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Effect of dietary probiotic, antibiotic or combination on broiler performance, cecum microbial population and ileal development

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Original Article *Nutrition*

Effect of dietary probiotic, antibiotic or combination on broiler performance, cecum microbial population and ileal development

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ABSTRACT

Objective: To investigate the effect of antibiotics and/or probiotics on performance, some serum metabolites, cecum microflora composition, and ileum histomorphology in broilers under the Egyptian conditions.
Design: Randomized controlled experimental study.
Animals: Two hundred forty 1-day-old Ross (308) chicks were reared till 35 days of age.
Procedures: The birds were randomly allocated into four main groups: a control diet without additives (CON); probiotic (Lactobacillus acidophilus) supplemented diet (PRO); antibiotic (Avilamycin) supplemented diet (ANT) and a mix group (AP) that received antibiotic in the diet form 1 to 4 days of age and treated during the rest of the experimental period with probiotics.
Results: Chickens fed on probiotic or antibiotic diets had linear improvement in live body weight (LBW) and feed conversion ratio (FCR) compared with the control group, while the best LBW and FCR were in the AP group. An improvement in the nutrient digestibility was observed in the probiotic added groups (PRO and AP). Serum cholesterol and low-density lipoprotein cholesterol contents decreased when antimicrobial (probiotic or antibiotic) supplementations were used, while there was an increase in high-density lipoprotein cholesterol contents, serum total protein, and albumin levels. Among all groups, cecum *Clostridium perfringens* and *Escherichia coli* counts decreased; however, there was an increase in Lactobacillus count compared to the control group. In probiotic supplemented groups (PRO and AP), a significant ($P<0.05$) improvement in ilea architecture.
Conclusion and clinical relevance: Using probiotic after initial treatment with an antibiotic in broiler diets had a positive effect on broiler growth performance, gut health (improved cecum microbial populations and ileum histomorphology), and nutrient digestibility.

Keywords: Probiotic, Antibiotic, Broiler, Nutrient digestibility, Cecal microbial population and Ileum histomorphology.

1. INTRODUCTION

An antimicrobial agent is a chemical, biological or natural substance that is used for killing microbes, stopping their growth or limiting their activity. The most popular antimicrobial agents used in the poultry industry are antibiotics and/or their recent alternatives such as probiotics. Antibiotics are widely used as growth promoters [1] or in control diseases such as intestinal infections and respiratory diseases [2]. One of the most important results of antibiotic usage is the alteration of the microorganism environment in the digestive tract, which has led to an improvement in growth and increasing feed efficiency [3]. However, the main problem of the improper use of antibiotics by farmers is the fear to meet some of strains of pathogens microbes resistant to antibiotics [4; 5] as well as antibiotic residues in bird's meat, which forced the European Union [6] to ban the use of antibiotics as a growth promoter.

Intestinal microbes have a significant impact on maintaining birds and human health, through preventing colonization by pathogens, facilitating nutrient uptake and metabolism, development of immunity, and disease resistance [7; 8]. Therefore, it is important to understand the role of gut microbes to the bird, and how to manage the gut microbial. Many factors influence gut microbes such as the age of birds, microbial environment, diet and antimicrobials. The most important factor of these is using antimicrobials, which affects the microbial content of the intestines [9]. Microbial changes in the cecal environment are, usually, monitored because the microbial system in the cecum is the primary culprit for food fermentation and produces some useful substances for birds such as organic acids [10: 11]. However, several viral and bacterial challenges facing broiler breeders made it hard to use antibiotics as growth promoters or taking into account their withdrawal period. The antibiotic alternatives (probiotics) do not have the same effects in eradicating the microbes or their activity.

Probiotics are beneficial live microorganisms that help in enhancing the bird (host) health. The most important roles of probiotic's in poultry industry is maintaining normal gut microflora by competitive exclusion, beneficial effects on metabolism by increasing digestive enzyme activities, improving nutrient digestibility and enhancing the immune system [12]. The mode of action of probiotics is the competitive exclusion of gut microbes. They influence them through their products (e.g. organic acids and volatile fatty acids) and also create a lower pH in gut environment which creates an unfavorable atmosphere that inhibits the growth of pathogenic bacteria and fungi [13: 10]. It might be concluded that probiotics reduce the pathogens and do not eliminate them, compared to the role played by the antibiotics. This shows that antibiotic substitutes cannot be used fully, especially in the case of some diseases (e.g. Clostridium or salmonella) which are difficult to be eliminated by the antibiotic substitutes .

Therefore, an antibiotic replacement following the use of antibiotic (as a treatment) is preferable. This investigation aimed to explore a new method to use both of antibiotics with probiotics to reduce the microbial load inside the bird without affecting the meat quality.

2. MATERIALS AND METHODS

2.1. Diets, Birds and Design

A total of 240 unsexed Ross 308 broiler chickens were randomly allocated in four groups each had 60 chicks arranged in six replicates (10 chicks each). The 1st group served as a control (CON) and received the basal diet without additives. The 2nd group received a diet supplemented with a probiotic (PRO), 3rd group received a diet supplemented with an antibiotic (ANT), while the 4th group received a diet supplemented with the antibiotic for the first four days of age; the probiotic was introduced, thereafter, for the rest of the experimental period. The antibiotic used in the study was Avilamycin at the level of 1000g/ton, while *Lactobacillus acidophilus* (ATCC 700396) was used as the probiotic (2×109 CFU/kg of diet). The L. acidophilus spores were obtained from the microbiology department, faculty of agriculture, Ain Shams University. The birds were randomly allocated in four treatments for 35 days. Room temperature was maintained at 330C during the first 2 day of age, then, gradually reduced 0.2oC every day until the age of 14 days. Chicks were raised until the end of the experimental period under the natural summer conditions (June). The experimental diets were formulated according to the nutrient requirements for broiler chickens (Ross 308) of the NRC [14] are shown in **Table (1)**.

2.2. Growth Performance Traits

Feed intake (FI) and live body weight (LBW) were recorded at the end of the experiment period (at 35 days) and the feed conversion ratio (FCR) was calculated (FCR; as a gram of feed intake per gram of weight gain). The mortality rate was recorded during the trial period and the ratio was calculated for each treatment.

2.3. Blood Biochemical

At the age of 35 days, three birds from each replicate were randomly selected to determine serum biochemical contents following the slaughter in a tube without anticoagulant to separate serum. Serum was collected after blood centrifugation at 3000xg for 15 min and placed in the freezer at - 10 °C till analysis. Parameters measured were total protein, albumin, globulin, glucose, triglycerides and cholesterol (total, HDL and LDL) using a spectrophotometer.

2.4. Digestibility Trial

Six chickens were taken from each treatment (1 bird from each replicate at the age of 35 days) for a four-day digestion experiment. Birds were subjected to starvation for 24 hours before sampling to ensure adequate clearing of the gastrointestinal tract. Excreta was collected 3 times a day (every 8 hours) from each cage for 4 days, taking into account the removal of feathers from excreta (Dry at 70 ° C for 20 hours) and weighed then stored in sealed bags at −20°C till analysis. Gross energy (for both of diets and excreta) was determined via Bomb Calorimetry (C5001- IKA- WERKE). To determine nutrient content (dry matter, crude fiber, crude protein) the methods of AOAC [15] were used.

2.5. Cecal Bacterial Counts

Lactobacillus spp., *Escherichia coli* and *Clostridium* counts in the cecal samples (around 1 g/sample) were enumerated using serial 10-fold dilutions with 1% buffer solution and streaked on appropriate selective media for enumeration of each bacteria (Rogosa and Sharpe (MRS) agar for *lactobacilli., Eosin* Methylene Blue agar for *E. coli* and Perfringens agar for *Clostridium*). These plates were incubated under anaerobic conditions at 37°C for 72 h for *Clostridium* and *lactobacilli* spp., but 48 h at 39˚C under aerobic conditions for E. coli. Microbial groups were enumerated using the poured plate's technique.

2.6. Ileum Histomorphology

The samples of ileum collected as segments of approximately 3 cm were taken from the mid-point of the ileum. Segments were fixed in 10% neutral buffered formalin solution and embedded in paraffin wax. All histological studies were performed, stained by haematoxylin and eosin which were examined under the light microscope (ZEISS Axio Imager .A2) fitted with a digital camera.

2.7. Statistical Analysis

The experiment was conducted according to the completely random design. Statistical analysis was carried out using the General liner Model (GLM) procedure using SAS [16] and the differences among means (p<0.05) were evaluated via Duncan's multiple range tests.

3. RESULTS

3.1. Performance

The performance parameters of broiler chickens' growth as affected by the dietary treatments during 1-35 days are in **Table (2)**. Results revealed that there was an improvement in LBW and an enhancement in the feed conversion ratio (FCR) in all treatments compared to the CON group. FI was not affected by the diets during this experiment. There was a significant (P<0.05) increase in LBW in AP compared to PRO and ANT groups. The PRO and ANT groups had similar FCR, while the AP group was better at the end of the experiment. Moreover, the AP group significantly reduced the mortality percentage compared to other groups.

Table 1. Composition and calculated analysis of experimental basal diets.

Ingredients (%)	Starter (1-21 d)	Grower (22-35 d)
Yellow corn	53.35	58.40
Soybean meal (44%)	33.14	28.90
Corn gluten meal (62%)	6.35	5.35
Soybean oil	3.00	3.10
Calcium carbonate	1.23	1.39
Di-calcium phosphate	1.93	1.96
Broiler premix*	0.35	0.35
Salt	0.45	0.45
DL-methionine	0.20	0.10
Total	100	100
Calculated chemical analysis		
ME (kcal kgG1)	3050	3100
Crude protein	23.00	21.00
Calcium	1.00	1.05
Av. phosphorus	0.48	0.48

*Each 3 kilogram of the premix contains the followings: 120000 I.U: VIT. A, 20000 I.U: VIT. D3, 10000 mg: VIT. E, 2000 mg: VIT. K3, 1000 mg: VIT. B1, 5000 mg: VIT. B2, 1500 mg: VIT. B6, 10 mg: VIT. B12, 10000 mg: Ca D-Pantothenate, 30000 mg, Niacin 1000 mg: Folic acid, 50 mg: Biotin, 250000 g: Choline Chloride, 60000 mg: Mn, 50000 mg Zn: 30000 mg: Iron, 10000 mg: Cu, 1000 mg: Iodine, and 100 mg: Se.

3.2. *Nutrient digestibility*

Nutrient digestibility data showed that using probiotic and antibiotic (AP group) in the broiler chickens' diet had a positive effect on the digestibility of dry matter; crude protein and crude fiber. However, the digestibility of energy was not affected (**Table 3**). Crude protein digestibility was higher (P<0.05) in AP and PRO groups in comparison to other groups. The digestibility of DM and CF improved in all treated groups compared to control.

Table 2. Effect of experimental treatments on growth performance.

Item	CON	PRO	ANT	AP	SEM	*P* -value
LBW	1728^{c}	1817^{b}	1809^{b}	1884^{a}	14.38	0.00
FI	3124	3139	3132	3129	2.386	0.14
FCR	1.808^{a}	1.728^{b}	1.731^{b}	1.661^{c}	0.014	0.00
Mort (%)	8.33	6.67	5.00	1.67	-	-

a ,b ,c Means within the same row with different superscripts are significantly (P < 0.05). SEM =Standard Error Mean. LBW=live body weight, FI= feed intake, FCR= feed conversion ratio, Mort= mortality (%), treatment groups: CON – corn-based diet without additives, PRO – based diet with adding probiotic, ANT – based diet with adding antibiotic, AP – that received antibiotic in the diet form 1 to 4 days of age and treated during the rest of the experimental period with probiotics.

Table3. Effect of experimental treatments on nutrient digestibility (%) and Energy content.

Item	CON	PRO	ANT	AP	SEM	*P* -value
DM	70.8^{b}	75.3^{a}	74.0^{a}	76.6^{a}	0.61	0.02
CF	39.7^{b}	47.6^{a}	43.2^{ab}	48.3^{a}	0.55	0.03
CP	64.6^{b}	67.7^{a}	65.1^{b}	68.6^{a}	1.06	0.04
GE	75.62	76.25	76.04	76.31	2.40	0.38

a ,b Means with different superscripts within the same row differ significantly (P < 0.05). SEM =Standard Error Mean. DM= dry matter, CF= crude fiber, CP= crude protein, GE=Gross energy, treatment groups: CON – corn-based diet without additives, PRO – based diet with adding probiotic, ANT – based diet with adding antibiotic, AP – that received antibiotic in the diet form 1 to 4 days of age and treated during the rest of the experimental period with probiotics.

3.3. *Biochemical serum analysis*

At the 35 day of age, the concentration of glucose, globulin, and triglycerides did not differ among experimental groups (**Table 4**). Irrespective of treatment, serum cholesterol and LDL concentrations decreased significantly (P<0.05) compared to those of control, while serum HDL concentration significantly was ameliorated in PRO and AP groups. Using probiotic and antibiotic led to an increase in serum concentrations of total protein and albumin compared to the control group. However, the serum level of globulin, triglycerides, and glucose were not affected.

3.4. Microbial population and histomorphology.

The composition of cecum microflora at the age of 35 days is shown in **Table (5).** Regardless the dietary treatment, there was a significant (P<0.05) increase in Lactobacillus in the microbial populations examined. On the contrary, the concentration of Escherichia coli and Clostridium were low in all treatments compared to control. The count of Escherichia coli (harmful microbe) in the AP group was clearly lower than of ANT, PRO and CON groups.

Histomorphology data (**Table 5**) for the ileum showed that probiotic supplemented broilers diets either alone or in a combination with the antibiotic had higher (p < 0.05) villus height (VH) and villus height: crypt depth ratio (VH/CD) in the ileum in comparison with other treatments. Crypt depth was not affected in all experimental groups

Table 4. Effect of experimental treatments on some serum biochemical parameters.

Item	CON	PRO	ANT	AP	SEM	*P* - value
Glucose (mmol/L)	12.61	12.83	12.95	12.74	3.54	0.28
Cholesterol (g/dl)	0.163[a]	0.141[b]	0.147[b]	0.143[b]	4.03	0.01
Total protein (g/dl)	3.19[c]	4.36[b]	4.20[b]	4.98[a]	0.67	0.04
Albumin (g/dl)	1.267[b]	1.383[a]	1.365[a]	1.353[a]	0.22	0.03
Globulin (g/dl)	1.406	1.422	1.410	1.419	0.16	0.35
Triglycerides (g/dl)	0.121	0.118	0.121	0.121	7.39	0.19
HDL (g/dl)	0.081[b]	0.091[a]	0.086[ab]	0.089[a]	2.16	0.02
LDL (g/dl)	0.073[a]	0.058[b]	0.058[b]	0.059[b]	5.52	0.01

[a,b,c:] Means within the same row with different superscripts are significantly different (P < 0.05) . SEM =Standard Error Mean. HDL= High-density lipoprotein: LDL= Low-density lipoprotein, treatment groups: CON – corn-based diet without additives, PRO – based diet with adding probiotic, ANT – based diet with adding antibiotic, AP – that received antibiotic in the diet form 1 to 4 days of age and treated during the rest of the experimental period with probiotics

.4. DISCUSSION

Feeding on a diet containing antimicrobial, whether antibiotics or probiotics, led to improving the performance of broiler chicks (LBW and FCR). Similar improvements in LBW and FCR have been reported in broiler fed with probiotic supplemented diets [17: 18: 19: 20]. As expected, the best productive performance was in the AP group (antibiotic followed by probiotics). This can be explained through the changes in the microbial ecosystem in the gut resulting in an improvement in digestive function and enhanced immune system which resulted in enhanced growth performance, broiler health, and reduced mortality. The same finding was reported in [21: 22] who concluded that the mortality percentage was significantly affected by using the antimicrobial (probiotic or antibiotic).

Table5. Effect of experimental treatments on cecum microbial counts (log CFU g^{-1}) and ileum histomorphology.

Item	CON	PRO	ANT	AP	SEM	*P* - value
Microbial count						
Lactobacillus spp.	1.758[d]	3.120[b]	2.605[c]	3.780[a]	0.224	0.00
Escherichia coli	3.682[a]	2.350[b]	2.036[b]	1.485[c]	0.247	0.00
Clostridium	2.326[a]	1.895[b]	0.807[c]	0.615[c]	0.219	0.00
Morphology						
Villus height (µm)	542.18[b]	699.18[a]	576.41[b]	714.91[a]	24.69	0.02
Crypt depth (µm)	140.28	137.59	133.15	135.09	3.503	0.92
VH/ CD ratio	3.93[b]	5.05[a]	4.36[ab]	5.31[a]	00.43	0.01

[a,b,c] Means within the same row with different superscripts are significantly (P < 0.05). SEM =Standard Error Mean. VH= Villus height, CD= Crypt depth, VH/CD = villus height to crypt depth ratio, treatment groups: CON – corn-based diet without additives, PRO – based diet with adding probiotic, ANT – based diet with adding antibiotic, AP – that received antibiotic in the diet form 1 to 4 days of age and treated during the rest of the experimental period with probiotics.

The energy value was insignificantly affected in all treated groups. Nevertheless, CP, DM, and CF values increased in AP and PRO groups compared to the CON group. The obtained results of nutrient digestibility in the chickens that were fed diets containing the probiotic and/or the antibiotic are similar to Apata, [23]; Li et al., [24]. Previous investigations proposed that probiotics can enhance nutrient absorption by changing the gut bacterial population [25]. Lei et al., [26] reported that using diets containing probiotic or antibiotic lead to better apparent digestibilities of DM, CP, and GE. There was a significant improvement in LBW and FCR due to the enhancement of digestion and utilization of nutrients. In a previous study, Apata, [23] reported that the supplement of probiotics could improve the nutrient digestibility of DM in the broiler. The earlier report of Edens, [27] also showed that adding probiotic in broiler diet improved digestion, absorption, and availability of nutrients associated with a positive effect on intestinal activity. The improvement in diet digestion and nutrient absorption can be explained on basis of the improvement in intestinal health (through decreasing pathogenic burden in gut and intestinal thickness [28] as well as the increase in the activity of some enzymes such as amylase, protease, and lipase due to adding probiotics in the diet [29]. The improvements in digestion of starch, proteins, and fats are due to some amelioration in enzymatic activity, which is reflected in the improvement of growth and feed conversion ratio.

Feeding probiotic or/and antibiotic to broilers led to significantly more decrements in serum cholesterol and LDL along with a significant increase in serum HDL levels than control birds. Similar results have been obtained by Islam et al., [30] when probiotic was added to broiler chickens diet. Panda et al., [31] and Elbaz et al., [20] also reported reductions in serum cholesterol levels in broiler fed diets containing probiotics. These negative influences of *Lactobacillus* cells on blood cholesterols may be due to their ability to utilize cholesterol for their own metabolism [32] or the incorporation of cholesterol into their cellular membrane [33]. The increase in total protein and albumin can be due to the addition of probiotics, which act to improve the utilization of dietary proteins through the competitive process between the probiotic and pathogenic microorganisms (i.e. reduction of protein breakdown into nitrogen) on the surface of the intestinal epithelial cells and, thus, increasing the surface area exposed to absorption of nutrients [7: 34].

Supplementing the diet with antimicrobial (probiotic and/or antibiotic) led to a decreased pathogenic population of Clostridium and Escherichia coli counts and it also had a significant effect in increasing Lactobacillus count especially in AP group compared to other experimental groups. Similar findings were also demonstrated by Kabir, [35]; Mahajan et al., [36]; Zhang et al., [37]. In this research, VH showed a significant increase in the probiotics fed chickens compared to the ANT and control groups. These results are in accordance with those obtained by Murakami et al., [38]; Chichlowski et al., [39]. The histomorphological ileum changes were found only in the probiotic groups (PRO and AP) which explain the observed improved productive performance of birds in this study. The increase in VH led to increments in the surface area exposed to nutrient absorption [40] causing an improvement in digestive and absorptive function [41]. This would justify the improvement in both LBW and FCR of chickens fed the probiotic and antibiotic (AP group) supplemented diet. From a practical point of view, this research showed the necessity for an adequate regulation for the use of antimicrobials in the broiler chicken diets to maximize the bird's health and performance as well as the breeder's profit, without any deleterious effects to the consumer.

It can be concluded that using probiotics in conjunction with antibiotics could enhance the nutrient digestibility and improve the growth performance of broiler. By reducing the effects of the harm caused by antibiotics, and maximizing the benefits of the probiotic directly in the gut (through the manipulation of intestinal microbes). This may lead to the elimination of harm to the consumer (the antibiotic residues that may still be in meat). Therefore, broiler breeders are recommended to use antibiotics only at an early age (1st week) and supplement probiotics after that to maximize economic benefits.

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Conflict of interest statement

The authors declare that there is no conflict of interest in the current research work.

Research ethics committee permission

The current research work is permitted to be executed according to standards of Research Ethics committee, Desert Research Center.

Authors' contribution

A. E. and S. E. conducted the experiment, analytical procedures; A. E. conducted the experiment design, analyzed the data, wrote and revised the manuscript; S. E. revised the manuscript.

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