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GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY, AND BLOOD PARAMETERS OF BROILER CHICKENS FED A DIET SUPPLEMENTED WITH ORGANIC ACIDS

Asmaa Sh. ELnaggar[1] and M. I. El-Kelawy [2]

[1] Dep. of Anim. and Poult. Prod,, Fac. of Agric., Damanhour Univ.

[2] Dep. of Poult. Prod., Fac. of Agric., New Valley Univ.

*** Corresponding author:** M. I. El-Kelawy Email: m.elkelawy@gmail.com

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ABSTRACT: The study aimed to assess the impact of different levels of organic acids (formic, acetic, and citric) on broiler chickens' growth performance, nutrient digestibility, carcass traits, blood parameters, bacterial count, antioxidant status, immune response, and economic efficiency. Two hundred and ten unsexed (*Cobb* 500) day-old chicks were randomly divided into seven groups, each with five replicates of six birds. The first group was used as the control for comparison purposes. Birds in groups 2 and 3 were fed a diet containing formic acid (0.5% and 1.0%), while birds in groups 4 and 5 were fed a diet containing acetic acid (0.5% and 1.0%). Birds in groups 6 and 7 were fed a diet containing citric acid (2.0% and 3.0%). Weekly weighing, feed consumption recording, and calculation of growth parameters were recorded. Additionally, biochemical, hematological, and immune parameters were analyzed, along with evaluating the microbial activity of the digestive system. Enhanced growth parameters, including final weight and body weight gain, were noted with formic, acetic, and citric acid supplementation, along with improved feed conversion ratios. Dressing percentage was increased, while abdominal fat was decreased with all supplementation groups. Hematological analysis revealed improved blood parameters, albeit with reduced red blood cell count and hemoglobin levels, in chickens supplemented with acetic and citric acid. Lipid and protein profiles were positively influenced, with lowered serum lipids and increased protein levels. Additionally, antioxidant and immunological status were enhanced, characterized by heightened antioxidant enzyme activity and immune responses. Moreover, supplementation led to favorable shifts in the gut microbiota, with increased Lactobacillus levels and decreased bacterial counts, including *Escherichia coli* and Proteus. In conclusion, incorporating organic acids as alternatives to antibiotics in broiler chicken diets significantly improves production performance and enhances economic efficiency while maintaining optimal health.

Keywords: Productive performance, broiler chickens, organic acids, growth promoters.

INTRODUCTION

The poultry industry is experiencing rapid growth globally, with feed additives being recognized as essential for optimizing performance and productivity in modern poultry production (Shahid et al., 2015). Consequently, there is a continuous search within the poultry sector for new feed additives aimed at enhancing feed efficiency and the health of poultry birds.

This quest intensified after the prohibition of antibiotic growth promoters in the EU since 2006 due to concerns over emerging microbial resistance and residues in meat and eggs (Leeson, 2007; Cakir et al., 2008; Dhama et al., 2015; Ullah et al., 2022).

In response, researchers have sought alternatives to antibiotic growth promoters to maintain growth and feed efficiency in farm animals (Attia et al., 2012; Alzawqari et al., 2016; Abudabos et al., 2018; Scicutella et al., 2021). Studies have suggested that organic acids, bacteriophages, organic minerals, probiotics, prebiotics, and enzymes could serve as viable substitutes for antibiotic growth promoters. The consumption of these feeds has been suggested as a suitable dietary option for offsetting the decline in performance effectiveness that occurs when antibiotic growth promoters are removed from animal diets (Jackson et al., 2004; Yan et al., 2012).

One such alternative is the use of organic acids as feed additives in animal production. Research has shown that organic acid supplementation improves the performance of Japanese quails (Fouladi et al., 2018) and broilers (Ishfaq et al., 2015; Emami et al., 2017; Tomar et al., 2017). Furthermore, Onunkwo et al. (2021) discovered that organic acids positively influence the growth of both animals and broiler chickens. Specifically, organic acids such as butyric acid, acetic acid, citric acid, formic acid, fumaric acid, and propionic acid were found to have a beneficial impact on growth in these species.

Organic acids have been observed to impact various aspects of animal growth and nutrition, including final weight gain, average daily weight gain, total feed intake, feed-to-gain ratio, daily protein intake, protein efficiency ratio, total water consumption, average daily water intake, and water-to-feed ratio. Additionally, studies have shown that organic acids can enhance nutrient utilization, promote growth, and improve feed conversion efficiency (Denli et al., 2003). Furthermore, organic acids have been found to stimulate pancreatic juice secretion and promote the growth of epithelial cells in the intestinal wall (Langhout and Sus, 2005). Additionally, they have been shown to modify gut morphology by increasing villi height, thereby enhancing the absorption area for nutrients (Dibner and Buttin, 2002). Therefore, the aim of this study was to assess the impact of varying levels of organic acids (citric, formic, and acetic) on the growth performance, digestibility of nutrients, carcass characteristics, certain blood parameters, bacterial count, antioxidant status, immune response, and economic efficiency of broiler chickens.

MATERIALS AND METHODS:

This study was carried out at the Poultry Research Unit (El-Bostan Farm), Department of Animal and Poultry Production, Faculty of Agriculture, Damanhour University, Damanhour, Egypt during year 2023. The main objective was to evaluate different levels of organic acids (OA) on growth performance, nutrient digestibility, carcass traits, some blood parameters, bacterial count, antioxidant status, the immune response, and the economic efficiency of broilers. Two hundred and ten unsexed day-old chicks obtained from a commercial hatchery, were randomly distributed into seven groups; each group contained five replicates, six birds each. Chickens were allocated to the following dietary treatments: the first group was fed a basal diet without supplementation (control), the 2nd and 3rd groups were fed basal diets supplemented with 0.5 and 1.0% of formic acid (FA), the 4th and 5th groups were fed the same basal diets supplemented with 0.5 and 1.0% acetic acid (AA), and the 6th and 7th groups were fed the same basal diets supplemented with 2.0 and 3.0% citric acid (CA). The experimental diets were formulated according to the NRC (1994). Ingredients and chemical composition of the experimental basal diets (% as fed basis) fed during the two phases (starter from days 7 to 20 and grower from days 21 to 35) are shown in Table 1.

Chicks were housed in wire cages (60 cm length × 50 cm depth × 40 cm height) provided with galvanized feeders and automatic nipple drinkers in a semi-opened room equipped with two exhaust fans to maintain normal ventilation.

Chicks were fed the experimental diets *ad libtium* and given free access to water. A light schedule similar to commercial conditions was applied until the 7th day, with 23 h of light followed by 20 h of light from the 8th day until 3 days before the slaughter test (8-32 days of age). The brooding temperature (indoor) was 32, 30, 27, and 24-21 °C during 1-7, 8-14, 15-20, and 21-35 days of age (declined gradually). Chicks in each replicate were weighed (g) weekly between 7 and 35 days of age, and the BWG (g/chick) was calculated. Feed consumption was recorded for each replicate (g/chick), and thereby FCR (g feed/g gain) was calculated. The economic evaluation for all experimental treatments was made (Zeweil, 1996) as below:

$$\text{Economic efficiency} = \frac{\text{Total revenue} - \text{Total cost}}{\text{Total cost}} \times 100$$

Where:

Total revenue = BW × Meat Price

Total cost = Feed cost + Addition cost + Other cost

The European production efficiency index (EPEI) was measured throughout the experimental period (7-35 days of age), according to the Hubbard broiler management guide (1999), as below.

$$\text{EPEI} = \frac{\text{BW (kg)}\ x\ \text{SR}}{\text{PP}\ x\ \text{FCR}}\ x\ 100$$

Where:

EPEI = European Production Efficiency Index; BW = body weight (kg).

SR = survival rate (100% - mortality); PP = production period (days).

FCR = feed conversion ratio (kg feed/ kg gain).

At 35 days of age, the apparent digestibility of nutrients and ash retention were measured using five birds per treatment housed individually in metabolic cages or treatments using the total collection method as cited by Abou-Raya and Galal (1971). The DM, CP and EE of feed and excrement were determined according to (AOAC, 2004) and expressed on dry matter basis.

At the end of experiment, five chicks were taken randomly from each group and slaughtered after 8 hours of fasting, processed, and the weight of the carcass and internal organs (dressing, total edible parts, abdominal fat, spleen, bursa, and thymus) was taken and expressed as the percentage of live BW. Five blood samples (about 3 ml) from each treatment were collected before slaughter from the wing vein for hemato-biochemical analysis. Heparin was utilized as an anticoagulant; however, a portion of the samples was maintained without heparin to acquire serum. Non-coagulated blood was used to test shortly after collection for estimating blood picture. Serum was separated by centrifuging the blood at 3,000 rpm for 20 minutes and then stored at −20°C until biochemical analysis.

Red blood cells, White blood cells, and different subclasses of WBC's (lymphocytes, heterocytes percentages) were counted according to Feldman *et al*. (2000). Packed cell volume (PCV) was measured by a microhaematocrit capillary tube using a Hemocrit reader.

The transaminase enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the calorimetric method of Retiman and Frankel (1957). Alkaline phosphatase (ALP) concentration was determined according to the colorimetric method of Belfield and Goldberg (1971). Kidney functional enzyme (creatinine) was determined according to Fabiny and Ertingshausen, (1971), while uric acid was determined according to the method of Patton and Crouch (1977). In addition, serum samples were assigned for the determination of creatinine and uric acid (Bartles *et al.,* 1972). Serum total lipids and triglyceride concentrations were determined by means of a spectrophotometer according to Chabrol and Charonnat (1973) while total cholesterol was determined according to the recommendation of Stein (1986). High-density lipoprotein (HDL) was measured according to Lopez-Virella (1977), and low-density lipoprotein (LDL) was calculated by the formula of Friedwald *et al*. (1972). Total protein (g/dl) (Henry *et al.*, 1974), albumin (g/dl) (Doumas, 1971), globulin (g/dl) (Coles, 1974), and different types of globulins (α-globulin, β-globulin, and γ-globulin) were determined according to Bossuyt *et al.* (2003). Glucose concentration (mg/dl) was measured according to Trinder (1969). Thyroid hormones: tri-iodothyronine (T3) and thyroxin (T4) were measured according to Sharp et al. (1987). The activity of malondialdehyde (MDA) in the blood was measured using the method of Placer *et al.* (1966). Total antioxidant capacity (TAC) was determined according to Koracevic *et al.* (2001), superoxide dismutase activity (SOD) (Misra and Fridovich, 1972), glutathione peroxidase activity (GSH-Px) (Paglia and Valentine, 1967) and glutathione activity (GSH) (Ellman, 1959).

Measurements were conducted according to the manufacturer's instructions. The lymphocyte transformation test (LTT) was determined following the method described by Balhaa *et al.* (1985). Serum bactericidal activity (BA) of the *Aeromonas hydrophila* strain was determined according to Rainger and Rowley (1993). Serum lysozyme activity (LA) was measured with the turbidimetric method described by Engstad *et al.* (1992), and the results are expressed as one unit of lysozyme activity that is defined as a reduction in absorbance at 0.001/min. Lysozyme activity = (A0 - A)/A.

Phagocytic activity and index were determined according to Kawahara *et al.* (1991). Phagocytic activity (PA) = percentage of phagocytic cells containing yeast cells.

Phagocytic index (PI)= number of yeast cells phagocytized/number of phagocytic cells. Also, immunoglobulins (IgA, IgG, and IgM) were determined using commercial ELISA according to Bianchi *et al*. (1995).

The effect of dietary treatments on the microbial activity of the digestive system was evaluated by measuring the total bacterial count (TBC) and also counting some pathogenic bacteria harboring the intestine, such as *Salmonella*, *Lactobacillus*, *E.coli*, and Proteus spp., according to methods described by ICMSF (1980).

Data obtained were analyzed using the GLM procedure of the Statistical Analysis System (SAS, 2002), using one-way ANOVA as in the following model:

$$Y_{ik} = \mu + T_i + e_{ik}$$

Where Y is the dependent variable; µ is the general mean; T is the effect of experimental treatments; and e is the experimental random error. Before analysis, all percentages were subjected to a logarithmic transformation ($\log_{10}x+1$) to normalize the data distribution. The differences among means were determined using Duncan's new multiple range test (Duncan, 1955).

RESULTS

Growth Performance

The influence of various concentrations of OA on the production performance of broiler chickens is summarized in Table 2. In the overall phase of the study, dietary supplementation with varying concentrations of FA (0.5% and 1.0%), AA (0.5% and 1.0%), and CA (2.0% and 3.0%) led to increases ($p < 0.05$) in final weight by 13.51% and 9.73%, 12.43% and 2.70%, and 11.89% and 3.78%, respectively. These supplements also resulted in notable enhancements ($P < 0.05$) of BWG by 14.95% and 10.72%, 13.82% and 3.04%, and 13.04% and 4.23%, respectively. Furthermore, FCR was significantly improved ($P < 0.05$) by 11.11% and 11.62%, 15.15% and 6.57%, and 9.60% and 7.07%, respectively. Nevertheless, there were no notable variations in feed consumption among the different levels of supplementation. Additionally, broiler chickens supplemented with these additives in the basal diet showed markedly improved economic efficiency and production index compared to the control group. Particularly noteworthy, broilers fed the basal diet with 2% CA exhibited the highest economic efficiency and production index among all experimental groups.

The apparent digestibility of the nutrients and ash retention

The impact of varying concentrations of OA on the apparent digestibility of essential nutrients in broiler chickens is outlined in Table 3. Incorporating organic acid supplements into the diet resulted in notable increases in the digestibility of OM, DM, CP, and EE compared to the control group. Nevertheless, the analysis revealed no significant influence of different supplement levels on the digestibility of CF or apparent ash retention.

Carcass characteristics and relative weight of immune organs

The impact of different levels of OA on the carcass characteristics of broiler chickens is presented in Table 4. Incorporating organic acid supplements into the diet led to an enhanced dressing percentage, while concurrently reducing the percentage of abdominal fat compared to the control group. In contrast, it was observed that chickens receiving the basal diet supplemented with 1% AA or 3% CA exhibited significantly lower dressing% compared to other supplemented groups. Furthermore, no significant effects of different supplement levels were observed on the percentages of spleen, bursa, and thymus.

Hematological traits and liver and kidney functions

The impact of different levels of OA on the hematological traits of broiler chickens is summarized in Table 5. Incorporating different supplements into the diet led to increases in RBC, HB levels, WBC, and lymphocyte count while concurrently decreasing the heterophil to lymphocyte ratio compared to the control group. Furthermore, chickens receiving the basal diet supplemented with 1% AA and 3% CA exhibited significantly lower RBC and HB levels than other supplemented groups. However, no significant effects of different supplement levels were observed on PCV percentage and heterophils percentage. Additionally, no significant effects of varying supplement levels were observed on liver and kidney function, as depicted in Table 6.

Blood biochemical analysis

The impact of different levels of OA on the lipid and protein profiles, as well as the blood glucose and thyroid hormones of broiler chickens, is detailed in Tables 7, 8, and 9. All feed supplements utilized in this study resulted in decreased serum levels of total lipids, cholesterol, and LDL, alongside increased serum triglycerides compared to the control group. Notably, chicks fed a basal diet supplemented with 0.5% and 1.0% FA, 0.5% AA, and 2.0% CA exhibited significantly lower ($P \leq 0.01$) levels of total lipids, cholesterol, and LDL, followed by those fed a basal diet supplemented with 0.5% FA and 3% CA, compared to the control group. However, no significant effects of different supplement levels were observed on HDL levels (Table 7). Furthermore, diets supplemented with OA led to increased levels of total protein, globulin, and γ-globulin compared to the control group. Conversely, chickens fed a basal diet supplemented with 1% AA and 3% CA displayed significantly lower levels of total protein, globulin, and γ-globulin than other supplemented groups. Nonetheless, no significant effects of different supplement levels were detected on albumin, α-globulin, and β-globulin levels (Table 8). Moreover, supplementation with OA resulted in elevated levels of glucose, T3, and T4 compared to the control group. Additionally, chickens fed a basal diet supplemented with 1% AA and 3% CA exhibited significantly lower levels of T3 and T4 than other supplemented groups (Table 9).

Indicators of antioxidant status and immunological status

The impact of different levels of OA on indicators of antioxidative and immunological status in broiler chickens is summarized in Tables 10 and 11. Incorporating different supplements of OA into the diet resulted in increased levels of TAC, reduced GSH, GSH-Px, and SOD, while concurrently decreasing levels of MDA compared to the control group. Notably, chickens fed a basal diet supplemented with 1% AA and 3% CA exhibited significantly lower levels of TAC, GSH, and SOD than other supplemented groups (Table 10). Additionally, diets supplemented with OA led to increased levels of lysozyme activity (LA), bactericidal activity (BA), lymphocyte transformation test (LTT), phagocytic activity, phagocytic index, and immunoglobulins (IgG, IgM, and IgA) compared to the control group. Furthermore, chicks fed a basal diet supplemented with 0.5% and 1.0% FA, 0.5% AA, and 2.0% CA displayed significantly higher ($P \leq 0.01$) levels of BA, phagocytic activity, phagocytic index, and IgA, followed by those fed a basal diet supplemented with 0.5% FA and 3% CA, compared to the control group (Table 11).

Bacterial count

The impact of different levels of OA on the bacterial count of broiler chickens gut microbiota is detailed in Table 12. Incorporating different organic acid supplements into the diet resulted in increased levels of Lactobacillus and decreased levels of total bacterial count, *Escherichia coli* (*E. coli*), and Proteus compared to the control group.

DISCUSSION

The current study elucidates the significant efficacy of incorporating OA-specifically formic, acetic, and citric acid—into broiler diets, substantially enhancing performance metrics. This enhancement is particularly evident in growth rates, FCR, economic efficiency, and production indices as compared to control groups. These findings are in harmony with prior research conducted by Sheikh et al. (2011), Ghazalah et al. (2011), Hassan et al. (2016), and Hossain and Nargis (2016), which collectively reinforce the premise that dietary inclusion of OA is beneficial in augmenting broiler performance. Additionally, the research conducted by ELnaggar and Abo EL-Maaty (2017) underscores that ducklings consuming a basal diet supplemented with OA demonstrated a notable increase in BW, BWG, economic efficiency, and improved feed conversion relative to the control cohort. Specifically focusing on FA, studies by Zhang et al. (2019) and Liu et al. (2020) reported marked enhancements in BW and BWG among broilers, thus underscoring the potential of formic acid in fostering growth and feed efficiency. Correspondingly, Li et al. (2018) found that broilers supplemented with acetic acid exhibited significant improvements in BW and BWG, coupled with an enhanced FCR, indicative of more effective nutrient utilization. However, the literature presents varied outcomes regarding CA supplementation. While Lee et al. (2017) observed an increase in BW and BWG in broilers, other studies reported no significant effects. The study hypothesizes that the observed increase in BWG among ducklings is attributable to the beneficial impact of OA on gut flora. These acids likely disrupt microbial cell membrane integrity, interfere with nutrient transport, and modulate energy metabolism, thereby exerting a bactericidal effect (Ricke, 2003). The acidity introduced into the gastrointestinal tract by these OA bolsters the stomach's defensive barrier against pathogens and enhances digestive enzyme activity. These acidifiers stimulate gastric acid secretion, lower gastrointestinal tract pH, and curtail pathogenic bacteria such as Salmonella and *E. coli* (Hume et al., 1993).

The antimicrobial and pH-altering capabilities of OA are instrumental in suppressing pathogenic intestinal bacteria, thereby reducing their metabolic demands and increasing nutrient availability for the host. This decrease in toxic bacterial metabolites, due to reduced bacterial fermentation, leads to enhanced protein and energy digestibility, culminating in improved weight gain and overall performance (Ghazalah et al., 2011). Furthermore, OA alters the intestinal microbial balance (Al-Kassie, 2009), fostering the predominance of beneficial microbes like Lactobacillus spp. and Bifidobacterium spp., which are integral to gut health. Moreover, the microbial fermentation of OA yields short-chain fatty acids (SCFAs), such as butyrate, which possess antioxidant and anti-inflammatory properties, thereby safeguarding the intestinal mucosa (Abdelqader et al., 2013). The acid-induced low pH environment augments pancreatic enzyme secretion, including amylase, lipase, and protease, thereby facilitating nutrient breakdown into absorbable forms. This acidic milieu also enhances serum calcium and phosphorus levels (Dhawale, 2005), promoting the absorption of essential minerals like Ca, P, Cu, and Zn. Additionally, the acidic environment in the intestines favors the absorption of vitamins A and D. OA also plays a pivotal role in improving villus architecture and functionality, mitigating oxidative damage, and optimizing villus height, surface area, and goblet cell numbers, thereby augmenting nutrient absorption (Abbas et al., 2012).

The influence of OA on animal nutrition extends beyond mere pH reduction. Extensive research has elucidated the multifaceted roles these compounds play in enhancing nutrient uptake and overall health. For instance, citric acid has been identified as a potent enhancer of phosphorus bioavailability in poultry. This is achieved through its ability to chelate calcium ions, thereby mitigating the formation of insoluble calcium phytate complexes, a reaction detailed in studies by Angel et al. (2001) and Snow et al. (2004). Such a mechanism underscores the nuanced role of OA in nutrition beyond simple acidification. Further, the observed improvements in feed conversion ratios (FCR) have been partially attributed to the selective promotion of beneficial gut microbiota by OA, as reported by Jin et al. (2000). This selective enhancement of gut health is believed to facilitate more efficient nutrient absorption and metabolism, leading to improved growth performance metrics such as body weight gain. Naghmeh and Jahanian (2012) have supported this notion, suggesting that the enhancement in FCR is likely a result of improved nutrient utilization efficiency. Additionally, the antibacterial properties of OA, particularly against pathogenic strains such as *E. coli* and Salmonella in the gastrointestinal tract, further contribute to their beneficial effects on animal health and nutrient utilization (Dhawale, 2005). This body of evidence collectively highlights the complex interplay between OA and animal nutrition, pointing to mechanisms that extend well beyond pH modulation. Through their capacity to chelate minerals, selectively modulate gut microbiota, and exert antibacterial effects, OA emerge as valuable dietary supplements in poultry nutrition, offering a multifaceted approach to improving growth performance and feed efficiency.

Incorporating organic acid supplements into the diet has shown a significant increase in OM, DM, CP, and EE levels compared to control groups. This enhancement aligns with the findings of Nourmohammadi et al. (2012), who observed that a 3% citric acid supplementation, in conjunction with microbial phytase, improved ileal nutrient digestibility (including CP, apparent metabolizable energy (AME), calcium, and total phosphorus) and mineral retention in broiler chickens. Similarly, Ghazalah et al. (2011) reported that the addition of fumaric, formic, acetic, and citric acids to broiler diets notably improved metabolizable energy and nutrient digestibility metrics such as CP, EE, CF, and nitrogen-free extract (NFE). Van Der Sluis (2006) suggested that the digestion-enhancing effects of OA are linked to a slowed feed passage through the digestive tract, facilitating improved nutrient absorption and resulting in drier droppings. This observation is supported by a substantial body of literature, with numerous studies corroborating the positive impact of organic acid supplementation on nutrient digestibility in broiler feed (Hernández et al., 2006; García et al., 2007; Rodjan et al., 2017; ELnaggar and Abo EL-Maaty, 2017; Sureshkumar et al., 2021). Organic acids contribute to this improvement by lowering the digesta's pH and enhancing gastric proteolytic activity, as detailed by Khan et al. (2016). The specific pH modulation within different intestinal segments plays a crucial role in promoting beneficial microbial populations, which are pivotal for efficient digestion and nutrient absorption. This modulation is particularly relevant given that most pathogenic bacteria thrive at a pH close to neutral (7.0), whereas beneficial bacteria prefer slightly acidic conditions (pH 5.8–6.2). By reducing the intestinal pH, OA creates an environment conducive to beneficial bacterial growth while suppressing pathogenic microbes (Haque et al., 2009), thereby optimizing nutrient digestion and absorption. Furthermore, OA is thought to stimulate pepsin activity, facilitating protein proteolysis into simpler peptides. This process triggers the release of digestive hormones such as gastrin and cholecystokinin, and promotes the secretion of pancreatic juice enriched with digestive enzymes like procarboxypeptidases, chymotrypsinogen, and trypsin (Adil et al., 2010). The resultant slower digesta passage rates, in the presence of OA, enhance nutrient absorption efficiency from the intestines (Abudabos et al., 2017). Additionally, the acidic environment reduces the production of bacterial metabolites such as ammonia and amines (Samanta et al., 2010), further improving digestibility. The efficacy of OA in enhancing nutrient digestibility is also linked to its role in augmenting the release of digestive enzymes, activating microbial phytase, and increasing pancreatic activity within the gut (Hernández et al., 2006). Collectively, these mechanisms underscore the multifaceted benefits of OA supplementation in poultry nutrition, highlighting its capacity to improve growth performance through enhanced feed efficiency and nutrient utilization. Dietary supplementation with various OA has been observed to enhance dressing percentages and total edible parts while concurrently reducing abdominal fat percentages in comparison to control groups. These findings are consistent with the work of Talebi et al. (2010), who noted improvements in the relative weights of carcass, giblets, and dressing in birds fed diets supplemented with citric acid over those in the control group. Similarly, Ghazalah et al. (2011) demonstrated that dietary inclusion of OA enhanced the relative weights of carcass, giblets, and dressing in birds supplemented with citric acid at a dosage of 2 g/kg relative to control birds. Further supporting these observations, ELnaggar and Abo EL-Maaty (2017) reported significant increases in the percentages of dressing and total edible parts, alongside a reduction in abdominal fat, with supplementation of either formic or citric acids at tested levels compared to controls. In addition to physical characteristics, dietary supplementation with OA has been linked to improvements in various blood parameters, including increases in glucose, thyroid hormones (T3 and T4), total protein, globulin fractions (α-globulin, γ-globulin), immunoglobulins (IgA, IgM, and IgG), lysozyme activity (LA), bactericidal activity (BA), lymphocyte transformation test (LTT), phagocytic activity, phagocytic index, RBCs, HB, WBCs, and triglycerides. Conversely, a decrease in serum total lipids, cholesterol, and low-density lipoprotein (LDL) levels was observed when compared to the control group. These results align with findings from studies on broiler chicks and ducks by Ghazalah et al. (2011) & ELnaggar and Abo EL-Maaty (2017), respectively, which highlighted that dietary OA led to an increased concentration of total protein and globulin, indicative of an enhanced immune response and disease resistance. The observed increase in globulin levels, a key indicator of immune responses and a source of antibodies, suggests that supplemental OA may bolster immune function. This proposition is supported by Rahmani and Speer (2005), who found an elevated percentage of gamma globulin in broilers receiving OA compared to those in the control group. The improvement in immune response attributed to dietary acidification may stem from the inhibitory effects of these compounds against pathogenic microorganisms throughout the gastrointestinal tract. Furthermore, the adjustments in serum lipid profiles and indicators of antioxidative status corroborate the findings of Kamal and Ragaa (2014) and ELnaggar and Abo EL-Maaty (2017), who reported significant reductions in blood total lipids, triglycerides, and cholesterol following dietary acidification. The beneficial impact of OA on blood lipid profiles may be elucidated through their role in diminishing microbial intracellular pH, as suggested by Abdel-Fattah et al. (2008).

The dietary inclusion of OA also led to an increase in Lactobacillus counts while decreasing the total bacterial count of *E. coli* and Proteus spp. in comparison to control groups. These results are in line with those reported by ELnaggar and Abo EL-Maaty (2017), who noted a reduction in total bacterial count, Salmonella, *E. coli*, and Proteus spp., with all dietary supplements compared to control groups. Such findings highlight the significant role of OA in reducing both total bacterial and gram-negative bacterial counts in broiler chickens, as demonstrated by Gunal et al. (2006). Abdel-Fattah et al. (2008) further elucidated that the lowered pH fosters the proliferation of beneficial bacteria while inhibiting the growth of pathogenic bacteria, which thrive at relatively higher pH levels.

In conclusion, incorporating organic acids as alternatives to antibiotics in broiler chicken diets significantly improves production performance, enhances digestibility, reduces abdominal fat, and enhances immune response. The study recommends the use of organic acid supplements to promote economic efficiency and production indices in poultry farming while maintaining optimal health.

Table (1): Ingredients and chemical composition of the experimental basal diets.

Ingredients (%)	**Starter**	**Grower**
Yellow corn	53.85	61.63
Soybean meal (44% CP)	34.28	27.50
Vegetable oil	3.00	3.00
Gluten meal	5.00	4.00
Dicalcium phosphate	1.69	1.69
Limestone	1.45	1.45
L-Lysine	0.03	0.03
DL-Methionine	0.10	0.10
Vit+min premix[1]	0.30	0.30
NaCl	0.30	0.30
Total	100	100
Calculated and determined composition,		
DM,%[2]	86.16	86.26
DM,%[3]	86.34	86.33
ME (Cal/kg)[3]	3016	3116
CP,%[2]	22.89	19.96
CP,%[3]	23.01	20.09
Crude Fat, %[2]	5.32	5.53
Crude Fat, %[3]	5.45	5.66
Crude fiber, %[2]	3.83	3.42
Crude fiber, %[3]	3.72	3.39
Lysine, %[3]	1.13	0.96
Methionine, %[3]	0.50	0.46
Calcium, %[3]	1.06	1.04
Av. Phosphorus, %[3]	0.46	0.45
Ash, %[2]	5.10	5.34

[1]Vit+Min mix. provides per kilogram of the diet: Vit. A, 12000 IU, vit. E (dl-α-tocopherol acetate) 20 mg, menadione 2.3 mg, Vit. D3, 2200 ICU, riboflavin 5.5 mg, calcium pantothenate 12 mg, nicotinic acid 50 mg, Choline 250 mg, vit. B_{12} 10 μg, vit. B_6 3 mg, thiamine 3 mg, folic acid 1 mg, d-biotin 0.05 mg. Trace mineral (mg/ kg of diet): Mn 80 Zn 60, Fe 35, Cu 8, and Selenium 0.1 mg. [2]Analyzed values. [3]Calculated values.

Table (2): Effect of dietary inclusion with different levels of organic acid (citric, formic, and acetic) on productive performance, economic efficiency and production index of *Cobb* 500 broiler chicks

Traits		BW 7 d	BW 35d	BWG (7-35 d)	FC (7-35d)	FCR (7-35d)	EEF*	REE (%) **	EPEI ***
Control	0	171	1850^{c}	1679^{c}	3320	1.98^{a}	0.359^{c}	100	267^{d}
Formic	0.5 %	170	2100^{a}	1930^{a}	3400	1.76^{c}	0.457^{b}	127	341^{b}
	1.0 %	171	2030^{a}	1859^{a}	3260	1.75^{c}	0.448^{b}	125	331^{b}
Acetic	0.5%	169	2080^{a}	1911^{a}	3210	1.68^{c}	0.539^{b}	150	354^{b}
	1%	170	1900^{b}	1730^{b}	3200	1.85^{b}	0.417^{b}	116	293^{c}
Citric	2.0 %	172	2070^{a}	1898^{a}	3390	1.79^{c}	0.745^{a}	207	401^{a}
	3.0 %	170	1920^{b}	1750^{b}	3220	1.84^{b}	0.415^{b}	115	298^{c}
SEM		2.09	18.98	12.98	16.90	0.087	0.070	--	4.01
P value		0.087	0.001	0.002	0.072	0.001	0.001	--	0.001

[a,b] Means in the same column followed by different letters are significantly different at $P \leq 0.05$. SEM; Standard error of mean. BW: Body weight, BWG: Body weight gain, FC: Feed consumption, FCR: Feed conversion ratio, * EEF: Economic efficiency= Net Revenue/ Total cost, ** REE: Relative economic efficiency; Assuming the REE of the control= 100, *** EPEI = European Production Efficiency Index

Table (3): Effect of dietary inclusion with different levels of organic acid (citric, formic, and acetic) on the apparent digestibility of the nutrients and ash retention of broiler chicks.

Dietary supplementations		OM	DM	CP	EE	CF	Apparent Ash retention
Control	0	59.5^{c}	63.90^{b}	72.87^{c}	68.13^{c}	18.00	34.00
Formic	0.5 %	66.4^{b}	68.30^{a}	83.00^{a}	88.70^{a}	16.20	33.90
	1.0 %	68.6^{b}	69.11^{a}	87.12^{a}	89.71^{a}	17.88	32.48
Acetic	0.5%	67.9^{b}	69.90^{a}	89.90^{a}	86.61^{a}	18.89	34.93
	1%	66.9^{b}	64.76^{ab}	79.30^{b}	78.89^{b}	18.01	30.02
Citric	2.0 %	71.7^{a}	70.76^{a}	87.98^{a}	86.67a	16.89	32.09
	3.0 %	65.3^{b}	65.9^{ab}	89.4^{a}	77.18^{b}	17.09	33.00
SEM		2.09	1.98	2.77	2.66	1.89	5.09
P value		0.002	0.003	0.001	0.002	0.072	0.087

[a, b] Means in the same column followed by different letters are significantly different at $P \leq 0.05$; SEM, Standard error of means. OM: Organic matter, DM: Dry matter, CF= Crude fiber, EE= Ether extract, CP= Crude protein,

Table (4): Effect of dietary inclusion with different levels of organic acid (citric- formic- acetic) on carcass characteristics and relative weight of immune organs to live body weight of broiler chickens

Traits		Dressing, %	Abdominal fat, %	Spleen, %	Bursa, %	Thymus, %
Control	0	63.71[c]	0.157[a]	0.188	0.617	0.617
Formic	0.5 %	71.00[a]	0.139[b]	0.143	0.569	0.569
	1.0 %	73.20[a]	0.127[b]	0.184	0.675	0.575
Acetic	0.5%	69.00[a]	0.119[b]	0.162	0.644	0.600
	1%	65.12[b]	0.120[b]	0.177	0.555	0.605
Citric	2.0 %	72.70[a]	0.111[b]	0.167	0.601	0.608
	3.0 %	67.00[ab]	0.112[b]	0.198	0.611	0.589
SEM		0.602	0.087	0.087	0.087	0.098
P value		0.001	0.001	0.065	0.076	0.088

[a, b] Means in the same column followed by different letters are significantly different at $P \leq 0.05$; SEM, Standard error of means.

Table (5): Effect of dietary inclusion with different levels of organic acid (citric- formic- acetic) on hematological traits of broiler chicks.

Dietary supplementations		Hematological parameters			White blood cells and differential leukocytes counts			
		RBCs ($10^6/mm^3$)	Hb (g/dl)	PCV %	WBCs ($10^3/mm^3$)	Hetero. (%)	Lympho. (%)	H/L ratio
Control	0.00	3.25[c]	9.89[c]	23.89	21.88[b]	13.88	41.90[b]	0.331[a]
Formic	0.5 %	4.14[a]	12.56[a]	25.98	24.89[a]	12.98	44.98[a]	0.289[b]
	1.0 %	4.26[a]	13.01[a]	27.98	27.87[a]	11.99	43.89[a]	0.273[b]
Acetic	0.5 %	3.99[a]	12.97[a]	28.88	26.89[a]	12.09	44.98[a]	0.269[b]
	1.0 %	3.54[b]	11.76[b]	24.89	25.89[a]	12.12	43.09[a]	0.281[b]
Citric	2.0 %	4.01[a]	12.34[a]	26.89	27.76[a]	12.34	46.00[a]	0.268[b]
	3.0 %	3.76[b]	11.56[b]	24.09	26.93[a]	12.78	45.67[a]	0.280[b]
SEM		0.987	4.90	6.99	8.65	1.23	4.99	0.087
P value		0.001	0.002	0.087	0.003	0.087	0.002	0.003

[a, b] Means in the same column followed by different letters are significantly different at $P \leq 0.05$; SEM, Standard error of means. HB: Hemoglobin; RBCs: red blood cell; PCV: packed cell volume; WBCs: white blood cells

Table (6): Effect of dietary inclusion with different levels of organic acid (citric- formic- acetic) on liver and kidney function of broiler chicken.

Dietary supplementations		Liver function			Kidney function	
		AST (U/L)	ALT (U/L)	ALK (U/L)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	0	60.98	40.09	12.09	0.780	2.44
Formic	0.5 %	61.11	38.90	11.89	0.809	2.30
	1.0 %	59.89	36.49	12.34	0.766	1.99
Acetic	0.5%	62.89	35.55	12.15	0.801	2.09
	1%	60.43	36.91	11.98	0.776	2.17
Citric	2.0 %	61.00	37.87	12.04	0.811	2.10
	3.0 %	58.79	39.00	12.25	0.821	2.21
SEM		4.89	2.67	4.98	0.017	0. 981
P value		0.098	0.076	0.098	0.076	0.076

[a,b,c] Means in the same row followed by different letters are significantly different at $P \leq 0.05$;.
SEM= Standard error of means. AST=aspartate amino transferase; ALT=alanine amino transferase; Alk =Alkaline phosphatase;

Table (7): Effect of dietary inclusion with different levels of organic acid (citric- formic- acetic) on lipid profile of broiler chickens

Dietary supplementations		Total lipids (mg/ dl)	Cholesterol (mg/ dl)	Triglycerides (mg/ dl)	HDL (mg/ dl)	LDL (mg/ dl)
Control	0.0%	411[a]	199[a]	86.98[b]	45.90	131.70[a]
Formic	0.5 %	356[c]	123[c]	99.98[a]	60.99	42.01[c]
	1.0 %	362[c]	120[c]	109.9[a]	61.56	36.46[c]
Acetic	0.5%	350[c]	119[c]	104.98[a]	59.99	38.01[c]
	1.0 %	397[b]	160[b]	94.67[a]	50.98	90.08[b]
Citric	2.0 %	344[c]	132[c]	100.98[a]	59.44	52.36[c]
	3.0 %	398[b]	189[b]	95.99[a]	52.34	117.46[b]
SEM		12.89	13.90	21.09	9.98	29.89
P value		0.001	0.002	0.001	0.081	0.001

[a,b,c] Means in the same row followed by different superscripts are significantly different at $P \leq 0.05$; SEM= Standard error of means, Chol.= total cholesterol; TG= triglycerides; HDL=high-density lipoprotein; LDL=low-density lipoprotein,

Table (8): Effect of dietary inclusion with different levels of organic acid (citric- formic- acetic) on Protein profile (g/dl) of broiler chickens.

Dietary supplementations		**Total protein**	**Albumin**	**Globulin**	**α–globulin (μg/dl)**	**β – globulin (μg/dl)**	**γ - globulin (μg/dl)**
Control	0.0%	3.59[c]	1.11	2.48[c]	0.970	0.956	0.954[c]
Formic	0.5 %	4.44[a]	1.09	3.35[a]	0.840	0.644	1.86[ab]
	1.0 %	4.98[a]	1.04	3.94[a]	0.899	0.899	2.14[a]
Acetic	0.5%	4.97[a]	1.02	3.95[a]	0.766	0.820	2.36[a]
	1%	4.21[b]	1.01	3.20[ab]	0.780	0.799	1.62[b]
Citric	2.0 %	4.89[a]	1.12	3.77[a]	0.690	0.760	2.32[a]
	3.0 %	4.09[b]	1.14	2.95[b]	0.654	0.822	1.47[b]
SEM		1.11	0.987	0.987	0.092	0.022	0.920
P value		0.001	0.087	0.002	0.0871	0.0917	0.001

[a,b,]Means in the same row followed by different letters are significantly different at $P \leq 0.05$; SEM, Standard error of mean.

Table (9): Effect of dietary inclusion with different levels of organic acid (citric- formic- acetic) on blood glucose and thyroid hormones of broiler chicks.

Dietary supplementations		Glucose (mg/dl)	T3 (ng/dl)	T4 (ng/dl)
Control	0.0	176[b]	2.59[c]	9.91[c]
Formic	0.5 %	199[a]	3.99[a]	12.01[a]
	1.0 %	201[a]	3.87[a]	11.90[a]
Acetic	0.5%	189[a]	3.90[a]	12.03[a]
	1%	186[a]	3.00[b]	10.9[b]
Citric	2.0 %	192[a]	3.82[a]	12.34[a]
	3.0 %	189[a]	3.01[b]	11.01[b]
SEM		11.90	0.980	2.98
P value		0.001	0.001	0.002

[a, b] Means in the same column followed by different letters are significantly different at $P \leq 0.05$; SEM, Standard error of means. T3= triiodothyronine; T4=thyroxine.

Table (10): Effect of dietary inclusion with different levels of organic acid (citric- formic- acetic) on indicators of antioxidative status of broiler chicks.

Dietary supplementations		Indicators of antioxidative status in blood (mg/dl)				
		MDA (µmol/L)	**TAC (nmol/L)**	**GSH (mmol/L)**	**GSH-Px (mmol/L)**	**SOD (U/ml)**
Control	0	35.90^{a}	2.09^{c}	6.43^{c}	2.98^{b}	1.09^{c}
Formic	0.5 %	32.11^{b}	3.02^{a}	9.11^{a}	3.66^{a}	1.98^{a}
	1.0 %	31.76^{b}	2.99^{a}	9.98^{a}	3.76^{a}	1.89^{a}
Acetic	0.5%	30.09^{b}	3.04^{a}	10.02^{a}	3.80^{a}	1.95^{a}
	1%	29.99^{b}	2.39^{b}	8.90^{b}	3.66^{a}	1.56^{b}
Citric	2.0 %	32.81^{b}	2.98^{a}	9.87^{a}	3.23^{a}	1.97^{a}
	3.0 %	33.04^{b}	2.50^{b}	8.67^{b}	3.55^{a}	1.70^{b}
SEM		5.90	0.987	1.98	0.981	0.087
P value		0.001	0.002	0.001	0.002	0.002

a, b Means in the same column followed by different letters are significantly different at $P \leq 0.05$; SEM, Standard error of means. TAC=total antioxidant capacity; ; GSH-Px =glutathione peroxidase; SOD=superoxide dismutase, MDA= malondialdehyde

Table (11): Effect of dietary inclusion with different levels of organic acid (citric- formic- acetic) on immunological status of broiler chicks

		Immunological status					**Immunoglobulines (mg/dl)**		
Dietary supplementations		**LA**	**BA**	**LTT**	**PA**	**PI**	**IgG**	**IgM**	**IgA**
Control	0	0.680^{b}	31.11^{c}	19.99^{b}	17.09^{c}	1.56^{c}	801.93^{c}	211.23^{b}	62.98^{c}
Formic	0.5 %	0.820^{a}	37.87^{a}	22.01^{a}	25.09^{a}	1.76^{a}	884.01^{a}	229.0^{a}	90.91^{a}
	1.0 %	0.823^{a}	38.9^{a}	23.42^{a}	26.91^{a}	1.79^{a}	896.26^{a}	228.0^{a}	89.76^{a}
Acetic	0.5%	0.798^{a}	39.00^{a}	24.09^{a}	24.22^{a}	1.81^{a}	876.40^{b}	249.5^{a}	87.76^{a}
	1%	0.790^{a}	33.09^{b}	24.00^{a}	20.19^{b}	1.68^{b}	881.80^{a}	236.9^{a}	76.5^{b}
Citric	2.0 %	0.811^{a}	37.61^{a}	23.98^{a}	25.76^{a}	1.80^{a}	842.56^{b}	245.8^{a}	86.5^{a}
	3.0 %	0.802^{a}	34.01^{b}	25.02^{a}	21.99^{b}	1.69^{b}	890.90^{a}	240.9^{a}	70.98^{b}
SEM		0.065	3.90	4.09	3.98	0.098	5.78	3.92	4.01
P value		0.001	0.001	0.001	0.002	0.002	0.001	0.002	0.001

[a, b] Means in the same column followed by different letters are significantly different at $P \leq 0.05$; SEM, Standard error of means. IgG= Immunoglobulin G; IgA= Immunoglobulin A IgM= Immunoglobulin M . LA= Lysosome activity; BA= Bactriocide activity ; LTT= Lymphocyte transformation test; PA= Phagocyte activity; PI = Phagocytic index.

Table (12): Effect of dietary inclusion with different levels of organic acid (citric- formic-acetic) **on bacterial count of broiler chicks.**

Dietary supplementations		TBC (cfu x 10^6)	Lactobacillus (cfu x 10^3)	*E.Coli* (cfu x 10^3)	*Proteus.* (cfu x 10^3)
Control	0.0	4.23[a]	1.66[c]	1.70[a]	0.99[a]
Formic	0.5 %	3.59[b]	2.13[a]	0.94[b]	0.76[b]
	1.0 %	3.51[b]	2.39[a]	0.92[b]	0.66[b]
Acetic	0.5%	3.37[b]	2.60[a]	0.68[c]	0.31[c]
	1%	3.43[b]	2.16[a]	0.97[b]	0.68[b]
Citric	2.0 %	3.20[c]	2.11[b]	0.68[c]	0..45[c]
	3.0 %	3.19[c]	2.03[b]	0.98[b]	0.65[b]
SEM		0.156	0.087	0.078	0.064
P value		0.001	0.002	0.002	0.002

a, b Means in the same column followed by different letters are significantly different at $P \leq 0.05$; SEM, Standard error of means. TBC = Total Bacterial Count

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الملخص العربى

أداء النمو ومعاملات هضم العناصر الغذائية وقياسات الدم لدجاج اللحم المغذى على عليقة تحتوي على الأحماض العضوية

أسماء شوقي النجار [1]، محمود إبراهيم الكيلاوي[2]
[1] قسم الانتاج الحيواني والداجني – كليه الزراعة – جامعه دمنهور.
[2] قسم إنتاج الدواجن - كلية الزراعة - جامعة الوادي الجديد

تهدف الدراسة إلى تقييم تأثير مستويات مختلفة من الأحماض العضوية (الفورميك، الخليك، الستريك) على أداء نمو دجاج اللحم، وهضم العناصر الغذائية، وصفات الذبيحة، وبعض معايير الدم، وعدد البكتيريا، وحالة مضادات الأكسدة، والاستجابة المناعية، والكفاءة الاقتصادية. تم توزيع 210 كتكوت عمر يوم غير مجنس عشوائيًا على سبع مجموعات، حيث كانت كل مجموعة تحتوي على 5 تكرارات بكل منها 6 كتاكيت. تم استخدام المجموعة الأولى كمجموعة مقارنة. تم تغذية الطيور في المجموعات 2 و 3 بعليقة تحتوي على حمض الفورميك (0.5% و 1.0%)، بينما تم تغذية الطيور في المجموعات 4 و 5 بعليقة تحتوي على حمض الخليك (0.5% و 1.0%). وأما الطيور في المجموعات 6 و 7 فتم تغذيتها بعليقة تحتوي على حمض الستريك (2.0% و 3.0%). تم الوزن الأسبوعي وتسجيل استهلاك العلف وتقدير الأداء الانتاجي. بالإضافة إلى ذلك، تم تحليل المعايير البيوكيميائية والهيماتولوجية والمناعية، إلى جانب تقييم النشاط الميكروبي للجهاز الهضمي. وقد لوحظ تحسن في الأداء الانتاجي، بما في ذلك الوزن النهائي ومعدل زيادة في وزن الجسم، مع استخدام اضافات حمض الفورميك والخليك والستريك، بالإضافة إلى تحسن في معدل تحويل العلف. زادت نسبة الذبيحة بينما قلت نسبة الدهون البطنية مع الاستخدام. كشف التحليل الهيماتولوجي عن تحسن في مقايس الدم. كما أثرت الاضافات بشكل إيجابي على مكونات الدم من البروتينات والدهون. بالإضافة إلى ذلك، تم تعزيز الحالة المضادة للأكسدة والمناعية، مما يتجلى في زيادة نشاط الإنزيمات المضادة للأكسدة والاستجابة المناعية. علاوة على ذلك، أدت المكملات إلى تغييرات إيجابية في تركيبة البكتيريا في الأمعاء، مع زيادة في مستويات اللاكتوباسيلوس وانخفاض في عدد البكتيريا، بما في ذلك *E. Coli* والبروتيوس. في الختام، فإن إضافة الأحماض العضوية كبديل للمضادات الحيوية في تغذية دجاج اللحم يعزز بشكل كبير أداء الإنتاج وتعزز الكفاءة الاقتصادية مع الحفاظ على الصحة الأمثل.