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Effects of Dietary Inclusion of Probiotics and Organic Acids on Performance, Intestinal Microbiology, Serum Biochemistry and Carcass Traits of Broiler Chickens

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ABSTRACT

This study was conducted to evaluate the effects of probiotics and organic acids, as alternative feed additives to antibiotics, on productive performance of broilers. Two different types of probiotics varying in the microbial content were tested and organic acids blend was compared against a single organic acid (lactic acid). One hundred and ninety eight broiler chicks were randomly allocated into six treatments, each with 33 chicks. Every treatment consisted of 3 replicates with 11 birds per replicate. The dietary treatments were a control diet without any feed additives or the same control diet supplemented either with a commercial antibiotic (Maxus®G200), probiotics (Bactocell® or Biopellet-S®) or acidifiers (Salmo-Nil Dry® or lactic acid). The antibiotic was added to the diet at the rate of 0.005%, whereas the probiotics were used at 0.01%. The product Salmo-Nil Dry® was provided to the diet at a level of 0.4%, whereas the lactic acid was used at 0.20 %. It was found that the antibiotic, probiotics and lactic acid increased the body weight. All dietary supplements improved the FCR compared to the control. The additives reduced the serum cholesterol level and the pH of small intestine but did not affect the carcass yield, breast or organ weights. The feed supplements showed a numerical decrease in intestinal aerobes, fecal coliforms and *E. coli* counts. In addition, all additives significantly reduced total aerobic and staphylococcus counts in the carcass meat, with a numerical decrease in *E. coli* count. In conclusions, probiotics and acidifiers can be used as potential alternatives to antibiotics in broiler diets. No difference between the used types of probiotics was detected. Lactic acid alone seems to produce better performance results than the organic acid mixture. The effect of lactic acid produced by bacteria might be similar to that of the chemical one.

Key words: Broilers, Probiotic, Organic acids, Performance, Lactic acid, Carcass

INTRODUCTION

The efficiency of poultry digestion depends on the microorganisms which live naturally in the digestive tract. Certain feed additives can be added to the diet to create favourable conditions in the intestinal tract for the digestion of feed. Antibiotics have been extensively used in poultry diets to control diseases and improve the productive performance. However, the use of antibiotics resulted in several complications such as development of drug- resistant bacteria (Sorum and Sunde, 2001), drug residues in the birds' body (Burgat, 1999), and imbalance of normal intestinal microflora (Andremont, 2000). Therefore, many countries in Europe have been banned the antibiotics usage as feed additives. As a result, there is an increasing interest in finding alternatives to antibiotics in poultry industry. Among these alternatives are the use of probiotics and organic acids in the animal nutrition. These additives are generally recognized as safe and are commonly used in recent years.

Probiotics are live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). These additives

are acting through maintaining a beneficial microbial population by competitive exclusion and antagonism (Fuller, 1989; Katoch et al., 2017), enhancing feed intake and digestion (Nahanshon et al., 1993; Hossein et al., 2017), and modifying bacterial metabolism (Jin et al., 1997; Pourakbari et al., 2016). Lactobacilli and enterococci are among the wide variety of microbial species that have been used extensively in poultry diets as probiotics (Patterson and Burkholder, 2003). The feeding of probiotics has been reported to improve growth performance and feed efficiency in broiler chickens (Kabir et al., 2004; Mountzouris et al., 2007; Samli et al., 2007, Awad et al., 2009; Tabidi et al, 2013; Nawaz et al., 2016).

The organic acids have antimicrobial activity as they are undissociated and can penetrate the bacterial cell wall and upset the growth of certain types of bacteria (Dhawale, 2005). Additionally, these acids can diminish the pH values of digesta and have trophic impacts on the mucosa of digestive tract (Dibner and Buttin, 2002). Furthermore, organic acids supplementations have been found to reduce colonization of pathogens on the intestinal wall and production of bacterial toxins, thus preventing the damage to the intestinal epithelial cells (Langhout, 2000). These acids can also improve the digestibility of protein and minerals such as Ca, P, Mg and Zn (Kirchgessner and Roth, 1988; Waseem Mirza et al., 2016). The use of organic acids and probiotics has been reported to protect the chicks by competitive exclusion (La Ragione and Woodward, 2003; Hassan et al., 2010), increase the nutrient utilization and performance (Denli et al., 2003; Adil et al., 2010; Khan and Iqbal, 2016).

Therefore, the present study was conducted to evaluate the effects of probiotics and organic acids, as alternative feed additives to antibiotics, on productive performance of broilers. Furthermore, two different types of probiotics varying in the microbial content were tested. The first probiotic type consisted of *Pediococcus acidilactici* bacteria, whereas the second one composed of *Bacillus subtilis* and *Enterococcus faecium*. In addition, a commercial acidifier product, which consists of organic acids blend, was investigated against a single organic acid (lactic acid). Moreover, the impacts of natural lactic acid produced by ‘’probiotic’’ bacteria (*Pediococcus acidilactici)* were compared with the ‘’chemical’’ lactic acid per se.

MATERIALS AND METHODS

Birds and diets

One hundred and ninety eight, one-day-old, broiler chicks (Cobb 500) were obtained from a local commercial hatchery. The birds were randomly allocated into six treatments, each with 33 chicks. Every treatment consisted of 3 replicates with 11 birds per replicate. The chicks were housed in pens (1.10 x 1.0 m^2 per replicate pen) with a bedding of wood shavings. The experiment lasted for 42 days. The initial brooding temperature was 33°C in the first week of age and reduced gradually 2°C per week until reaching about 20 °C at the end of experiment. A lightening period of 23 h per day was provided throughout the experimental period. The dietary treatments were a control diet without any feed additives or the same control diet supplemented either with a commercial antibiotic (Maxus®G200), probiotics (Bactocell® or Biopellet-S®) or acidifiers (Salmo-Nil Dry® or lactic acid). The antibiotic (Elanco Animal Health, USA) contained 200g of avilamycin activity per kg. Both types of probiotics were different and varied in the microbial composition. Bactocell® (Lallemand S.A.S, France) consisted of lactic acid producing bacteria, *Pediococcus acidilactici* 1.0 x10^{10} CFU /g, and dextrose as a carrier up to 1g, while Biopellet-S® (Samu Median Co., LTD, Korea) comprised of *Bacillus subtilis* 3.0 x 10^{10} CFU and *Enterococcus faecium* 3.0 x 10^{10} CFU per kg, and dextrose up to 1 kg. Also, two different kinds of acidifiers were used; Salmo-Nil Dry® (Nutri- AD International, Belgium) which is a commercial by-product containing a group of acids (Ca – formate 60%, Ca – propionate 10%, Ca - lactate 10%, Ca - citrate 20%), whereas the second one consisted of one type of acid (lactic acid powder-food grade 88%, ICIS, UK).The antibiotic was added to the diet at the rate of 0.005%, whereas the probiotics were used at 0.01%. The product Salmo-Nil Dry® was provided to the diet at a level of 0.4%, whereas the lactic acid was used at 0.20 %.The dietary doses of the tested feed additives, except lactic acid, were used according to the recommended levels of the produced companies.

During the experiment, the birds were fed on a starter diet for 21 days, and then switched to a grower diet from day 22 up to day 42. The diets were calculated to meet or exceed the nutrient requirements for broiler chickens recommended by NRC for poultry (1994). The control and experimental diets were formulated to have the same nutrient contents. The experimental diets were supplemented without (control) or with the tested feed additives. The antibiotic and probiotics were added to the diets in very small proportions by substituting equal amounts of corn, while the acidifier diets were formulated by adjusting the amounts of corn, vegetable oil and soybean meal (SBM) to maintain the energy density and protein level. Ingredients and chemical composition of the diets are shown in Table 1 and 2. The used ingredients

were analyzed for their proximate composition using the standard laboratory methods according to AOAC (2005). The diets were formulated based on the nutrient contents of the ingredients. Feed and water were offered to the birds *ad libitum* during the experiment.

Ethical approval

All animal procedures were approved by the Animal Ethics Committee at Faculty of Veterinary Medicine, Beni-Suef University, Egypt.

Table 1. Physical and chemical composition (%) of the starter diets (as fed)

Composition	Group					
	Control	Antibiotic	Probiotics		Acidifiers	
	(-)	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid
Dietary ingredients						
Yellow corn	46.93	46.925	46.92	46.92	46.06	46.49
Soybean meal	36.00	36.00	36.00	36.00	36.17	36.09
Sunflower oil	7.08	7.08	7.08	7.08	7.38	7.23
Corn gluten	6.00	6.00	6.00	6.00	6.00	6.00
Dicalcium phosphate	1.70	1.70	1.70	1.70	1.70	1.70
Limestone	1.41	1.41	1.41	1.41	1.41	1.41
Common salt	0.47	0.47	0.47	0.47	0.47	0.47
DL- methionine	0.11	0.11	0.11	0.11	0.11	0.11
Vit. and min. premix[1)]	0.30	0.30	0.30	0.30	0.30	0.30
Feed additives	-	0.005	0.01	0.01	0.40	0.20
Chemical composition						
Metabolizable energy, kcal/kg	3200.0	3199.8	3199.6	3199.6	3200.1	3200.0
Dry matter	91.39	91.39	91.38	91.38	91.07	91.23
Crude protein	23.0	23.0	23.0	23.0	23.0	23.0
Methionine	0.51	0.51	0.51	0.51	0.51	0.51
Methionine + Cystine	0.90	0.90	0.90	0.90	0.90	0.90
Lysine	1.15	1.15	1.15	1.15	1.15	1.15
Ether extract	9.43	9.43	9.43	9.43	9.71	9.57
Crude fiber	3.13	3.13	3.13	3.13	3.13	3.13
Ash	6.39	6.39	6.39	6.39	6.40	6.40
Calcium	1.00	1.00	1.00	1.00	1.00	1.00
Phosphorus, available	0.45	0.45	0.45	0.45	0.45	0.45
Sodium	0.20	0.20	0.20	0.20	0.20	0.20

[1)] Vitamins and minerals premix (Agri-Vet Company, Egypt): each 3.0 kg contain Vit. A, 12000000 IU; Vit.D_3 2000000 IU; Vit.E, 10000 mg; Vit.K_3, 2000 mg; Vit.B_1, 1000 mg; Vit.B_2, 5000 mg; Vit. B_6, 1500 mg; Vit.B_{12}, 10mg; biotin, 50mg; pantothenic acid, 10000 mg; nicotinic acid, 30000 mg; folic acid, 1000 mg; choline chloride, 250000 mg; Mn, 60000 mg; Zn, 50000 mg; Fe, 30000 mg; Cu, 10000 mg; I, 1000 mg; Se, 100mg; Co, 100mgand complete to 3.0 kg by calcium carbonate.

Table 2. Physical and chemical composition (%) of the grower diets (as fed)

Composition	Group					
	Control	Antibiotic	Probiotics		Acidifiers	
	(-)	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid
Dietary ingredients						
Yellow corn	53.35	53.345	53.34	53.34	52.52	52.96
Soybean meal	36.15	36.15	36.15	36.15	36.29	36.20
Sunflower oil	7.14	7.14	7.14	7.14	7.43	7.28
Limestone	1.46	1.46	1.46	1.46	1.46	1.46
Dicalcium phosphate	1.18	1.18	1.18	1.18	1.18	1.18
Common salt	0.35	0.35	0.35	0.35	0.35	0.35
DL- methionine	0.07	0.07	0.07	0.07	0.07	0.07
Vit. and min. premix[1)]	0.30	0.30	0.30	0.30	0.30	0.30
Feed additives	-	0.005	0.01	0.01	0.40	0.20
Chemical composition						
Metabolizable energy, kcal/kg	3200.3	3200.1	3199.9	3199.9	3200.2	3200.2
Dry matter	91.21	91.20	91.20	91.20	90.88	91.04
Crude protein	20.01	20.01	20.01	20.01	20.00	20.00
Methionine	0.39	0.39	0.39	0.39	0.39	0.39
Methionine + Cystine	0.73	0.73	0.73	0.73	0.73	0.73
Lysine	1.11	1.11	1.11	1.11	1.11	1.11
Ether extract	9.58	9.58	9.58	9.58	9.84	9.70
Crude fiber	3.20	3.20	3.20	3.20	3.19	3.20
Ash	5.80	5.80	5.80	5.80	5.80	5.80
Calcium	0.90	0.90	0.90	0.90	0.90	0.90
Phosphorus, available	0.35	0.35	0.35	0.35	0.35	0.35
Sodium	0.15	0.15	0.15	0.15	0.15	0.15

[1)] Vitamins and minerals premix (Agri-Vet Company, Egypt): each 3.0 kg contain Vit. A, 12000000 IU; Vit.D_3 2000000 IU; Vit.E, 10000 mg; Vit.K_3, 2000 mg; Vit.B_1, 1000 mg; Vit.B_2, 5000 mg; Vit. B_6, 1500 mg; Vit.B_{12}, 10mg; biotin, 50mg; pantothenic acid, 10000 mg; nicotinic acid, 30000 mg; folic acid, 1000 mg; choline chloride, 250000 mg; Mn, 60000 mg; Zn, 50000 mg; Fe, 30000 mg; Cu, 10000 mg; I, 1000 mg; Se, 100mg; Co, 100 mg and complete to 3.0 kg by calcium carbonate.

Growth performance

The diets were offered to the chicks daily and feed intake/day was calculated after removal of the refused feed. The total feed consumption per day was divided by the number of birds in each pen to obtain the average daily feed intake / bird. All the birds were individually weighed at the start and end of the experiment as well as at weekly intervals throughout the experiment. Accordingly, the weekly weight gain of the birds was measured. Based on the feed intake and weight gain, the feed conversion ratio was estimated and corrected for mortality on a bird day basis. The mortality rate was recorded daily throughout the experiment.

Excreta quality

The quality of excreta from each dietary treatment was evaluated by measuring its dry matter (DM) content. The excreta of six birds per treatment (two birds / replicate) were collected at the end of starter (d 21) and grower (d 42) periods. The collected fresh excreta from each bird were taken, thoroughly mixed and then dried at 105 ºC in hot air oven for 24h to determine the DM content.

Blood parameters

Blood samples of six birds per treatment (two birds /replicate) were collected at the end of starter (d 21) and grower (d 42) periods. The birds were sacrificed and the

samples of blood were taken from the neck of birds and then collected in blood tubes. The samples were centrifuged at 3.000 rpm for 15 minutes for separating the serum. Afterwards, the obtained serum was stored at –20ºC until analysis. The serum samples were analyzed for some biochemical parameters, including glutamic pyruvic transaminase (GPT), glutamic oxaloacetate transaminase (GOT), creatinine, glucose and cholesterol by using chemical kits.

pH values of intestinal contents

Immediately after sacrificing the selected birds (at day 21 and 42) for obtaining the blood samples, the digesta of small intestine and caecum of only three birds / treatment (one bird per replicate) were individually isolated in tubes. These samples were diluted with water at the rate of 1:5, and then thoroughly mixed. Thereafter, the samples were measured for pH values using pH meter (Youssef et al., 2012).

Carcass characteristics:

Six birds from each treatment (two birds / replicate), close to the average live body weight, were selected at the end of experiment. Birds were weighed, subjected to 24h-feed withdrawal with free access to water, reweighed and slaughtered by neck cutting. The birds were scalded, defeathered, and eviscerated after removal of head, neck and legs. The carcass without giblets was weighed, expressed as a percentage of its live weight and considered as the carcass yield. In addition, the weight of the breast, proventriculus, gizzard, liver and heart was recorded and its relation to the live body weight of the birds, in percentages, was calculated.

Microbiological examination

Intestinal digesta

At the end of the experiment, the contents of small intestine (mixed contents of duodenum, jejunum and ileum) and caecum of 3 birds / treatment were individually collected directly after slaughter in separate sterile Petri dishes. Afterwards, one gram from each sample was mixed with 9 ml of 0.1% sterile peptone water and then ten- fold serial dilution up to 10^{-6} was prepared. One ml from each serial dilution of intestinal contents was separately pipetted into double set of Petri dishes and mixed with 15 ml of melted deMan, Rogosa and Sharpe (MRS) agar (Biolife) then incubated at 42˚C under microaerophilic conditions (5% CO_2) for 48h for lactobacilli count; another 1 ml was mixed with 15 ml of melted standard plate count agar (SPCA, Oxoid, CM325) and incubated at 35˚C for 48h for total aerobic bacterial count; another 1 ml was inoculated into three replicate tubes of Lauryl Sulphate Tryptose Broth (LST, Oxoid, CM451) with inverted Durham's tubes and incubated at 35˚C for 48 hours for determination of the most probable number (MPN) of coliforms. A loopful from each positive LST tubes showing gas was transferred into tubes containing Brilliant Green Bile Lactose broth (Oxoid, CM31) with inverted Durham's tubes and incubated at 35˚C for 48 hours. Positive tubes showing gas production were recorded and MPN of coliforms was estimated. A loopful from each positive brilliant green bile lactose broth was inoculated into tubes of *E. coli* broth (Biolife, 401425), and then incubated at 44˚C for 48 hours for determination of MPN of faecal coliforms. Positive tubes showing gas production were calculated as MPN of faecal coliforms. A loopful from each positive *E. coli* broth tubes was streaked onto plates of eosin methylene blue agar (Oxoid, CM69) and incubated at 35˚C for 24 hours for determination of MPN of *E. coli*. Typical colonies appear as greenish metallic nucleated with dark purple center with or without sheen.

Carcass meat

After slaughtering and dressing of broiler chickens at the end of experiment, 3 birds per treatment (one bird / replicate) were used for microbiological examination of the muscles. The muscle samples were prepared according to the muscle maceration technique recommended by ICMSF (1978). The muscle surface was sterilized by hot spatula, and then ten grams of breast and thigh muscles (5 g from each) were taken from deep muscle under aseptic conditions. Then, the samples were transferred to a sterile homogenizing jar to which 90 ml of 0.1 % sterile peptone water were added. The contents were thoroughly homogenized for 2 minutes at 2000 r.p.m. using a sterile homogenizer. Such homogenate was serially diluted as in the intestinal contents. Total aerobic, coliform, faecal coliform and *E. coli* counts were estimated as previously mentioned for intestinal samples. *Staphylococcus aureus* count was done according to APHA (1992) by spreading 100 µl from each dilution over a dry surface of Baird-Parker medium (BP, Oxoid, CM275) plates. Inoculated plates were incubated at 35˚C for 24 hours. Suspected colonies were recorded and *Staphylococcus aureus* count was calculated.

Statistical analyses

The results were analysed statistically using Statistical Package for Social Science (SPSS for Windows (IBM), version 20, Chicago, USA, 2011). The data were analysed by using one-way ANOVA and subsequent

Duncan's multiple range test to determine the differences between the treatments. Results are expressed as means ± SEM. Probability values of less than 0.05 ($P < 0.05$) were considered significant.

RESULTS

The antibiotic, probiotics and lactic acid increased significantly ($P < 0.05$) the body weight during the grower period (3-6 weeks) compared to the control, but had no significant effect ($P > 0.05$) on the birds' weight during the starter period (0-3 week). However, salmo-nil treatment did not influence the body weight comparing to the control group throughout the experiment.

During the starter period, the feed intake and weight gain were not affected by the dietary treatments (Table 3). Nevertheless, the feed conversion ratio (FCR) for birds fed diets containing the tested feed additives (1.44) was lower ($P < 0.001$) than the control (1.56). Among the tested additives, the biopellet-s group was found to have the lowest FCR (1.39), whereas the highest one (1.49) was in the salmo-nil treatment. No difference ($P > 0.05$) in FCR was detected between the birds fed diets supplemented with bactocell and acidifiers (salmo-nil and lactic acid). The mortality rate was lower in biopellet-s and acidifiers groups (3.03%) than the other treatments (6.06%).

During the grower period, the feed intake was lower in biopellet-s and acidifiers treatments than the control while that of other groups was not affected. Compared to the control, the weight gain of birds fed diets supplemented with antibiotic and bactocell was higher ($P<0.05$), but did not differ in other treatments. However, the weight gain in biopellet-s and lactic acid groups was similar to that of antibiotic and bactocell treatments. The FCR in all feed additives supplemented groups (about 1.93) was lower ($P < 0.05$) than the control group (2.16). Moreover, there was no difference ($P > 0.05$) in FCR between these additives. The mortality rate was null in control and acidifiers groups, but was about 3.20% in other treatments.

All over the experimental period, it was found that the feed intake, as in the grower period, was lower ($P<0.05$) in biopellet-s and acidifiers groups compared to the control. The weight gain of birds fed diets supplemented with antibiotic, probiotics and lactic acid was similar ($P> 0.05$) and higher ($P < 0.05$) than the control birds. However, no difference in the weight gain between salmo-nil and control was found. Moreover, the feed additives improved ($P < 0.05$) the feed conversion ratio compared to the control (1.78 vs. 1.97). However, there was no difference ($P> 0.05$) in FCR between the feed additive groups. The mortality rate was 3.03% in acidifiers, 6.06% in control and biopellet-s, and 9.09% in antibiotic and bactocell groups. The excreta of the birds were analysed for the DM content at the end of starter and grower periods. There were no differences ($P > 0.05$) in DM values between the different dietary treatments, indicating that the dietary supplements did not affect the excreta quality.

Supplementation of the feed additives exhibited no significant ($P > 0.05$) differences in the serum concentration of SGPT, SGOT, creatinine, glucose, and cholesterol at the end of starter period (Table 4). The same findings in the serum constituents were found at the end of grower phase, with exception of cholesterol level which was lower ($P < 0.05$) in all birds fed the dietary supplements compared with those fed the control diet.

The effect of dietary treatments on the pH values of the intestinal contents is presented in table 5. It was found that supplementation of antibiotic and salmo-nil significantly ($P < 0.05$) reduced the pH of small intestine contents (5.84) at the starter period, but with insignificant ($P > 0.05$) decrease in other dietary supplements (6.07) when compared with the control (6.29). Moreover, the feed additives numerically decreased the pH values (6.06 vs. 6.59) of small intestine at the grower period. However, the dietary additives did not influence ($P > 0.05$) the pH of the caecum digesta at the starter or grower period compared to the control.

The carcass characteristics of the birds fed different diets are demonstrated in table 6. The carcass yield percentage did not show any significant differences ($P > 0.05$) among the dietary treatments, but exhibited a numerical increase in probiotics (72.84 %), followed by antibiotic and lactic acid groups (about 71.45%) compared to the control (70.35%). Moreover, the relative weights of breast, proventriculus, gizzard, liver and heart were not affected ($P > 0.05$) by the dietary supplements when compared with the control.

No significant ($P > 0.05$) effect of dietary treatments on the intestinal bacterial count was observed (Table 7). However, the feed additives numerically decreased the total aerobic, coliforms, faecal coliforms and *E. coli* counts in both small intestine and caecum. On the other hand, there was a numerical increase in *Lactobacilli* count in acidifiers and probiotics, but it decreased in the antibiotic group. Concerning the microbial examination of carcass meat, all additives significantly ($P <0.001$) reduced the total aerobic and *Staphylococcus* counts (Table 8). Moreover, a numerical decrease in *E. coli* count in all treatments was noticed.

Table 3. Growth performance and excreta DM content of broilers fed different dietary treatments throughout the experiment

Period	Group						P-value
	Control	Antibiotic	Probiotics		Acidifiers		
	(-)	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid	
Starter period (0-3 wk)							
Feed intake, g	832.68 ^{a}±23.24	830.62 ^{a}±11.44	821.38 ^{a}±23.25	814.16 ^{a}±19.70	803.26 ^{a}±3.87	802.52 ^{a}±7.82	0.693
Weight gain, g	535.42 ^{a}±24.83	582.60 ^{a}±11.00	559.04 ^{a}±18.34	587.41 ^{a}±21.33	540.00 ^{a}±3.36	556.97 ^{a}±8.92	0.212
FCR, g/g	1.56 ^{a}±0.03	1.43^{cd}±0.01	1.47^{bc}±0.01	1.39^{d}±0.02	1.49^{b}±0.002	1.44^{bc}±0.01	0.0001
Excreta DM content,%	14.69 ^{a}±1.49	15.59 ^{a}±1.32	15.52 ^{a}±2.09	15.37 ^{a}±1.67	16.29 ^{a}±0.69	15.28 ^{a}±1.72	0.981
Mortality, %	6.06	6.06	6.06	3.03	3.03	3.03	
Grower period (3-6 wk)							
Feed intake, g	2513.8^{a}±69.89	2457.0^{ab}±31.88	2464.7^{ab}±12.85	2375.7^{b}±25.22	2263.5^{c}±11.15	2265.4^{c}±7.93	0.001
Weight gain, g	1163.3^{bc}±16.17	1265.0^{a}±13.36	1278.3^{a}±39.14	1231.1^{ab}±19.50	1143.0^{c}±16.24	1208.4^{ab}±19.98	0.006
FCR, g/g	2.16 ^{a}±0.12	1.94^{b}±0.06	1.93^{b}±0.07	1.93^{b}±0.02	1.98^{b}±0.03	1.87^{b}±0.04	0.011
Excreta DM content,%	12.08 ^{a}±2.07	12.52 ^{a}±1.14	14.38 ^{a}±1.13	13.63 ^{a}±1.40	13.99 ^{a}±0.63	13.68 ^{a}±1.16	0.830
Mortality, %	0.0	3.23	3.23	3.13	0.0	0.0	
Total period (0-6 wk)							
Feed intake, g	3346.4^{a}±93.13	3287.7^{ab}±20.44	3286.1^{ab}±10.40	3189.8^{bc}±44.92	3066.8^{c}±7.28	3067.9^{c}±15.75	0.002
Weight gain, g	1698.7^{b}±8.67	1847.6^{a}±24.36	1837.3^{a}±20.80	1818.5^{a}±40.82	1683.0^{b}±12.89	1765.4^{a}±28.90	0.002
FCR, g/g	1.97 ^{a}±0.05	1.78^{b}±0.04	1.79^{b}±0.03	1.75^{b}±0.02	1.82^{b}±0.01	1.74^{b}±0.03	0.001
Mortality, %	6.06	9.09	9.09	6.06	3.03	3.03	

$^{a, b, c}$ Means within the same row with different superscripts are significantly different (P< 0.05).

Table 4. Serum constituents of broiler chickens fed different diets at the end of starter (d 21) and grower (d 42) periods

Period	Parameter	Group						P-value
		Control	Antibiotic	Probiotics		Acidifiers		
		(-)	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid	
Starter	SGPT (µ/L)	5.33 [a]±0.33	6.00 [a]±0.58	4.33 [a]±0.33	4.00 [a]±0.58	5.00 [a]±1.15	5.67 [a]±0.67	0.312
	SGOT (µ/L)	187.7 [a]±15.81	174.0 [a]±31.77	211.0 [a]±39.80	187.7 [a]±11.35	173.3 [a]±13.93	167.7 [a]±3.38	0.794
	Creatinine (mg/dL)	0.28 [a]±0.02	0.34 [a]±0.02	0.36 [a]±0.02	0.28 [a]±0.01	0.34 [a]±0.08	0.37 [a]±0.07	0.659
	Glucose (mg/dL)	238.7 [a]±21.18	230.0 [a]±12.58	239.7 [a]±22.15	236.3 [a]±21.84	232.3 [a]±12.67	223.3 [a]±21.31	0.989
	Cholesterol (mg/dL)	142.3 [a]±12.17	121.0 [a]±34.77	142.3 [a]±12.17	123.7 [a]±17.70	140.0 [a]±11.24	140.3 [a]±13.68	0.919
Grower	SGPT (µ/L)	4.60 [a]±0.51	3.80 [a]±0.37	5.00 [a]±0.45	4.40 [a]±0.51	3.80 [a]±0.37	5.00 [a]±0.55	0.274
	SGOT (µ/L)	291.3 [a]±5.85	258.8 [a]±15.73	268.5 [a]±16.42	264.2 [a]±18.52	257.5 [a]±29.94	284.2 [a]±22.97	0.793
	Creatinine (mg/dL)	0.42 [a]±0.04	0.32 [a]±0.01	0.32 [a]±0.02	0.41 [a]±0.04	0.37 [a]±0.03	0.42 [a]±0.05	0.143
	Glucose (mg/dL)	196.2 [a]±16.55	216.0 [a]±8.01	208.0 [a]±8.76	170.5 [a]±18.34	217.4 [a]±18.05	167.5 [a]±21.06	0.161
	Cholesterol (mg/dL)	175.8[a]±14.41	112.6[b]±11.33	123.0 [b]±17.68	101.0 [b]±16.94	111.0 [b]±8.67	116.8 [b]±9.31	0.010

[a, b] Means within the same row with different superscripts are significantly different (P< 0.05).

Table 5. pH values of intestinal contents of birds fed different diets at the end of starter (d 21) and grower (d 42) periods

Period	Intestinal Segment	Group						P-Value
		Control	Antibiotic	Probiotics		Acidifiers		
		(-)	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid	
Starter	Small intestine	6.29 [a]± 0.06	5.80 [c]± 0.04	6.04 [abc]± 0.01	6.13[ab]± 0.05	5.88 [bc]± 0.19	6.03 [abc]± 0.01	0.021
	Caecum	7.02 [a]± 0.08	6.56 [a]± 0.07	7.44 [a]± 0.39	6.99 [a]± 0.08	7.25 [a]± 0.43	6.45 [a]± 0.33	0.164
Grower	Small intestine	6.59 [a]± 0.19	6.12 [a]± 0.04	6.00 [a]± 0.11	5.96 [a]± 0.25	6.05 [a]± 0.05	6.16 [a]± 0.06	0.073
	Caecum	6.60 [a]± 0.20	7.33 [a]± 0.17	7.20 [a]± 0.35	6.89 [a]± 0.49	7.27 [a]± 0.12	6.76 [a]± 0.33	0.329

[a, b, c] Means within the same row with different superscripts are significantly different (P< 0.05).

Table 6. Carcass and organ weights relative to BW (%) of broiler chickens fed different experimental diets

Parameter	Group						P-value
	Control	Antibiotic	Probiotics		Acidifiers		
	(-)	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid	
Carcass yield	70.35±0.89	71.24±0.81	73.03±0.96	72.65±0.29	70.81±0.80	71.66±0.94	0.186
Breast	22.03±0.79	22.45±0.64	23.34±0.49	23.05±0.65	22.16±0.53	22.88±0.95	0.213
Proventriculus	0.43±0.03	0.42±0.05	0.45±0.04	0.46±0.03	0.40±0.03	0.41±0.04	0.881
Gizzard	2.38±0.11	2.20±0.09	2.42±0.10	2.49±0.10	2.34±0.06	2.35±0.05	0.397
Liver	2.42±0.10	2.50±0.13	2.66±0.06	2.76±0.11	2.49±0.14	2.56±0.06	0.250
Heart	0.55±0.07	0.50±0.09	0.58±0.06	0.57±0.07	0.49±0.07	0.53±0.08	0.948

Table 7. Effect of dietary treatments on intestinal bacterial count (log cfu/g) of broilers at the end of the experiment

Intestinal bacteria	Segment	Group						P-value
		Control	Antibiotic	Probiotics		Acidifiers		
		(-)	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid	
Total aerobic count	S. intestine	8.10±0.74	7.06±0.41	7.46±0.67	6.92±0.30	7.15±0.49	7.87±0.55	0.457
	Caecum	8.88±0.25	8.15±0.29	8.18±0.50	8.31±0.42	8.05±0.25	8.80±0.31	0.204
Lactobacilli	S. intestine	6.19±0.48	5.82±0.60	6.39±0.05	6.53±0.56	6.85±0.54	6.49±0.80	0.632
	Caecum	6.84±0.34	6.30±0.68	6.93±0.34	6.97±0.40	7.04±0.29	7.05±0.40	0.785
Coliforms	S. intestine	5.35±0.66	3.98±0.41	4.54±0.69	3.92±0.28	4.23±0.54	5.10±0.52	0.339
	Caecum	5.76±0.47	5.18±0.29	5.35±0.44	5.06±0.54	4.48±0.44	5.56±0.44	0.174
F. coliform	S. intestine	5.30±0.68	3.89±0.39	3.60±0.29	3.92±0.28	4.11±0.47	4.98±0.58	0.117
	Caecum	5.71±0.51	5.47±0.34	5.02±0.39	4.89±0.55	4.40±0.40	5.31±0.54	0.436
E. coli	S. intestine	3.75±0.09	3.41±0.21	3.60±0.29	3.51±0.13	3.45±0.09	3.48±0.13	0.203
	Caecum	4.06±0.25	3.33±0.22	3.61±0.25	3.44±0.14	3.38±0.08	3.63±0.24	0.156

Table 8. Effect of dietary treatments on bacterial count (log cfu/g) in carcass meat of broilers at the end of the experiment

Bacterial type	Group						P-value
	Control	Antibiotic	Probiotics		Acidifiers		
	(-)	Maxus	Bactocell	Biopellet-s	Salmo-Nil	Lactic acid	
Total aerobic count	5.10[a]±0.10	3.10[b]±0.10	3.39 [b]±0.12	3.54[b]±0.16	3.46[b]±0.09	3.38[b]±0.25	0.0001
Coliforms	2.48 [a]±0.21	2.06 [a]±0.29	2.55 [a]±0.28	2.27 [a]±0.33	2.93 [a]±0.27	2.96 [a]±0.27	0.193
F. coliform	2.01 [a]±0.10	1.51 [a]±0.03	1.81 [a]±0.20	1.80 [a]±0.20	2.35 [a]±0.22	1.98 [a]±0.35	0.166
E. coli	1.71 [a]±0.23	1.32 [a]±0.21	1.51 [a]±0.15	1.44 [a]±0.22	1.39 [a]±0.20	1.60 [a]±0.13	0.540
Staphylococcus	3.42 [a]±0.06	1.39[c]±0.21	1.65[c]±0.25	1.49[c]±0.29	1.43[c]±0.30	2.33[b]±0.33	0.0001

[a, b, c] Means within the same row with different superscripts are significantly different (P< 0.05).

DISCUSSION

Growth performance

Feed additives are considered an indispensable part of feed manufacture and animal nutrition. Substitution of conventional antibiotic growth promoters with alternative feed additives has received great attention in the recent past since the European Union and many countries have banned using antibiotics as growth promoters in poultry nutrition (Reid and Friendship, 2002). Improvement in body weight and weight gain of birds fed diets supplemented with probiotics and lactic acid, especially during the grower period, is thought to be induced by their effects on maintenance of beneficial bacteria population, and improving nutrient digestion (Jin et al., 1997; Adil et al., 2010; Getachew, 2016; Khan and Iqbal, 2016). However, salmo-nil treatment did not affect the body weight or weight gain in this study. The beneficial observations of organic acids are not consistent because the benefits of these acids are related to several variables including the kind of organic acid used, dosage, buffering capacity of dietary ingredients, as well as sanitation level of the production environment (Dibner and Buttin, 2002). In addition, feed palatability may be affected by the sources and inclusion levels of dietary organic acids, and therefore seems to affect the efficacy of these acids (Kim et al., 2015). Moreover, the feed intake was not affected by the feed additives during the starter period, but reduced (about 8 %) in biopellet-s and acidifiers treatments than the control throughout the grower period. However, all the tested feed additives improved the feed conversion efficiency in starter and grower periods. All over the experimental period (0-6 week), the performance indices were similar to that observed during the grower period. The positive effect of probiotics on growth performance in the present study is also reported in other studies (Mountzouris et al., 2007; Samli et al., 2007; Awad et al., 2009; Pourakbari et al., 2016). Furthermore, the influence of lactic acid on performance is consistent with the findings of other researchers (Runho et al., 1997; Adil et al., 2010; Bhanjat et al., 2010).The improved growth performance by lactic acid is probably due to the beneficial effect of the acid on the intestinal flora. The organic acids may affect the integrity of microbial cell membrane or hinder the nutrient transport and energy metabolism causing the bactericidal effect (Ricke, 2003). Besides, butyric acid has been reported to decrease the colonization of bacteria in the caeca of broiler chicken (Van Immerseel et al., 2004). In addition, the increased feed conversion efficiency in lactic acid group could be due to the enhanced utilization of nutrients resulting in increased body weight gain (Adil et al., 2010). Moreover, the obtained results indicate that the effect of feed additives on performance becomes more pronounced within the grower period, but their effects appear to be cumulative commenced at the starter phase. However, the effect of salmo-nil on the performance data is supported by the findings reported by Paul et al. (2007) who found that the use of the organic acid salts in broiler diets reduced feed intake, but the body weight gain was similar to control birds and thus improved FCR. Based on performance indices, no difference between both types of probiotics was detected, indicating that the mode of action of different probiotics is nearly identical. The same finding was also found between lactic acid producing bacteria (bactocell) and lactic acid per se, suggesting that the effect of lactic acid produced by bacteria is comparable to the chemical one. In addition, lactic acid appears to have more beneficial effect than the organic acid mixture. Moreover, the effect of probiotics and lactic acid on performance seems to be identical to that of antibiotic growth promoters. The same findings were reported in previous studies with probiotics (Bai et al., 2013; Tabidi et al., 2013) and lactic acid (Bhanjat et al., 2010).

Throughout the experiment, the feed additives had no effect on the excreta quality as observed by no change in its moisture content. This finding indicates that these compounds have a potential effect on modulation of intestinal microflora and pathogen inhibition (Mountzouris et al., 2007; Hassan et al., 2010).

Blood parameters

Supplementation of the diets with antibiotic, probiotics and acidifiers did not affect the serum glucose concentration as well as the liver and kidney functions as indicated by no change in the serum levels of SGPT, SGOT, and creatinine. The same findings were reported in previous studies which tested the effect of probiotics (Gheith, 2008; Salim et al., 2011) or organic acids (Hernandez et al., 2006; Abdel Fattah et al., 2008; Adil et al., 2010) on blood metabolites. However, all the additives reduced the cholesterol level in the grower period only. It is reported that the probiotic supplementation significantly reduced the serum cholesterol level of the chickens (Ashayerizadeh et al., 2011, Beski and Al-Sardary, 2015; Pourakbari et al., 2016). The most important way of cholesterol excretion is through synthesis of bile acids from cholesterol in the liver (Wilson et al., 1998). The use of probiotics can degenerate bile salts and de-conjugate production of enzymes by the activity of lactic acid bacteria, as well as reduction of the pH in the intestinal tract can be effective in decreasing the cholesterol concentration. Solvability of non-conjugate bile acids is reduced at a low pH and consequently, they are absorbed less from the intestine and are excreted more in the excreta (Klaver and Van der Meer, 1993). Consequently, the liver, for re-establishment of the hepatic cycle of bile acids, coverts more cholesterol into the tissues and therefore its concentration in the blood is reduced (Ros, 2000). Also, the effect of antibiotic and organic acids on serum cholesterol could be attributed to the reduction of the intestinal pH that was observed in our study. Kamal and Ragaa (2014) reported that blood total lipids and cholesterol decreased significantly by organic acids.

pH values of intestinal digesta

The tested feed additives can reduce the pH of small intestine contents. Probiotics were found to modify the intestinal environment by reducing the pH (Kabir, 2009). Moreover, organic acids supplementation has pH diminishing property, although non-significant, in various gastrointestinal segments of the broilers (Abdel-fattah et al., 2008). The reduced pH is helpful for the growth of favourable bacteria by simultaneously hindering the growth of pathogenic bacteria which grow at a relatively higher pH. Nevertheless, the feed additives did not influence the pH of caecum. It is possible that the effects of organic acids in the distal part of the digestive tract decrease because of the reduction in concentration of acids as a result of absorption and metabolism (Bolton and Dewar, 1964). Thus, it can be assumed that the effect of organic acids in the distal segments of the intestinal tract could be due to the reduced entry of pathogenic bacteria from the upper portions of intestinal tract as a compensatory mechanism but no valid literature concerning such mechanism was found.

Carcass characteristics

In this study, the antibiotic, probiotics and lactic acid showed an insignificant increase in the carcass yield percentage (1 to 3 %) when compared to the control. This is may be attributed to the greater live body weight of these birds. Recently, Falaki et al. (2011) reported that probiotic supplementation significantly improved the carcass weight, but without any significant influence on the carcass yield. Moreover, the relative weights of breast and internal organs were not affected by the dietary treatments. The impact of probiotics on the relative weights of tested organs is consistent with that noticed by Awad et al. (2009). Concerning to acidifiers, their effects are supported by the results of other investigations which found that the organic acids did not affect dressing yield and carcass characteristics of broiler chicken (Adil et al., 2010; Kopecký et al., 2012; Attia et al., 2013; Ghasemi et al., 2014).

Based on the results of carcass characteristics, no significant difference between both types of probiotics was recorded. The same observation was found between lactic acid and salmo-nil treatments. Likewise, the effect of lactic acid produced by bacteria tended to be similar to that of lactic acid per se. However, probiotics seem to produce more beneficial effects in carcass yield among the treatments, followed by lactic acid and antibiotic products.

Microbiological examination

The antibiotic, probiotics and acidifiers reduced the count of pathogenic bacteria especially total aerobes, coliforms and *E. coli*. The inhibitory effect of probiotics and acidifiers could be attributed to a decrease in intestinal pH (Fuller, 1989; Boroojeni et al., 2014). Sakata et al. (2003) reported that probiotic bacteria actually increase the production rates of volatile fatty acids and lactic acid. Several mechanisms related to the antagonistic effects of probiotics on various microorganisms include secretion of antimicrobial substances, competitive adherence to the intestinal mucosa, and stimulating the immune system

(Collado et al., 2010). Organic acids can perforate the bacteria cell wall and upset normal cellular functions (Davidson, 2001). The increased count of *Lactobacilli* in probiotics and acidifiers could be attributed to their effect in stimulating the growth of beneficial bacteria and suppressing the pathogenic one (Van Immerseel et al., 2006; Getachew, 2016; Waseem Mirza et al., 2016).

The used feed additives have the ability to improve the keeping quality of the carcass meat through its role in reducing the total aerobes, *Staphylococcus* and *E. coli* counts. Kabir (2009) reported that probiotics improved the meat quality via diminishing *Staphylococcus* and *E. coli* counts in broiler meat. Moreover, organic acids have been observed to have bactericidal effects on pathogenic bacteria (Kim et al., 2015; Khan and Iqbal, 2016). In addition, lactic acid can be used to reduce the bacterial contamination of broiler carcasses (Byrd et al., 2001). The effect of tested additives on the bacterial count of meat could be also attributed to its activity in lowering the count of intestinal pathogens.

CONCLUSIONS

Probiotics and lactic acid increased the body weight of broilers as the antibiotic growth promoters. Moreover, the tested probiotics and acidifiers provided a better feed conversion which was similar to that produced by antibiotic. Also, their effects on intestinal and meat pathogens were similar to that of antibiotic, but with stimulating effect on beneficial bacteria. No difference between the used kinds of probiotics was observed. Lactic acid alone seems to produce better performance results than the organic acids blend. The natural lactic acid produced by bacteria could have a comparable effect to that of the chemical one. Finally, the obtained results indicate that the probiotics and organic acids are promising alternatives to antibiotics in diets of broilers.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors participated in making the design, performing the experiment, analyses of the data, and writing the paper.

REFERENCES

Abdel-Fattah SA, El-Sanhoury MH, El-Mednay NM and Abdel-Azeem F (2008). Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. International Journal of Poultry Science, 7: 215–222. DOI: 10.3923/ijps.2008.215.222.

Adil S, Banday T, Ahmad B, hat G, Saleem Mir M and Rehman M (2010). Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken.Veterinary Medicine International, Article ID 479485, 7 pages. doi:10.4061/2010/479485.

Andremont A (2000). Consequences of antibiotic therapy to the intestinal ecosystem. Annales Françaises d'Anesthésie et de Réanimation 19: 395–402.doi.org/10.1016/S0750-7658(00)90209-0.

AOAC (2005). Official Methods of Analysis of AOAC International, 18th ed. Association of Official Analytical Chemists, Gaithersburg, MD.

APHA (1992). Standard Methods for Examination of Water and Wastewater, 18th Edition. American Public Health Association. Washington DC.

Ashayerizadeh A, Dabiri N, Mirzadeh KH and Ghorbani MR (2011). Effect of dietary supplementation of probiotic and prebiotic on growth indices and serum biochemical parameters of broiler chickens. Journal of Cell and Animal Biology, 5: 152-156.doi: 1A53EB113509.

Attia YA, Abd El-Hamid AE, Ellakany HF, Bovera F, Al- Harthi MA and Ghazaly SA (2013). Growing and laying performance of Japanese quail fed diet supplemented with different concentrations of acetic acid. Italian Journal of Animal Science,12: 222-230.doi.org/10.4081/ijas.2013.e37.

Awad WA, Ghareeb K, Abdel-Raheem S and Böhm J (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry Science, 88:49–55. DOI: https://doi.org/10.3382/ps.2008-00244.

Bai SP, Wu AM, Ding XM,Lei Y, Bai J, Zhang KY and Chio JS (2013). Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. Poultry Science, 92:663–670.http://dx.doi.org/10.3382/ps.2012-02813.

Beski SSM and Al-Sardary SYT (2015). Effects of dietary supplementation of probiotic and synbiotic on broiler chickens hematology and intestinal integrity. International Journal of Poultry Science, 14: 31-36.DOI: 10.3923/ijps.2015.31.36.

Bhanjat SK, Anjali DC, Shyam SG and Panda AK (2010). Effect of lactic acid and antibiotic on the performance of broiler chickens. Indian Journal of Animal Nutrition,27 (3): 290-293.

Bolton W and Dewar WA (1964). The digestibility of acetic, propionic and butyric acids by the fowl. British Poultry Science, 6: 103–105. http://dx.doi.org/10.1080/00071666508415562.

Boroojeni FG, Vahjen W, Mader A, Knorr F, Ruhnke I, Röhe I, Hafeez A, Villodre C, Männer K and Zentek J (2014).The effects of different thermal treatments and organic acid levels in feed on microbial composition and activity in gastrointestinal tract of broilers. Poultry Science, 93: 1440–1452.DOI: 10.3382/ps.2013-03763.

Burgat V (1999).Residues of drugs of veterinary use in food.La Revue Du Praticien, 41: 985–990.PMID:2063112

Byrd JA, Hargis BM, Caldwell DJ, Bailey RH, Herron KL, McReynolds JL, Brewer RL, Anderson RC, Bischoff KM, Callaway TR and Kubena LF (2001). Effect of lactic acid administration in the drinking water during pre-slaughter feed withdrawal on Salmonella and Campylobacter contamination of broilers. Poultry Science, 80:278–283.

Collado MC, Gueimonde M and Salminen S (2010). Probiotics in adhesion of pathogens: mechanisms of action; in Watson RR, Preedy VR (eds): Bioactive Foods in Promoting Health, Chennai, Academic Press, Elsevier 23: 353–370.DOI: 10.1016/B978-0-12-374938-3.00023-2

Davidson PM (2001). Chemical preservatives and natural antimicrobial compounds. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food microbiology-fundamentals and frontiers.2nd ed. Washington, DC: American society for microbiology, p.593-627.DOI: 10.1128/9781555815912.ch33.

Denli M, Okan F and Celik K (2003).Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield.Pakistan Journal of Nutrition, 2: 89–91.doi=pjn.2003.89.91

Dhawale A (2005). Better eggshell quality with a gut acidifier. Poultry International, 44: 18–21.https://www.researchgate.net/.../286331926

Dibner JJ and Buttin P (2002). Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. Journal of Applied Poultry Research,11:453–463. https://doi.org/10.1093/japr/11.4.453

Falaki M, Shams Shargh M, Dastar B and Zerehdaran S (2011). Effect of different levels of probiotic and prebiotic on performance and carcass characteristics of broiler chickens. Journal of Animal and Veterinary Advances,10:378-384. DOI: 10.3923/javaa.2010.2390.2395

Fuller R (1989). Probiotic in man and animal. Journal of Applied Bacteriology, 66: 365–378.DOI: 10.1111/j.1365-2672.1989.tb05105.x.

Getachew T (2016). A review on effects of probiotic supplementation in poultry performance and cholesterol levels of egg and meat. Journal of World Poultry Research, 6(1): 31-36. PII: S2322455X1600006-6.

Ghasemi HA, Akhavan-Salamat H, Hajkhodadadi I and Khaltabadi-Farahani AH (2014). Effects of dietary organic acid blend supplementation on performance, intestinal morphology and antibody-mediated immunity in broiler chickens. Acta Advances in Agricultural Sciences, 2: 64-74. doi.AAAS-2014-50344376.

Gheith IM (2008). Clinicopathological studies on the effect of probiotic in broilers. M.V.Sc. Thesis, Fac. Vet. Med., Benha University. www.bvmj.bu.edu.eg/issues/22-2/5.

HassanHMA, MohamedMA, YoussefAW and HassanER (2010).Effect of using organic acids to substitute antibiotic growth promoters on performance and intestinal microflora of broilers. Asian-Australian Journal of Animal Science, 23: 1348 – 1353.DOI: 10.5713/ajas.2010.10085.

Hernandez F,Garcia V, Madrid J, Orengo J, Catala P and Megias MD (2006). Effect of formic acid on performance, digestibility, intestinal histomorphology and plasma metabolite levels of broiler chickens. British Poultry Science, 47: 50–56. DOI: 10.1080/00071660500475574

Hossein G, Farshid K and Mostafa F (2017). Effect of using protexin probiotic and ephedra funereal powder supplementation on performance and some carcass traits on broiler chicks. Scholarly Journal of Agricultural Science, 7(1): 6-10. Available online at http://www.scholarly-journals.com/SJAS.

ICMSF (1978). International commission on microbiological specifications for foods. Microorganisms in foods. 2. Sampling for microbiological analysis: Principles and specific

applications. University of Toronto Press, Toronto.

Jin LZ, Ho YW, Abdullah N and Jalaludin S (1997). Probiotics in poultry: Modes of action. World's Poultry Science Journal, 53: 352–368.DOI: 10.1079/WPS19970028.

Kabir SML, Rahman MM, RahmanMB, Rahman MM and AhmedSU (2004).The dynamics of probiotics on growth performance and immune response in broilers. International Journal of Poultry Science, 3: 361–364.DOI: 10.3923/ijps.2004.361.364

Kabir SML (2009). The role of probiotics in the poultry industry. International Journal of Molecular Science, 10: 3531-3546.doi: 10.3390/ijms10083531.

Kamal AM and Ragaa NM (2014). Effect of dietary supplementation of organic acids on performance and serum biochemistry of broiler chicken. Nature and Science, 12: 38-45.doi:10.7537/marsnsj120214.06.

Katoch S, Thakur S and Rajput R (2017). Effect of probiotic supplementation in broiler birds offered feed formulated with lower protein densities. International Journal of Livestock Research,7(2):90-102. doi:10.5455/ijlr.20170205013927.

Khan SH and Iqbal J (2016). Recent advances in the role of organic acids in poultry nutrition. Journal of Applied Animal Research, 44 (1): 359-369. DOI: 10.1080/09712119.2015.1079527.

Kim JW, Kim JH and Kil DY (2015). Dietary organic acids for broiler chickens: a review. Revista Colombiana de Ciencias Pecuarias, 28: 109-123.doi: 10.17533/udea.rccp.v28n2a01.

Kirchgessner M and Roth FX (1988). Ergotrope effekte durch organische sauren in der fekelaufzucht und schweinemast. Ubersichten zur Tiereren¨ahrung, 16: 93–108. ISSN 0303-6340.

Klaver FAM and van der Meer R (1993). The assumed assimilation of cholesterol by Lactobacilli and Bifidobacterium bifidum is due to their bile salt deconjugating activity. Applied Environmental Microbiology, 59: 1120–1124. Doi:0099-2240/93/041120-05$02.00

Kopecký J, Hrnčár C and Weis J (2012). Effect of organic acids supplement on performance of broiler chickens. Animal Sciences and Biotechnologies, 45: 51-54.ISSN online 2344 - 4576

La Ragione RM and Woodward MJ (2003). Competitive exclusion by *Bacillus subtilis*spores of *Salmonella enterica*serotype Enteritidis and *Clostridium perfringens*in young chickens. Veterinary Microbiology, 94:245–256.DOI: 10.1016/S0378-1135(03)00077-4.

Langhout P (2000). New additives for broiler chickens. World Poultry,16: 22–27.doi:20001416551.

Mountzouris KC, Tsistsikos P, Kalamara E, Nitsh S, Schatzmayr G and Fegeros K (2007). Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poultry Science, 86: 309–317. https://www.ncbi.nlm.nih.gov/pubmed/17234844.

Nahanshon SN, Nakaue HS and Mirosh LW (1993). Effects of direct fed microbials on nutrient retention and parameters of Single White Leghorn pullets. Poultry Science, 72: (Suppl .2), 87. (Abstract)

National Research Council (1994). Nutrient requirements of poultry.9th revised edition, National Academy Press. Washington, D.C.

Nawaz H, Irshad MA, Ali M and Haq A (2016). Effect of probiotics on growth performance, nutrient digestibility and carcass characteristics in broilers. The Journal of Animal & Plant Sciences, 26(3): 599-604.

Patterson JA and Burkholder KM (2003). Application of prebiotics and probiotics in poultry production. Poultry Science, 82: 627–631. DOI: https://doi.org/10.1093/ps/82.4.627

Paul SK, Halder G, Mondal MK and Samanta G (2007). Effect of organic acid salt on the performance and gut health of broiler chicken. Japanese Poultry Science, 44: 389–395. http:// doi.org/ 10.2141/ jpsa.44.389

Pourakbari M, Seidavi A, Asadpour L and Martínez A (2016). Probiotic level effects on growth performance, carcass traits, blood parameters, cecal microbiota, and immune response of broilers. Anais da Academia Brasileira de Ciências, 88(2): 1011-1021.http://dx.doi.org/10.1590/0001-3765201620150071.

Reid G and Friendship R (2002). Alternatives to antibiotic use: Probiotics for the gut. Animal Biotechnology, 13: 97-112. http://dx.doi.org/ 10.1081/ABIO-120005773.

Ricke SC (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry Science, 82: 632–639. DOI: https://doi.org/10.1093/ps/82.4.632.

Ros E (2000). Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk. Atherosclerosis, 51: 357-379.https://doi.org/10.1016/S0021-9150(00)00456-1.

Runho RC, Sakomura N K, Kuana S, Banzatto D, Junqueira OM and Stringhini JH (1997). Use of an organic acid (fumaric acid) in broiler rations. Revista Brasileira de Zootecnia, 26: 1183–1191. http://hdl.handle.net/11449/35218.

Sakata T, Kojima T, Fujieda M, Takahashi M and Michibata M (2003). Influences of probiotic bacteria on organic acid production by pig caecal bacteria *in vitro*. Proceedings of the Nutrition Society, 62: 73–80. DOI:10.1079/PNS2002211.

Salim HA, Abd-Allah OA and Fararh KM (2011).Effect of feeding probiotic on hematological, biochemical properties and immune response in broiler. Benha Veterinary Medical Journal, 22: 35-43.

Samli HE, Senkoylu N, Koc F, Kanter M and Agma A (2007). Effects of *Enterococcus faecium* and dried whey on broiler performance, gut histomorphology and microbiota. Archives of Animal Nutrition, 61: 42–49. http://dx.doi.org/10.1080/17450390601106655.

Sorum H and Sunde M (2001). Resistance to antibiotics in the normal flora of animals. Veterinary Research, 32: 227–241.https://doi.org/10.1051/vetres:2001121.

Tabidi MH, Mukhtar AM and Hassan IM (2013). Effects of probiotic and antibiotic on performance and growth attributes of broiler chicks. Global Journal of Medicinal Plant Research,1(1):136-142.

Van Immerseel F, De Buck J, De Smet I, Pasmans F, Haesebrouck F and Ducatelle R (2004). Interactions of butyric acid- and acetic acid-treated Salmonella with chicken primary cecal epithelial cells in vitro. Avian Diseases, 48: 384–391. doi: http://dx.doi.org/10.1637/7094.

Van Immerseel F, Russell J, Flythe M, Gantois I, Timbermont L, Pasmans F, Haesebrouck F and Ducatelle R (2006). The use of organic acids to combat *Salmonella* in poultry: A mechanistic explanation of the efficacy. Avian Pathology, 35: 182–188. http://dx.doi.org/10.1080/03079450600711045

Waseem Mirza M, Rehman ZU and Mukhtar N (2016). Use of organic acids as potential feed additives in poultry production. Journal of World's Poultry Research, 6(3): 105-116. PII:S2322455X1600015-6.

Wilson TA, Nicolosi RJ, Rogers EJ, Sacchiero R and Goldberg DJ (1998). Studies of cholesterol and bile acid metabolism, and early atherogenesis in hamsters fed GT16-239, a novel bile acid sequestrant (BAS), Atherosclerosis, 40: 315-324. https://doi.org/10.1016/S0021-9150(98)00135-X.

Youssef IMI, Beineke A, Rohn K and Kamphues J (2012). Influences of increased levels of biotin, zinc or mannan-oligosaccharides in the diet on foot pad dermatitis in growing turkeys housed on dry and wet litter. Journal of Animal Physiology and Animal Nutrition, 96 (5): 747-761. DOI:10.1111/j.1439-0396.2010.01115.x