in the GIT by feeding diets containing organic acids to broiler chickens. Average reductions in the numbers of coliform bacteria or *E. coli* count were 0.86 ± 0.23 $\log_{10}$ CFU for the ileum and 0.82 ± 0.22 $\log_{10}$ CFU for the cecum. However, the average reductions in the numbers of coliform bacteria or *E. coli* count were greater than those of lactic acid-producing bacteria or *Lactobacilli* counts in the ileum or the cecum. The reason that lactic acid-producing bacteria or *Lactobacilli* may be less affected by dietary organic acids than coliform bacteria or *E. coli* may be related to the fact that coliform bacteria or *E. coli* are more sensitive to pH reductions than lactic acid-producing bacteria or *Lactobacilli* in the GIT. Because previous experiments have focused on the specific bacteria species, the effects of dietary organic acids on the change in the entire microbial populations in the GIT are still unknown. Therefore, further experiments are necessary to demonstrate the effects of dietary organic acids across the whole microbial populations. In addition, the application of molecular-based techniques such as real-time polymerase chain reaction (RT-PCR), and pyrosequencing procedures may yield more valuable and accurate results than conventional culture-based techniques (Kil and Swanson, 2011b).

Other possible effects

Previous experiments have reported that dietary organic acids can increase phosphorus utilization in corn-soybean meal diets fed to broiler chickens (Boling *et al.*, 2000; Esmaeilipour *et al.*, 2011). Phosphorus utilization may be increased due to the chelating properties of organic acids with calcium, which can result in increased phytate-phosphorus solubility, increasing their ability to be hydrolyzed (Centeno *et al.*, 2007). Some researchers have also proposed that organic acids may stimulate energy metabolism by providing energy sources for epithelial cells in the GIT (Ravindran and Kornegay, 1993; Partanen and Mroz, 1999). For instance, some organic acids such as fumaric and citric acids are intermediates of the tricarboxylic acid cycle, and butyric acid is the direct energy source for epithelial cells in the GIT (Partanen and Mroz, 1999; Pryde *et al.*, 2002). However, no data have elucidated the cellular roles of organic acids sin the energy metabolism of broiler chickens.

Table 3. Effects of dietary organic acids on the gastrointestinal microbial population in broiler chickens[1].

Organic acid	Inclusion g/kg	Changes in microbial counts[2], log_{10} CFU				References
		Ileum		Cecum		
		LAC[3]	COLI[4]	LAC[3]	COLI[4]	
Citric acid	30			-0.2	-0.2	Biggs and Parsons, 2008
Citric acid	30	0.2	-0.5*			Aydin *et al.*, 2010
Fumaric acid	5	0.2	-1.7	-0.4	-1.0	Pirgozliev *et al.*, 2008
	10	-0.2	-1.1	-0.3	-0.5	
	15	-0.8	-0.6	-0.5	-1.4	
Sorbic acid	5	-0.6	0.2	-0.5	-0.6	Pirgozliev *et al.*, 2008
	10	-1.2	-1.1	-0.4	-1.8	
	15	-0.7	-1.2	-0.8	-1.6	
Blend[5]	3			-0.1	-0.2*	Kim *et al.*, 2009
	5			-0.1	-0.1*	
Mean		-0.44	-0.86	-0.37	-0.82	
SE[6]		0.20	0.23	0.07	0.22	

[1]An asterisk mark (*) represents significant difference compared with the control group ($p<0.05$).

[2]Changes in microbial counts (log_{10} CFU, colony forming unit) = the number of microbes measured in broiler chickens fed diets containing organic acids minus the number of microbes measured in broiler chickens fed diets containing no organic acids.

[3]LAC = *Lactobacilli* or lactic acid-producing bacteria.

[4]COLI = total coliform bacteria or *Escherichia coli*.

[5]Blend = 40% lactic acid + 20% fumaric acid + 30% citric acid + 10% formic acid.

[6]Standard error.

Organic acids and growth performance

There has been accumulating evidence that broiler chickens fed diets containing various sources and levels of organic acids have improved growth performance. In the current review, we surveyed 31 recent publications and compared the effects of diets containing various organic acids on body weight gain, feed intake, and feed efficiency (gain to feed ratio) with those of control diets in broiler chickens (Table 4). The results for individual organic acid are detailed below.

Citric acid

Citric acid ($C_6H_8O_7$) is a weak organic acid and has been used as a natural preservative. Citric acid has been widely used as an organic acid supplement for pigs and chickens. We examined eight previous experiments using various inclusion levels of dietary citric acid (Table 5). The data indicated that dietary citric acid generally led to increased body weight gain and feed efficiency but decreased feed intake of broiler chickens; however, there was high variation in the ratio of the number of positive responses to the number of negative responses. The average improvements in body weight gain and feed efficiency were 4.7 and 6.0%, respectively. It is noteworthy that feed efficiency in most acid-supplemented groups was improved except for three treatment groups. Regardless of the inclusion levels of citric acid, half of the acid-supplemented groups reported significant increase in body weight gain (Chowdhury *et al.*, 2009; Haque *et al.*, 2010; Nourmohammadi *et al.*, 2010; Salgado-Tránsito *et al.*, 2011). However, only two experiments reported significant improvements in feed intake (Haque *et al.*, 2010; Nourmohammadi *et al.*, 2010) and feed efficiency (Chowdhury *et al.*, 2009; Salgado-Tránsito *et al.*, 2011). It is postulated that excessive amounts of dietary citric acid inclusion may compromise performance because two experiments using 60 g/kg citric acid in diets reported significant decreases in body weight gain.

Table 4. Overall effects of dietary organic acids on the growth performance of broiler chickens[1,2].

Organic acid	No. Exp.[3]	Inclusion, g/kg	BWG, % changes[4]				FI, % changes[4]				Gain: Feed, % changes[4]			
			Mean	Range	No. Sig. (p<0.05)[5]	+/-[6]	Mean	Range	No. Sig. (p<0.05)[5]	+/-[6]	Mean	Range	No. Sig. (p<0.05)[5]	+/-[6]
Citric acid	8	5~60	4.7	-16.7~25.2	9/14	8/6	-1.3	-24.9~13.1	4/14	6/7	6.0	-4.2~25.2	5/14	11/3
Fumaric acid	3	1.25~45	1.3	-2.3~4.0	1/6	5/1	1.9	-1.0~5.0	2/6	4/1	0.2	-2.2~3.1	0/9	4/5
Formic acid	5	1~10	2.8	-3.8~10.3	1/10	8/2	0.4	-1.0~4.1	1/4	1/3	5.3	0.5~18.2	2/11	11/0
Formate salt	2	3~28.9	2.6		0/1	1/0	-0.5		1/1	0/1	-11.5	-25.0~3.1	1/5	1/4
Butyric acid	5	1~25	1.9	0.3~4.0	2/10	10/0	-0.6	-4.5~2.1	0/10	4/6	2.5	-1.0~5.9	3/10	9/1
Propionic acid	1	2	11.2		1/1	1/0	5.1		1/1	1/0	6.1		1/1	1/0
Propionate salt	1	3	0.5		0/1	1/0	-6		1/1	0/1	6.5		1/1	1/0
Blend	6	1~6	0.3	-5.8~3.2	1/7	5/2	-1.7	-9.9~1.3	0/6	3/3	3.2	-2.3~12.4	2/7	5/2

[1]References: (Patten and Waldroup, 1988; Alçiçek *et al.*, 2004; Leeson *et al.*, 2005; Gunal *et al.*, 2006; Hernández *et al.*, 2006; García *et al.*, 2007; Paul *et al.*, 2007; Biggs and Parsons, 2008; Pirgozliev *et al.*, 2008; Samanta *et al.*, 2008; Al-Kassi and Mohssen, 2009; Ao *et al.*, 2009; Bozkurt *et al.*, 2009; Chowdhury *et al.*, 2009; Kim *et al.*, 2009; Mahdavi and Torki 2009; Panda *et al.*, 2009a,b; Haque *et al.*, 2010; Nourmohammadi *et al.*, 2010; Smulikowska *et al.*, 2010; Esmaeilipour *et al.*, 2011; Salgado-Tránsito *et al.*, 2011; Aghazadeh and Tahayazdi, 2012; Nourmohammadi *et al.*, 2012; Świątkiewicz and Arczewska-Wlosek, 2012).

[2]Detailed information for each organic acid was provided in the Tables 5 through 9.

[3]Total number of experiments testing each organic acid.

[4]The percentage increase or decrease in the growth performance (BWG, body weight gain; FI, feed intake) measured in the organic acid- supplemented groups relative to the control group.

[5]Number of organic acid-supplemented groups showing significant changes (p<0.05) vs. total number of organic acid-supplemented groups.

[6]Number of organic acid-supplemented groups showing the positive impact vs. number of organic acid-supplemented groups showing the negative impact.

Table 5. Effects of dietary citric acid on the growth performance of broiler chickens.

Source	Inclusion, g/kg	Overall change,[1,2] %			References
		BWG	FI	Gain:Feed	
Citric acid	30	7.7	3.6	4.1	Nourmohammadi *et al.*, 2012
	60	-16.6*	-24.9*	8.4	
Citric acid	5	19.5*	7.7	11.8*	Chowdhury *et al.*, 2009
Citric acid	6.25	7.9	-1.8	9.7*	Salgado-Tránsito *et al.*, 2011
	12.5	14.9*	5.2	9.8*	
	25	12.6*	3.5	9.1*	
	50	25.2*	0.0	25.2*	
Citric acid	5	16.6*	12.0*	4.6	Haque *et al.*, 2010
Citric acid	20	-1.1	-3.3	2.2	Esmaeilipour *et al.*, 2011
	40	-9.0	-13.4	4.4	
Citric acid	40	-5.5*	-2.1	-3.5	Biggs and Parsons, 2008
Citric acid	30	19.4*	13.1*	6.4	Nourmohammadi *et al.*, 2010
	60	-16.7*	-12.9*	-3.9	
Citric acid	20	-9.3	-5.2	-4.2	Ao *et al.*, 2009
Mean		4.7	-1.3	6.0	

[1]The percentage increase or decrease in the body weight gain (BWG), feed intake (FI), and gain to feed ratio (Gain:Feed) measured in the organic acid-supplemented groups relative to the control groups.

[2]An asterisk mark (*) represents significant difference at p<0.05.

Fumaric acid

Fumaric acid ($C_4H_4O_4$) is a weak organic acid with a fruit-like taste. Published data from three previous experiments (Patten and Waldroup, 1988; Skinner *et al.*, 1991; Biggs and Parsons, 2008) indicated that broiler chickens fed diets containing various inclusion levels of fumaric acid had increased body weight gain and feed intake, except for the data from 45 g/kg of fumaric acid-supplemented groups of one experiment (Biggs and Parsons, 2008; Table 6). On the contrary, Pirgozliev *et al.* (2008) reported that adding 5, 10, or 15 g/kg fumaric acid to broiler diets significantly reduced body weight gain by 7.9 to 25.7% and feed intake by 5.9 to 41.4% compared with the control groups. The reason for this large negative impact on broiler performance is unclear. As a result, we excluded the data from Pirgozliev *et al.* (2008) from our calculations of the average change in the growth performance to prevent the results of the current study obscuring the effects of dietary fumaric acid on the growth performance. Subsequently, the average improvements in body weight gain and feed intake were 1.3 and 1.9%, respectively. Feed efficiency was slightly improved by an average of 0.2%. However, it is difficult to conclude that dietary fumaric acid has positive effects on broiler performance because of the scarcity of data.

Formic acid and its salts

Formic acid (CH_2O_2) is the simplest carboxylic acid. Formic acid is very volatile and has a pungent smell. Therefore, the free form of formic acid has not been widely used as a dietary supplement, whereas its salts (as formates), which are less pungent and easier to handle, have been often added to broiler diets. We reviewed seven previous experiments using formic acids or formates as dietary supplements for broiler chickens (Table 7). Feeding broiler chickens with diets containing 1 to 10 g/kg of formic acid was reported to increase body weight gain, feed intake, and feed efficiency. The average improvements were 2.8, 0.4, and 5.3% for body weight gain, feed intake, and feed efficiency, respectively. With the exception of García *et al.* (2007) who reported that the birds fed diets containing 5 or 10 g/kg of formic acid had less body weight gain than those fed the control diets, positive effects on body weight gain were reported for all formic acid-supplemented groups. Furthermore, clear dose-dependent positive effects on body weight gain were also reported in some

Table 6. Effects of dietary fumaric acid on the growth performance of broiler chickens.

Source	Inclusion, g/kg	Overall Change,[1,2] %			References
		BWG	FI	Gain:Feed	
Fumaric acid	5	-7.9*	-5.9*	5.1	Pirgozliev *et al.*, 2008
	10	-25.7*	-41.4*	12.9	
	15	-21.0*	0.0	6.9	
Fumaric acid	1.25	4.0*	5.0*	-1.0	Skinner *et al.*, 1991
	2.5	1.5	1.7	-0.2	
	5	2.0	4.2*	-2.2	
Fumaric acid	15	1.3	1.5	-0.1	Biggs and Parsons, 2008
	30	1.3	0.0	1.3	
	45	-2.3	-1.0	-1.2	
Fumaric acid	5			3.1	Patten and Waldroup, 1988
	10			2.0	
	15			0.3	
Mean[3]		1.3	1.9	0.2	

[1]The percentage increase or decrease in the body weight gain (BWG), feed intake (FI), and gain to feed ratio (Gain:Feed) measured in the organic acid-supplemented groups relative to the control groups.

[2]An asterisk mark (*) represents significant difference at p<0.05.

[3]Values for the mean were calculated with the exclusion of the data from Pirgozliev *et al.*, (2008), in order to prevent the biased underestimation.

experiments (Hernández *et al.*, 2006; Panda *et al.*, 2009b). However, the results for dietary formates were inconsistent. Patten and Waldroup (1988) observed decreased feed efficiency by dietary supplementation of calcium formate from 7.2 to 28.9 g/kg, whereas Paul *et al.* (2007) reported improved body weight gain and feed efficiency with diets containing 3 g/kg ammonium formate. The differences in the form of formates and the inclusion levels among experiments may cause these inconsistent results. It may be reasonable to conclude that the free form of formic acid has positive effects on the growth performance of broiler chickens, but the effects of formates are questionable.

Table 7. Effects of dietary formic acid and its salts (formates) on the growth performance of broiler chickens.

Source	Inclusion, g/kg	Overall Change,[1,2] %			References
		BWG	FI	Gain:Feed	
Formic acid	5	-2.3		9.8*	García *et al.*, 2007
	10	-3.8		18.2*	
Formic acid	5	1.2	-1.0	2.4	Hernández *et al.*, 2006
	10	3.6	-1.0	4.6	
Formic acid	1	10.3*	4.1*	6.3	Al-Kassi and Mohssen, 2009
Formic acid	2	0.3		0.5	Panda *et al.*, 2009b
	4	1.6		2.2	
	6	3.3		3.3	
	8	7.1		5.0	
	10	6.6		3.9	
Formic acid	1		-0.4	2.4	Bozkurt *et al.*, 2009
Mean		2.8	0.4	5.3	
Calcium formate	7.2			-1.5	Patten and Waldroup, 1988
	14.8			-19.2	
	22.0			-25.0	
	28.9			-14.7	
Ammonium-formate	3	2.6	-0.5*	3.1*	Paul *et al.*, 2007
Mean		2.6	-0.5	-11.5	

[1]The percentage increase or decrease in the body weight gain (BWG), feed intake (FI), and gain to feed ratio (Gain:Feed) measured in the organic acid-supplementedgroups relative to the control groups.

[2]An asterisk mark (*) represents significant difference at p<0.05.

Butyric acid

In the past decade, butyric acid ($C_4H_8O_2$) has been the most intensively studied by many poultry researchers. Butyric acid is considered important for the normal development of epithelial cells because it can be used as a direct energy source by epithelial cells and has bactericidal activity in the GIT (Pryde *et al.*, 2002). We examined five previous experiments using butyric acid (Table 8). When butyric acid was added to broiler diets, body weight gain and feed efficiency were generally improved. Average percentage improvements were 1.9 and 2.5% for body weight gain and feed efficiency, respectively. However, the improvements in feed efficiency were likely caused by decreased feed intake along with no or little change in body weight gain because 6 of 10 butyric acid-supplemented groups showed decreased feed intake. No clear explanation for this anorexic effect has been postulated. In addition, high inclusion levels of butyric acid may have a negative effect on feed efficiency because Aghazadeh and TahaYazdi (2012) reported that 25 g/kg of dietary butyric acid decreased feed efficiency by 1.0%. Based on the current data, however, it appears that butyric acid at low inclusion levels may have the most promising effects on broiler performance among dietary organic acids.

Table 8. Effects of dietary butyric acid on the growth performance of broiler chickens.

Source	Inclusion, g/kg	Overall Change,[1,2] %			References
		BWG	FI	Gain:Feed	
Butyric acid	2	1.0	-1.4	2.4*	Panda *et al.*, 2009a
	4	4.0*	-1.9	5.9*	
	6	3.4*	-1.1	4.5*	
Butyric acid	2	2.3	0.7	1.6	Leeson *et al.*, 2005
	4	0.3	-1.5	1.8	
Butyric acid	1	0.6	-4.5	5.1	Leeson *et al.*, 2005
	2	0.6	-1.7	2.3	
Butyric acid	2	3.3	1.3	2.0	Mahdavi and Torki 2009
	3	2.6	2.1	0.6	
Butyric acid	25	0.6	1.6	-1.0	Aghazadeh and Tahayazdi, 2012
Mean		1.9	-0.6	2.5	

[1]The percentage increase or decrease in the body weight gain (BWG), feed intake (FI), and gain to feed ratio (Gain:Feed) measured in the organic acid-supplemented groups relative to the control group.

[2]An asterisk mark (*) represents significant difference at p<0.05.

Other organic acids

Other sources of organic acids and mixtures (or blends) of various organic acids have also been tested for their utilization in broiler diets (Table 9). Al-Kassi and Mohssen (2009) reported that adding 2 g/kg of propionic acid to broiler diets resulted in significant improvements in body weight gain, feed intake, and feed efficiency by 11.2, 5.1, and 6.1%, respectively. Likewise, Paul *et al.* (2007) also reported that broiler chickens fed diets containing 3 g/kg of calcium propionate had significantly improved feed efficiency by 6.5% compared with those fed control diets. In recent years, there has been increasing attention on the blending type (i.e., mixtures) of organic acids based on the assumption that synergistically positive effects of individual organic acid exist (Kil *et al.*, 2011a). We examined six previous experiments investigating this aspect. Alçiçek *et al.* (2004) reported that feeding broiler chickens with 2.5 g/kg of blends of lactic acid, formic acid, and citric acid improved growth performance although the improvements did not reach statistical significance. Gunal *et al.* (2006) also observed that birds fed diets containing 2 g/kg blends of propionate salts and formates had numerically greater body weight gain and feed intake than those fed the control diets. Similar improvements have also been reported by Samanta *et al.* (2008) who added 1 g/kg of acid blends of formic acid, propionic acid, calcium propionate, and ortho-phosphoric acid to broiler diets. Kim *et al.* (2009) also reported that body weight gain in acid blend-supplemented groups was increased by from 1.8 to 3.2%, whereas feed efficiency was improved by nearly 4.0%.

In contrast, two previous experiments observed negative effects of dietary acid blends on body weight gain of broiler chickens. Świątkiewicz and Arczewska-Wlosek (2012) reported that broiler chickens fed diets containing 4 g/kg of acid blends had less body weight gain and feed efficiency. Similarly, Smulikowska *et al.* (2010) reported decreased body weight gain and feed intake of broiler chickens fed diets containing 6 g/kg of acid blends. Considering the number of positive and negative responses, the effectiveness and synergism of acid blends for broiler chickens remain unclear.

Table 9. Effects of propionic acid and organic acid blends on the growth performance of broiler chickens.

Source	Inclusion, g/kg	Overall Change,[1,2] %			References
		BWG	FI	Gain:Feed	
Propionic acid	2	11.2*	5.1*	6.1*	Al-Kassi and Mohssen, 2009
Calcium propionate	3	0.5	-6.0*	6.5*	Paul *et al.*, 2007
Blend[3]	3	1.8	-2.2	4.0*	Kim *et al.*, 2009
	5	3.2*	-1.2	4.3*	
Blend[4]	4	-1.6	0.7	-2.3	Świątkiewicz and Arczewska-Wlosek, 2012
Blend[5]	6	-5.8	-9.9	4.1	Smulikowska *et al.*, 2010
Blend[6]	1	1.9		12.4	Samanta *et al.*, 2008
Blend[7]	2.5	1.5	1.3	0.2	Alçiçek *et al.*, 2004
Blend[8]	2	1.1	1.2	-0.1	Gunal *et al.*, 2006
Mean		1.5	-1.4	3.9	

[1]The percentage increase or decrease in the body weight gain (BWG), feed intake (FI), and gain to feed ratio (Gain:Feed) measured in organic acids supplemented groups relative to the control group.

[2]An asterisk mark (*) represents significant difference at $p<0.05$.

[3]Blend = 40% lactic acid + 20% fumaric acid + 30% citric acid + 10% formic acid.

[4]Blend = 37.5% formic acid+ 25% propionic acid + 37.5% acetic acid.

[5]Blend = lactic acid + formic acid + citric acid and their salt.

[6]Blend = formic acid + propionic acid + calcium propionate + ortho phosphoric acid.

[7]Blend = formic acid + lactic acid + citric acid.

[8]Blend = propionate salt+ formate salt.

Factors affecting inconsistent results

The responses of broiler chickens to dietary organic acids have shown considerable inconsistency. There have been many successful demonstrations of positive effects of dietary organic acids on growth performance, whereas other studies were unable to find beneficial effects or even reported negative effects on growth performance. The extent of the effects was also variable among the previous experiments using different inclusion levels and sources of organic acids. Several possible factors responsible for these variations can be identified.

One factor could be the variation in other dietary ingredients and their chemical properties such as buffering capacity (Mroz *et al.*, 1997; Partanen, 2001). The sources and amounts of dietary protein and minerals may affect the buffering capacity of diets, which can influence the degree of acidification that occurs with the inclusion of organic acids (Partanen and Mroz, 1999). Although the related data for broiler chickens have been limited, the effects of buffering capacity on the effectiveness of dietary organic acids have been reported in pigs. Ravindran and Kornegay (1993) reported that the positive effects of dietary organic acids on weanling pigs were greater for the diets of low buffering capacity (simple corn-soybean meal-based diets) than for the diets of high buffering capacity (complex diets containing various protein sources). Therefore, the inconsistent responses to dietary organic acids in broiler chickens are likely associated with the specific chemical properties of experimental diets such as buffering capacity. Another possible factor causing variation in results may be experimental conditions such as the sanitation level of the environment. Dietary organic acids may affect the microbial population in the GIT. It may be expected, therefore, that the antimicrobial effects of organic acids would be more pronounced when birds are exposed to less sanitary conditions (Kil *et al.*, 2010). Therefore, difference in sanitary conditions among experiments may be the possible reason for the inconsistent results. In addition, based on the data we have reviewed, feed palatability is likely affected by the sources and inclusion levels of dietary organic acids, and therefore appears to influence the efficacy of dietary organic acids. More research is required to determine the effects of dietary organic acids on feed palatability or feed choice in broiler chickens.

Conclusions

Dietary organic acids have been considered as potential alternatives to AGPs for improving growth performance and health status of broiler chickens. The possible mode of action of organic acids supports the notion that they could be effective in broiler chickens. The pH reduction in the GIT through diet acidification leads to an increase in nutrient utilization and inhibition of pathogenic bacterial growth. Direct growth-inhibiting effects on pathogenic bacteria have also been identified. However, the effects of various sources and inclusion levels of organic acids on the pH of the GIT appear to be limited to the upper part of the GIT because these acids are highly absorbable, and thus little amounts of dietary organic acids may reach the lower part of the GIT. A protected form of dietary organic acids may overcome this problem and further experiments are needed to study the effects of the protected forms of organic acids on the pH of the GIT. It is also difficult to confirm the effects of organic acids on nutrient utilization because the extent of improvements in nutrient retention appears to be smaller than anticipated. As previous reviews have reported, we observed that most dietary organic acids improve growth performance of broiler chickens, despite some inconsistent results. However, the appropriate inclusion levels are unknown, and no clear dose-dependent responses to dietary organic acids are available. Differences in dietary ingredients, physical and chemical properties of the diets, and rearing conditions are most likely responsible for these variations. Therefore, it is difficult to conclude whether dietary organic acids have consistently positive effects on growth performance, and whether they are promising alternatives to AGPs for broiler chickens. Further studies are needed to elucidate the mode of action of dietary organic acids and their effects on growth performance of broiler chickens.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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