IMPACTS OF PROBIOTICS WITH OR WITHOUT ORGANIC ACIDS AS DIETARY SUPPLEMENTS ON GROWTH PERFORMANCE, CARCASS QUALITY, DIGESTIBILITY, INTESTINAL DEVELOPMENT, AND GUT MICROBIOTA OF BROILER CHICKS †

[IMPACTO DE LOS PROBIOTICOS CON O SIN ÁCIDOS ORGÁNICOS COMO COMPLEMENTO DIETÉTICO EN EL CRECIMIENTO, CALIDAD DE LA CANAL, DIGESTIBILIDAD, DESARROLLO INTESTINAL Y LA MICROBIOTA DEL INTESTINO EN POLLOS DE ENGORDA]

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SUMMARY

Background. Due to the harmful effects resulting from the addition of antibiotics to treat diseases affecting poultry and their use as growth promoter, it was necessary to have safe alternatives such as organic acids and probiotics, which work to provide an appropriate pH for activity of beneficial bacteria and reducing the numbers of harmful pathogenic bacteria. **Objective.** To assess the effects of dietary supplementation of probiotics, with or without organic acids (acetic, propionic, and benzoic) on growth performance, carcass quality, digestibility, intestinal development, gut microbiota, and serum parameters of broiler chicks. **Methodology.** A total number of 250 one-day old, unsexed Ross (308) broiler chicks, were equally assigned to five feeding treatments (50 birds/treatment, five replicates of ten birds/treatment) as follows: T1 (control); fed a basal diet (BD), T2; fed BD + 2g commercial probiotics (PRO)/kg diet, T3: fed BD + 2g PRO/ kg diet + 10 ml acetic acid/kg diet, T4: fed BD + 2g PRO/kg diet + 10 ml propionic acid/ kg diet, and T5: fed BD + 2g PRO/ kg diet + 10 ml benzoic acid / kg diet. The trial prolonged from day 1 to day 42 of age. **Results.** Compared to the control, the results revealed that body weight and body weight gain were significantly improved at 3 and 6 wks of age as a result of supplementing the diets with probiotics and organic acids (especially benzoic acid). The positive impacts of the supplements on FCR were more pronounced from 4-6 weeks and for the overall period (0-6 wks). No mortalities occurred when organic acids were supplemented. The tested supplements also decreased bacterial counts of ileal *E. coli*. Moreover, probiotics plus benzoic acid treatment (T5) significantly ($P < 0.05$) increased the ileal *lactobacilli* count. Carcass traits, internal organs and GIT histological parameters were positively affected by different treatments, especially probiotics plus benzoic acid treatment. The supplements improved ALT, AST and ALP activity and decreased cholesterol and triglycerides content in blood serum ($P <0.05$). **Implications.** The study showed that the addition of organic acids to probiotics led to an increase in the *lactobacilli* count. **Conclusions.** The tested feed supplements displayed beneficial impacts and positive role on broilers productive performance and improved their gut health and function through enhancement of the intestinal microbiota.

Key words: Blood metabolites; feed acidifiers; growth promoters; intestinal morphology; meat-type chickens; meat yield.

RESUMEN

Antecedentes. Debido a los efectos nocivos derivados de la adición de antibióticos para el tratamiento de enfermedades que afectan a las aves y su uso como promotores del crecimiento, es necesario contar con alternativas seguras como los ácidos orgánicos y los probióticos, que funcionan para proporcionar un pH adecuado para la actividad de las bacterias beneficas y reducir el número de bacterias patógenas dañinas. **Objetivo.** Evaluar los efectos de la suplementación dietética con probióticos con o sin ácidos orgánicos (acético, propiónico y benzoico) sobre el crecimiento, calidad de la canal, la digestibilidad, el desarrollo intestinal, la microbiota del intestino y los parámetros

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séricos de pollos de engorde. **Metodología.** Se asignó un total de 250 pollos de engorde Ross (308) sin sexar de un día de edad a cinco tratamientos de alimentación (50 aves/tratamiento, cinco repeticiones de diez aves/tratamiento) de la siguiente manera: T1 (control); alimentado con dieta basal (BD), T2; alimentado con BD + 2 g de probióticos comerciales (PRO)/kg de dieta, T3: alimentado con BD + 2 g PRO/kg dieta + 10 ml de ácido acético/kg dieta, T4: alimentado con BD + 2 g PRO/kg dieta + 10 ml ácido propiónico/kg dieta, y T5: alimentado con BD + 2g PRO/kg dieta + 10 ml ácido benzoico/kg dieta. El ensayo se prolongó desde el día 1 hasta el día 42 de edad. **Resultados.** En comparación con el tratamiento control, los resultados revelaron que el peso corporal y la ganancia de peso corporal mejoraron significativamente a las 3 y 6 semanas de edad como resultado de la suplementación de las dietas con probióticos y ácidos orgánicos (especialmente ácido benzoico). Los impactos positivos de los suplementos en FCR fueron más pronunciados de 4 a 6 semanas y durante el período general (0 a 6 semanas). No observo mortalidad cuando se complementaron con ácidos orgánicos. Los suplementos probados también redujeron los recuentos bacterianos de *E. coli* ileal. Además, los probióticos más el tratamiento con ácido benzoico (T5) aumentaron significativamente ($P < 0.05$) el recuento de lactobacilos ileales. Las características de la canal, los órganos internos y los parámetros histológicos del GIT se vieron afectados positivamente por diferentes tratamientos, especialmente con probióticos más el tratamiento con ácido benzoico. Los suplementos mejoraron la actividad de ALT, AST y ALP y redujeron el contenido de colesterol y triglicéridos en el suero sanguíneo ($P < 0.05$). **Implicaciones.** El estudio mostró que la adición de ácidos orgánicos a los probióticos condujo a un aumento en el recuento de lactobacilos. **Conclusiones**. Los suplementos alimenticios probados mostraron impactos beneficiosos y un papel positivo en el rendimiento productivo de los pollos de engorde y mejoraron su salud y función intestinal a través de la mejora de la microbiota intestinal.
Palabras clave: Metabolitos sanguíneos; acidificantes de piensos; promotores del crecimiento; morfología intestinal; pollos de carne; rendimiento de carne.

INTRODUCTION

The usage of antibiotics in broilers' dietsas growth promoters were always debatable since their use has been pointed out as one of the possible causes of the increase in bacterial resistance, based on the possibility of the presence of residues in human foods, which can lead to problems when applying antibiotics in human therapy (Roe,2003; Silva *et al.*,2010). Therefore, since 2006, the European Union has banned antibiotics use in animal feeds (Huyghebaert *et al.*,2011). Due to this decision, some diseases related to enteritis, such as coccidiosis, worsened on farms causing the broilers to perform less (Kipper *et al.*,2013).

As alternatives, probiotics were proposed to replace antibiotics, since they are dietary supplements composed of live and non-pathogenic microbial agents that benefit host health through intestinal balance (Sanders,2008; Kabir,2009; Ayasan, 2013). On the other hand, dietary supplementation with organic acids shows specific antimicrobial activity when used as feed preservatives with additional effects, including pH reduction, trophic effects on the gastrointestinal tract mucosa, and increase of pancreatic secretions (Dibner and Buttin, 2002; Van Immerseel *et al.,* 2006; Kim, 2015). Thus, it is extremely important to evaluate the synergistic or antagonistic effects of probiotics together with organic acids (OA) when used in poultry diets. The aim of this study, therefore, was to evaluate the effect of dietary supplements of probiotics with or without organic acids (acetic, propionic, benzoic) on productive performance, carcass quality, serum analyses, digestibility, intestinal development and gut microbiota of broiler chicks.

MATERIALS AND METHODS

This study was carried out at the Poultry Research Farm, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt. This study has been approved by the Council of Poultry Production Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

Birds and experiment design

A total number of 250 1-day old unsexed Ross (308) broiler chicks, having a similar average body weight (BW), were used. All chicks were wing-banded, individually weighed, and randomly allotted into 25 equal replicates of 10 chicks each. Each replicate was housed in floor pen (2 m length × 0.75 m width). The 25 replicates were equally divided among five dietary treatments (5 replicates per treatment, N = 50 birds/treatment) as follows: T1 (control); fed a basal diet (BD), T2; fed BD + 2g commercial probiotics (PRO)/kg diet, T3: fed BD + 2g PRO/ kg diet + 10 ml acetic acid/kg diet, T4: fed BD + 2g PRO/kg diet + 10 ml propionic acid/ kg diet, and T5: fed BD + 2g PRO/ kg diet + 10 ml benzoic acid / kg diet. The trial prolonged from day 1 to day 42 of age. The tested commercial probiotic, EGAVET is a product of Bio Natural France Co., contains one strain of bacteria: *Pediococcus acidilactici* (10^9 CFU/g). The tested acetic acid is a product of LG Chem Company, Germany. The propionic acid used in this study is a product of SIGMA Company, USA. The benzoic acid material is a product of Dow Chemical Company, USA. The chicks were fed their diets in mash form (mixed on the farm) that met all the nutrients requirements recommended by NRC (1994). During the experimental period (0 – 6 wks.); they were fed a starter diet from 0 - 3 weeks and a grower diet from 4 - 6 weeksof age (Table 1). Supplementations of commercial probiotics, acetic, propionic, and benzoic acids were provided in the diets from day 1 to day 42 of age. All birds involved in the study were kept under similar managerial and hygienic conditions and were offered feed and water *ad libitum*. The chicks were maintained on a 24-h constant light regimen throughout the experimental period. The initial temperature at start was set at 32 °C and reduced by 2–3 degrees weekly until 23 °C at 21 days of age and thereafter. The humidity ranged between 55 and 60% throughout the experimental period.

Table 1. Composition and calculated analysis of the basal diets (starter and grower).

Ingredients (kg)	Starter	Grower
Yellow corn	571	606
Soybean meal(48%)	317	271
Gluten meal	65	61
Dicalcium phosphate	17	15
Limestone	12.2	11.3
Vit.& Min. Mix[1]	3	3
Common Salt	3	3
DL-Methionine	0.5	0.2
L-Lysine	1.3	1
Soybean oil	10	28.5
Total	1000	1000
Calculated analysis		
Crude protein,%	23	21
ME (Kcal/Kg)	2950	3100
Ca, %	1	0.9
$P_{av,}$ %	0.45	0.4
Lysine, %	1.2	1.05
Meth. &Cys, %	0.83	0.74
Crude fiber, %	3.56	3.31

[1]Vitamin-mineral mixture supplied per kg of diet: Vit. A, 12,000 1 IU, Vit. D3, 2,200 IU, Vit. E, 10 mg, Vit. K3, 2 mg, Vit. B1, 1 mg, Vit. B2, 4 mg, Vit. B6, 1.5 mg, Vit. B12, 10 ìg, Niacin, 20 mg, Pantothenic acid, 10 mg, Folic acid, 1 mg, Biotin, 50 mg, Copper, 10 mg, Iodine, 1 mg, Iron, 30 mg, Manganese, 55 mg, Zinc, 50 mg and Selenium, 0.1 mg, Phytase 500 U and xylam, 600 U. 2Calculated values based on feed composition Tables of NRC (1994)

Growth parameters

The initial and weekly body weights (BW) were recorded on an individual basis till 6 weeks of age. The feed consumption (FC) was weekly recorded on a replicate basis for the whole experimental period according to (Attia, 1995). The daily body weight gain (BWG) = [(Final BW − initial BW)/number of period days], and feed conversion ratio (FCR) = (FC / BWG) were calculated. The number of dead birds was recorded, and liveability rates (%) were calculated for each treatment.

Carcass traits and internal organs

At the end of the experiment (42 days of age), a slaughter test was performed inside the poultry farm of the Faculty of Agriculture, Assiut University using 5 birds from each treatment (1 bird from each replicate around the average LBW of each treatment). The birds were individually weighed to the nearest gram and slaughtered using a sharp, sterile knife.

The measurements recorded from the carcass were: giblets weight (liver + heart + empty gizzard), abdominal fat weight, eviscerated carcass weight, dressed carcass weight, and carcass parts weights (breast, thigh, and drumstick). All weights of such organs and parts were also expressed as a percent of pre-slaughter body weight.

Enumeration of intestinal bacteria

At the slaughter time of the 25 birds mentioned before, fresh digesta samples were collected from the ileum and caeca of each bird for bacterial enumeration. Samples were kept in the laboratory in sealed sterile tubes at 4 °C until the enumeration of microbial populations. One gram of each digesta sample was mixed with 9 ml of saline (v/v). The mixture was then serially diluted in 0.9% sterile saline solution. The dilutions from 10^{-3} to 10^{-5} were used for the enumeration of *E. coli* and total Coliforms, and the dilutions 10^{-5} to 10^{-7} were used in quantifying the *lactobacilli*. These dilutions were inoculated in selective agar media and counted following conventional microbiological techniques. All microbiological analyses were performed in duplicates, in which 100 µl from each diluted sample was inoculated on agar plates, and the average values were used for statistical analysis. Results were expressed as the log of colony-forming units (CFU) per gram of digesta.

Digestibility trial

Digestibility trial was carried out using 5 birds from each treatment at 42 days of age to determine the apparent digestibility of nutrients. The birds were housed in separate cages (43 × 45 × 37 cm) for 7 days and feed and fresh water were offered *ad-libitum* during the digestion trial. Birds were fed the grower diets for 3 days as a preliminary period followed by 4 days as a main experimental period. Feed consumption was determined, and the excreta was collected quantitatively at the end of the fourth day of the main period. Feathers and dropped feed were taken out of the excreta, then the excreta was dried at 60 °C till constant weight and ground. The excreta of the birds of the same replicate was mixed into one bag and kept in a dry place until carrying out proximate analysis and evaluation of uric acid in the excreta for nitrogen correction. The apparent digestion coefficients (ADC) were calculated as follows:

ADC = [(consumed nutrient – excreted nutrient) / consumed nutrient] *100

The apparent protein digestion coefficient was corrected for uric acid content in the excreta.

Histological examination of GIT and Intestinal development

For histological analysis, 2-cm tissue samples from the middle length of duodenum, jejunum and ileum were transacted; digesta washed away using normal saline and fixed in 10% buffered formalin. Tissues were dehydrated by transferring through a series of alcohols with increasing concentration (70, 80, 90 and 100%; respectively) then placed into xylo and embedded in paraffin. A microtome Leica RM 2235 (Leica Biosystems Nussloch GmbH, Germany) was used to make 3 cuts and stained with heamatoxylin and eosin by using a digital camera (ToupTek, Version x 64, 3. 7. 7892) and light microscopy (Olympus CX31, Olympus, Hicksville, New York, USA) at 4 x magnification. The photos were taken, and morphometric analysis was performed by means of an image analysis program (image J software) to measure the villi height and crypt depth by examining 6 random villi and 6 crypts' depths. Besides, the villi height/ crypt depth was calculated. Villi height was measured from top of villi until crypt mouth. Crypt depth was measured from crypt bottom until crypt mouth (Rezaian and Hamedi, 2007).

Serum analyses

Blood samples were collected from each of the 25 slaughtered birds in non-heparinized tubes (10.0 ml). Total cholesterol (TC), glucose (GLU), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alanine phosphatase (ALP) were measured. Samples were analysed by an auto-analyser (SHIMADZU CL-8000 automatic autoanalyzer Cairo, Egypt) and using commercial kits purchased from Shanghai Kehua Bio-engineering Co., Ltd (KHB), Cairo, Egypt.

Statistical analysis

The variables presented as percentages were arcsine-transformed before analysis. Each replicate was considered as the experimental unit. The influence of treatment on the measured variables was analyzed by ANOVA following a completely randomized design, and the statistical analysis was conducted using the General Linear Model (GLM) procedure, SAS version 9.4 (2012, SAS Institute Inc., Cary, NC, USA). Significant differences among treatment means were determined using Duncan multiple range tests (Duncan, 1955).

RESULTS

Growth performance

The results of broiler growth performance as affected by the different experimental treatments are shown in Table 2. The tested dietary treatments had a significant positive effect ($P < 0.05$). On broilers final BW, BWG, and FCR compared to the control group. Moreover, T5 (probiotics+ benzoic) was superior in BW by about 28.0, 12.3, 5.7, 8.0% and in BWG by about 28.7, 12.6, 5.8, 8.2% than T1, T2, T3, and T4; respectively ($P < 0.05$). It is clear that the effect was more pronounced when supplementing the diet with benzoic plus probiotics than the control or the other treatments. No mortalities were recorded, and the liveability of the chickens was utmost during the experimental period in those birds received OA together with probiotics. However, 14% and 6%mortality rates were recorded for the control group and the group that received only probiotics, respectively.

Carcass traits and internal organs

The carcass characteristics of broiler chickens as affected by supplements of probiotics and organic acids (acetic, propionic, and benzoic) are presented in Table 3. The results revealed slight improvement of carcass traits of T2 (probiotic) over T1 (control) but pronounced improvements were detected for the carcass traits of T5 (probiotic + benzoic) over the control. The chickens of T5 showed the highest ($P < 0.05$) eviscerated carcass and dressed carcass percent (72.39 and 77.09; respectively) compared to those of T1, T2, T3 and T4. The relative weights of the internal organs of broilers aged 42-days are shown in Table (3). The nutritional treatments showed a negative effect ($P < 0.05$) on the weights and proportions of the liver, heart and gizzard but less pronounced on spleen and bursa.

Histological examination of GIT and Intestinal development

Mean values of villus height (VH), crypt depth (CD), and villus height to crypt depth ratio (VH/CD) from duodenum, jejunum, and ileum at 42 days of age as affected by treatments are shown in Table (4). The results revealed significantly higher values ($P < 0.05$)

Table 2. Effect of dietary supplementation ofprobiotics (PRO) and organic acids on growth performance of broiler chicks aged 1 to 42 days.

Age	Treatment					SEM	*P*-value
	(Control) Basal diet (BD)	BD+PRO	BD+PRO + Acetic acid	BD+PRO + Propionic acid	BD+PRO + Benzoic acid		
Body weight(g)							
At hatch	39.7	39.4	39.5	39.7	39.5	0.21	0.7836
3 weeks	504.3^{c}	528.4^{b}	547.4^{b}	556.2^{b}	628.3^{a}	14.52	0.0318
6 weeks	1768.8^{d}	2016^{c}	2142.2^{b}	2095.8^{b}	2264^{a}	21.20	0.0531
Body weight gain(g/b/d)							
0-3 weeks	22.1^{b}	23.2^{b}	24.2^{b}	24.5^{b}	28.0^{a}	1.40	0.0327
4-6 weeks	60.5^{d}	70.8^{c}	75.9^{ab}	73.4^{b}	77.8^{a}	2.18	0.0169
0-6 weeks	41.3^{c}	47.0^{b}	50.0^{a}	48.9^{b}	52.9^{a}	3.27	0.0483
Feed consumption(g/b/d)							
0-3 weeks	42.6^{b}	42.5^{b}	44.2^{ab}	47.0^{a}	50.5^{a}	0.92	0.0495
4-6 weeks	131.9^{a}	121.1^{c}	126.7^{b}	129.0^{b}	131.7^{a}	0.64	0.0526
0-6 weeks	87.6^{a}	81.8^{b}	85.4^{ab}	88.0^{a}	91.1^{a}	5.68	0.0495
Feed conversion ratio (g feed: g gain)							
0-3 weeks	1.9^{a}	1.8^{b}	1.8^{b}	1.9^{a}	1.8^{b}	0.02	0.0462
4-6 weeks	2.1^{a}	1.7^{b}	1.6^{b}	1.7^{b}	1.7^{b}	0.03	0.0226
0-6 weeks	2.1^{a}	1.7^{b}	1.7^{b}	1.7^{b}	1.7^{b}	0.02	0.0842
Mortality (%)							
0-6 weeks (%)	14	6	0	0	0	0	0
Liveability (%)							
0-6 weeks (%)	86	94	100	100	100	-	-

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

of VH and VH/CD ratio together with lower values of CD in duodenum, jejunum and ileum of the group received probiotics mixed with benzoic acid (T5), followed by the treatment fed probiotics mixed with propionic acid (T4). Moreover, it is noteworthy to mention that the effect of probiotics plus organic acids on histological properties of different GIT areas was more pronounced than the probiotic alone.

Table 3. Effect of dietary supplementation of probiotics (PRO) and organic acids on carcass traits and internal organs of broilers aged 42 days.

Traits	Treatments					SEM	*P*-value
	(Control) Basal diet (BD)	BD+PRO	BD+PRO + Acetic acid	BD+PRO + Propionic acid	BD+PRO + Benzoic acid		
Carcass traits							
Eviscerated carcass, %	63.03^{c}	69.00^{b}	69.26^{b}	68.36^{b}	72.39^{a}	2.35	0.0427
Dressed carcass, %	68.93^{c}	73.51^{b}	73.90^{b}	72.79^{b}	77.09^{a}	3.14	0.0263
Breast, %	21.71^{b}	19.99^{b}	21.11^{b}	27.44^{a}	27.49^{a}	1.25	0.0374
Drumstick, %	10.98^{b}	10.98^{b}	11.82^{b}	11.03^{b}	12.58^{a}	1.36	0.0051
Thigh, %	15.62^{c}	16.47^{b}	16.66^{b}	16.42^{b}	17.35^{a}	0.61	0.0537
Abdominal fat, %	2.06^{a}	1.58^{b}	1.46^{b}	1.16^{b}	1.47^{b}	0.12	0.0147
Internal organs							
Liver %	3.17^{a}	2.38^{bc}	2.54^{b}	2.25^{c}	2.43^{bc}	0.69	0.0251
Spleen %	0.21^{a}	0.17^{b}	0.21^{a}	0.17^{b}	0.20^{ab}	0.01	0.0583
Bursa %	0.14^{ab}	0.12^{bc}	0.10^{c}	0.11^{c}	0.15^{a}	0.22	0.0147
Heart %	0.47^{a}	0.38^{b}	0.41^{b}	0.41^{b}	0.40^{b}	0.03	0.0263
Gizzard %	2.26^{a}	1.74^{bc}	1.70^{c}	1.78^{bc}	1.87^{b}	0.29	0.9476

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

Table 4. Effect of dietary supplementation of probiotics (PRO) and organic acids on GIT parameters of broiler aged 42 days.

Parameter	Treatments					*SEM*	*P-value*
	(Control) Basal diet (BD)	BD+PRO	BD+PRO + Acetic acid	BD+PRO + Propionic acid	BD+PRO + Benzoic acid		
Duodenum							
Villi height (µm)	1880.5^{c}	2100.5^{b}	$2215.6b^{b}$	2512.7^{b}	3371.9^{a}	32.5	0.0051
Crypt depth (µm)	462.1^{b}	513.1^{a}	325.2^{c}	335.7^{c}	362.4^{c}	28.4	0.0274
Villi height/ Crypt depth	4.0^{c}	4.1^{c}	6.8^{b}	7.4^{b}	9.3^{a}	0.8	0.0019
Jejunum							
Villi height (µm)	1654.2^{c}	1802.8^{b}	1950.9^{ab}	1922.5^{ab}	2032.9^{a}	27.6	0.0521
Crypt depth (µm)	465.3^{a}	355.5^{b}	213.3^{c}	217.5^{c}	235.8^{c}	19.3	0.0386
Villi height/ Crypt depth	3.5^{c}	5.0^{b}	9.1^{a}	8.8^{a}	8.6^{a}	0.6	0.0097
Ileum							
Villi height (µm)	982.0^{c}	1055.3^{b}	1111.7^{b}	1155.2^{b}	1368.2^{a}	34.2	0.0491
Crypt depth (µm)	465.7^{a}	455.6^{a}	380.2^{b}	382.6^{b}	475.6^{a}	37.8	0.0276
Villi height/ crypt depth	2.1^{b}	2.3^{b}	2.9^{a}	3.0^{a}	2.9^{a}	0.2	0.0052

a,b,c Means within the same row with different superscripts are significantly different (P ≤ 0.05)

Gut microbiota

The effects of the tested supplements on ileal and caecal bacterial count at 42 days of age are presented in Table 5. A highly significant trend towards increases of *lactobacilli* count and decrease of *E. coli* count in both ileum and caecum was proved (P <0.05) for the treatment fed probiotics plus benzoic acid followed by the treatment fed probiotics plus propionic and then by the treatments fed probiotic alone or with acetic acid. The control treatment displayed significantly higher ileal and caecal *E. coli* count together with significantly lower ileal and caecal *lactobacilli* count than the other tested treatments (P < 0.05).

Digestibility Coefficients

The effects of probiotics and organic acids supplements on excreta moisture and nutrients digestibility coefficients of broiler chickens at 42 days of age are presented in Table 6. The results revealed that supplying the experimental diets with probiotics plus organic acids (acetic, propionic, or benzoic) resulted in two significant effects (P <0.05): a) significant less wet excreta (less excreta moisture percent), b) significant higher digestibility coefficients of crude protein and NFE. The digestibility coefficient of ether extract was not significantly affected; however, there was an insignificant trend (numerical) towards enhancement of fat digestibility in T5 (probiotics plus benzoic) compared with the control (T1). The ability of organic acids to reduce excreta moisture can be attributed to the significant improvement in NFE digestibility.

Serum parameters

The results of the serum analysis are presented in Table 7. The different studied serum criteria were not significantly affected by probiotics supplementation. However, enriching the diets with both probiotics plus organic acids (especially benzoic acid) had attributed to a significant increase of glucose and the activity of ALT, AST and ALP enzymes, and a significant decrease in cholesterol and triglycerides compared to the control treatment (P < 0.05).

Table 5. Effect of dietary supplementation of probiotics (PRO) and organic acids on the GUT microbiota (CFU/g) of broiler aged 42 days.

Bacterial strain	Treatment					SEM	*P*-value
	(Control) Basal diet (BD)	BD+PRO	BD+PRO + Acetic acid	BD+PRO + Propionic acid	BD+PRO + Benzoic acid		
Ileal Bacteria							
Lactobacilli	1.63×10^{8c}	2.97×10^{8b}	2.18×10^{8b}	5.85×10^{8ab}	7.48×10^{8a}	0.35×10^{8}	0.0374
E. coli	6.32×10^{9a}	4.82×10^{8a}	2.64×10^{8b}	1.95×10^{8b}	1.81×10^{8b}	0.51×10^{8}	0.0065
Caecalbacteria							
Lactobacilli	1.22×10^{7c}	3.26×10^{8b}	4.33×10^{8a}	2.85×10^{8b}	3.95×10^{8a}	0.14×10^{8}	0.0085
E. coli	8.36×10^{8a}	7.84×10^{7b}	3.95×10^{7c}	5.42×10^{7c}	4.12×10^{7c}	0.21×10^{7}	0.0076

a,b,c Means within the same row with different superscripts are significantly different (P ≤ 0.05)

Table 6. Effect of dietary supplements of probiotics (PRO) and organic acids on digestibility coefficients (DM basis) of broiler aged 42 days.

Nutrient (%)	Treatments					SEM	*P*-value
	(Control) Basal diet (BD)	BD+PRO	BD+PRO + Acetic acid	BD+PRO + Propionic acid	BD+PRO + Benzoic acid		
Excreta moisture	54.51^{a}	47.31^{a}	29.47^{b}	35.21^{b}	31.16^{b}	3.45	0.0434
Organic matter	79.01	82.37	86.33	81.44	84.39	2.41	0.2834
Crude protein	70.34^{b}	72.35^{b}	80.54^{a}	82.45^{a}	85.67^{a}	2.62	0.0427
Crude fiber	40.36	31.47	35.67	33.19	37.12	1.15	0.2194
Ether extract	90.22	92.11	94.69	93.90	95.71	1.02	0.3092
Nitrogen free extract	82.41^{c}	90.64^{b}	90.52^{b}	93.71^{a}	95.62^{a}	1.29	0.0243

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

Table 7. Effect of dietary supplements of probiotics (PRO) and organic acids on serum parameters of broiler aged 42 days.

Parameters	Treatments					SEM	*P*-value
	(Control) Basal diet (BD)	BD+PRO	BD+PRO + Acetic acid	BD+PRO + Propionic acid	BD+PRO + Benzoic acid		
Glucose (mg/dl)	162.58^{b}	170.06^{b}	190.85^{b}	200^{b}	252.39^{a}	0.150	0.0392
Cholesterol (mg/dl)	123.83^{a}	104.28^{b}	90.54^{c}	92.71^{c}	94.05^{c}	0.009	0.0194
Triglycerides (mg/dl)	105.161^{a}	95.02^{a}	57.01^{b}	64.86^{b}	52.09^{b}	0.005	0.0151
ALT (u/l)	13.60^{b}	16.62^{ab}	19.15^{a}	20.06^{a}	20.44^{a}	1.035	0.0243
AST (u/l)	196.46^{c}	206.21^{c}	221.73^{b}	236.35^{a}	245.73^{a}	0.014	0.0051
ALP (u/l)	2078^{c}	2658^{bc}	3120.2^{ab}	3716^{a}	3783^{a}	0.006	0.0419

ALT, alanine aminotransferase, AST, aspartate aminotransferase, ALP, alkaline phosphatase.
[a,b,c,d]Means within the same row with different superscripts are significantly different ($P \leq 0.05$)

DISCUSSION

The results of growth performance obtained in the present study agree with those of Abdel-Raheem *et al.* (2012), Omar (2014) and Fallah (2016), who ascertain that the LBW was significantly increased due to probiotics supplementation. Moreover, these results are partially confirmed by those of Vinus *et al.* (2017) who found improvements in BWG and FCR during all experimental periods with increasing levels of blend of OA in the diets. In contrast to our results, findings reported by Wu *et al*. (2016), and Makled *et al*. (2019) indicated that the dietary supplementation with sodium butyrate had no effect on broilers final BW, BWG, FC, or FCR. In our study, the feed conversion ratio was improved by about 19% compared to the control due to supplementing the experimental diets with probiotics alone or together with organic acids ($P < 0.05$).

Concerning probiotics as a dietary feed additive, our results agreed with Al-Sultan *et al*. (2016) and Okuneye *et al.* (2016) who stated that probiotics had significant effects on body weight, feed consumption, and feed conversion ratio from 1 - 42 days of age. Also, Nguyen and Kim*et al*. (2020) and Dauksiene*et al.* (2021) obtained an increase in the average body weight, body weight gain, FC, and FCR due to probiotics supplementation in the broilers diet. Moreover, the livability of birds fed with probiotics in addition to organic acids (T3, T4, T5) was higher since the mortality rate decreased from 14% in T1 (control) to 6% in T2 (probiotics) to zero % in T3, T4, and T5 (organic acids plus probiotics). These findings agree with those of Riad *et al*. (2010) who stated that mortality rate decreased in response to dietary probiotics from 0-42 days of age compared to the controls.

The findings of carcass traits obtained in our study agree with the results of some previous studies (Attia, 2018; Dauksiene *et al.*, 2021; Saleem *et al.*,2020). Moreover, the present data showed that the dressed carcass % in the group fed probiotics + benzoic acid (T5) was higher than the control treatment by 8.16% and higher than T2 (probiotics) by 3.58%, and the percentage of breast was higher in T5 by 5.78% than the control and by 7.50% than T2. The same treatment (T5) achieved a significantly higher percentage of breast, thigh and drumstick ($P < 0.05$). Regarding carcass quality, the superiority of T5 over the other groups that received organic acids (T3, T4) was less pronounced. The higher percentage of meat yield in birds fed on probiotics mixed with benzoic acid is due to the increase in feed consumption and thus improvement in feed conversion ratio during the different growth periods (0-6 week) compared to other treatments, as the addition of organic acids increases the feed palatability (Biggs *et al.*, 2008). It is also noteworthy to mention that supplementing diets with probiotics plus organic acids decreased the percentage of abdominal fat.

The obtained findings of carcass quality here disagree with Nourmohammadi *et al.* (2010) who reported that there were no significant effects of dietary treatment on carcass characteristics. Partially dissimilar results were found by Zhang *et al*. (2011) and Ahsan-ul-Haq *et al*. (2014) who reported that breast meat and giblets weight of broiler chicks were not significantly affected by citric acid supplementation, but dressing percentage was significantly improved and abdominal fat weight was reduced with increasing acetic acid levels.

The tested feed supplements here showed a negative effect ($P < 0.05$) on the weights and proportions of the liver, heart and gizzard but less pronounced on spleen and bursa, as the addition of organic acids did not cause any damage to these organs and the percentages were in the normal range. These results disagree with those of Abdel-Raheem *et al.* (2012), Liu *et al*. (2020) and Attia *et al*. (2018); who reported that probiotics and organic acids significantly improved liver, spleen and bursa weights compared with the control. In contrast, Mallo *et al*. (2012) studied the effect of sodium butyrate on immunity organs and did not detect significant differences among the treatments in liver, spleen, and bursa weights.

Regarding the histological examination of GIT and intestinal development, the results here agree with those reported by Abdel-Raheem *et al*. (2012) and Al-Sultan*et al*. (2016) who stated that the addition of probiotics increased the VH and villus height: crypt depth ratio in duodenum, jejunum, and ileum in comparison with the control diet. Similarly, Dai *et al*. (2021) and Dauksiene*et al*. (2021) reported highly significant differences between the organic acid group and the control group in VH, CD, and villus/crypt ratio in the duodenum, jejunum, and ileum at 42 days of age. However, our results disagree with those reported on probiotics by Al-Sultan *et al.* (2016) and those reported on organic acids by Gao *et al*. (2019) and Liu *et al*. (2020) who stated no significant differences between organic acids and control groups in VH, CD and VH/CD of duodenum, jejunum, and ileum of broiler chickens.

Our findings on the effects of dietary supplementation with probiotics and organic acids on broilers gut microbiota agree with those of Goh *et al.* (2020) and Dauksiene *et al.* (2021) who proved that probiotics could enhance the amount of intestinal *Lactobacillus* and reduce the population of aerobe bacteria and *Escherichia coli* (*E. coli*) in duodenum and caeca. In confirmation of these results, using mixtures of organic acids and probiotics, Elbaz *et al.* (2021) and Galli *et al.* (2021) found that *Lactobacillus* population was increased significantly in probiotics and probiotics plus citron groups, and both *E. coli* and total coliform were significantly reduced in ileal contents compared with the control group.

Misuse of dietary carbohydrates has been reported to cause watery stools in broilers (Choct, 2009). According to Huyghebaert *et al.* (2011), increased nutrient digestibility can be affected by nutrient composition, feed formula regime, environment, and health status of animals. Organic acids are known to stimulate pancreatic secretions and increase the acidity of the gastro-intestinal tract, resulting in a reduced pH. Thus, resulting in improved digestibility of crude protein (Dibner and Buttin, 2002; Ndelekwute *et al*., 2019). The ability of the organic acids to reduce the faecal moisture could be due to the significant improvement in fibre digestibility. Poor utilization of fibre has been reported to cause watery faeces in broiler chickens (Choct, 2009). Undigested fibre in the gastrointestinal tract forms a substrate for bacteria and could be fermented by bacteria thereby generating water as one of the products. This culminates to high moisture content of faeces. Improving fibre digestibility means reduction in the quantity of undigested fibre which would have been fermented by bacteria. Organic acids are known to induce pancreatic secretion and also could acidify intestinal digesta leading to a reduction in gut pH (lesson *et al.* 2005). The acidic nature of the proventriculus which is the stomach of chickens could be linked to the improvement in crude protein digestibility.

The results of serum analyses in the present study partially agree with those of Ghazalah *et al.* (2011) and Abudabos *et al*. (2017). They reported significant increase in blood glucose, and triglyceride, however; they did not prove significant different activity of ALT between the control and treated birds. The findings of serum lipid profile are in agreement with Abdo and Zeinb, (2004), who reported that blood total lipids and cholesterol decreased significantly by dietary acidifiers. The beneficial role of organic acids in reducing the blood lipid profile may be interpreted through their influence in decreasing the microbial intracellular pH. Thus, inhibits the action of important microbial enzymes and forces the bacterial cell to use energy to release the acid protons, leading to an intracellular accumulation of acid anions (Young and Foegeding,1993).

CONCLUSION

The tested feed supplements specially probiotics plus benzoic or acetic acids displayed beneficial impacts and positive role in improving broilers productive performance and in enhancing intestinal health and function through regulation of the intestinal microbiota.

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Conflict of interest. The authors declare no conflict of interest.

Compliance with ethical standards. The care and use of experimental birds were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. This study has been approved by the council of Poultry Production Department, Faculty of Agriculture, Assiut University, in its session No. 102 on 5-10-2020. This study has been approved by ethical committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, according to the OIE standards for use of animals in research (06/2023/0090).

Data availability. The datasets generated during the current study are available from the corresponding author on reasonable request (*abollez@aun.edu.eg*)

Author contribution statement (CRediT). M.N. Makled. Conceptualization, project administration, supervision, writing-review and editing. **M.A. ELdeeb**. Writing-review and editing, supervision. **K. Abouelezz.** Supervision, investigation, project administration. **O.K. Amen -** supervision, investigation, Writing – review and editing. **M.A. Habib –**Writing-review and editing, investigation, data curation, formal analysis.

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