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Effect of some growth promoters on the cecal microflora of broiler chickens

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Abstract: This search was designed to study the effect of some growth promoters on the microflora of cecum broiler chickens. A total of 450 broiler chickens of the commercial Hubbard Flex strain were used in a randomized completely design with 5 groups, 3 replicates in each group, (30 birds / replicate). Birds were raised on floor pens for 42 days. Group (1) Basal diet-no additives (control), group (2) Basal diet + antibiotic, group (3) Basal diet + probiotic, group (4) Basal diet + prebiotic and group (5) Basal diet + organic acid. On days 21 and 42 of the experiment two birds from each replicate were selected and after that killed by severing the jugular vein taking (1) cm from the cecum contents to determine some microbial indicators in cecum. The results showed that there was no significant differences ($P > 0.05$) in the PH of cecal digesta in (21)d of age and it was significant decrease ($P < 0.05$) noted in the total count of bacteria in the second, third and fourth groups compared with control, while birds of third Group showed significantly higher *Lactobacillus* count compared with control, there was no significant difference ($P > 0.05$) between the groups in the *Bifidobacter* count while it was significant decrease ($P < 0.05$) in *Coliformes* count in second and third groups and significant decrease in *E.Coli* count in the last groups and in the fifth group also compared with control. In 42d of birds age significant decrease ($P < 0.05$) in cecal PH was observed in third, fourth and fifth groups. A significant decrease in the total count in cecum was observed in birds of second, fourth and fifth groups compared with control. A significant decrease noted in the *Lactobacillus* count in second group while showed birds fourth and fifth groups significantly higher compared with control, also a significant increase noted in the *Bifidobacter* count in all groups compared with control. There was significant decrease in *Coliforme* count in second and third groups as well as a significant decrease in *E.Coli* count in the last groups and in the fifth group also compared with control.
Key words: Growth promoters, Cecal microflora, Broiler.

Introduction:

Antibiotics have been used to protect the animal health and to improve growth for many years. Aim of using antibiotics in feed sector was to get advantage from feeding and increasing protection against some diseases, toxins, and making better the absorption of nutrients in intestines. Confidence has diminished about antibiotics used for improving performance and reducing stress factors because of the risk development of bioresistance against bacteria in human. The recent European Union ban on the prophylactic use of in-feed antibiotics has escalated the search for alternatives for use within the poultry industry[1] . Currently, Natural Alternatives like probiotic, prebiotic, plant extracts and the organic acids seem the most interesting alternative supplement in regard of minimizing economic loses [2,3].

Probiotics are defined as live bacteria-yeast cultures or biological products that are added to drinking water or feed to regulate the ecological balance of microflora in the digestive tract of animals. These substances prevent the harmful effects of potentially pathogenic microorganisms and allow animals to derive increased benefits from the feed [4,5].

A prebiotic defined as a nondigestible food ingredient that can be utilized by intestinal microflora, which beneficially affects the host [6,7]. Oligosaccharides are carbohydrates composed of short chains of monosaccharide. Some are thought to enhance the growth of beneficial organisms in the gut, and others are thought to function by competing with pathogenic bacteria for attachment sites in the lumen. In this way, prebiotic oligosaccharides may improve host health. Organic acids (for example, lactic acid, fumaric acid, propionic acid, citric acid, formic acid, and acetic acid) create an acidic environment by decreasing the pH in the digestive tract, which prevents the development of pathogenic microorganisms and increases enzyme activity. Moreover, this increases the digestibility and utility of minerals such as iron, calcium, phosphorous, magnesium, and zinc, as well as proteins and amino acids [8]. Therefore, the objectives of the present study were to comparing the effects of antibiotic, probiotic, prebiotic and organic acids on hematological parameters and serum biochemical of broiler chickens.

Materials and Methods

Experimental design:

In this study, 450 broiler chickens of the commercial Hubbard Flex strain were used in a randomized completely design with 5 groups: 4 treatment groups and a control group, and 3 replicates in each group, 30 birds replicates. Birds were raised on floor pens (300 × 300 × 100 cm) for 42 days and had free access to feed and water throughout the entire experimental period. The lighting program consisted of a period of 23 h light and 1 h of darkness.

Feeding:

The chicks were fed standard starter (from 1 to 14 d), grower (from 15 to 35 d) and finisher (from 36 to 42) diets according to Feed mixture followed in the Department of Animal Production at the University of Damascus (Table 1). Groups were randomly assigned to following treatment groups,

Group 1: Basal diet-no additives (control).
Group 2: Basal diet + (100 g/ ton) antibiotic (lincomaycin).
Group 3: Basal diet + (1000 g/ ton) probiotic (*Bacillus subtilis.spp)*.
Group 4: Basal diet + (1000 g/ ton) prebiotic (Mannan oligosaccharide MOS)
Group 5: Basal diet + (1000 g/ ton) organic acid (sorbic acid, propionic acid, benzoic acid).

Table 1: Composition of basal diet in different periods of the experiment

Ingredient (%)	Period (days)		
	Starter (0-14)	Grower (15-35)	Finisher (36-42)
Corn	60.2	69	74
Soybean meal (44%)	35.8	27	22
Dicalcium phosphate	2.2	2.2	2.2
Calcium carbonate	1	1	1
Salt	0.4	0.4	0.4
DL-Methionine	0.1	0.1	0.1
Colin chloride	0.1	0.1	0.1
Vitamin Premix	0.1	0.1	0.1
Mineral Premix	0.1	0.1	0.1
Total	100	100	100

Table 2 shows the analysis of nutrient material in testing portion.

Period (days)			
Nutrient content	**Starter (0-14)**	**Grower (15-35)**	**Finisher (36-42)**
ME (kcal/kg)	2866.8	2971	3031
Crude protein (%)	21.17	18.1	16.34
ME/CP	135.4	164.3	185.5
Crude Fat (%)	2.77	3.03	3.18
Calcium (%)	0.96	0.94	0.93
P available (%)	0.77	0.74	0.73
Methionine (%)	0.47	0.42	0.4
Lysine %	1.27	1.00	0.85

Cecal Microflora Composition

Six broilers per treatment (a male and a female from each replicate) at the age of 21 and 42 d were killed by severing the jugular vein. The carcasses were subsequently opened and the entire GI tract was removed aseptically. The GI tract was then divided into sections (ileum, ceca, and colon), after that taking (1) cm from the cecal contents ,the pH of cecal digesta was measured on fresh samples (1) g diluted with 10 ml of deionized water and using an electrode and a pH meter, cecal digesta contents were then aseptically emptied in a new sterile bag and were immediately diluted 10-fold (i.e., 10% wt/vol) with sterile ice-cold anoxic PBS (0.1 M; pH 7.0) and subsequently homogenized for 3 min in a stomacher. Each cecal digesta homogenate was serially diluted from 10^{-1} to 10^{-7}. Dilutions were subsequently plated on duplicate selective agar media for enumeration of target bacterial groups. In particular, Total count of bacteria, *Lactobacillus spp*., *Bifidobacteria spp*, coliforms and E.Coli were enumerated using nutrient agar, Rogosa agar, *Bifidobacterium agar,* MacConkey agar, Plates were then incubated at 38°C for 24 to 72 h aerobically (nutrient and MacConkey agars) or 48 to 120 h anaerobically (Rogosa agar) and colonies were counted.Results were expressed as $\log^{10}$ colony forming units per gram of cecal digesta.

Statistical Analysis

Data was subjected to a one-way analysis of variance (ANOVA) and the comparison of means was carried out through least significant differences (LSD) test. All statistical analyses were done using SPSS program [9].

Results and Discussion:

1-In (21) d of age

Table 3 shows the effect of different diets on the PH and cecal microflora at the (21) d of birds age, no significant differences were observed in the PH of cecal digesta at this age although they decreased at the birds of some groups, especially the birds of the fifth group, but this decrease was not significant (P> 0.05). These results are accordance with findings of [10] who did not find any change in the cecal PH by adding organic acids (such as maleic acid), while [11] indicate that weak effects of organic acids such as propionic and formic acid in cecal PH may be due to the strong effect of the solution regulator (buffering) resistant to changes in the PH in poultry intestines. It was also noted a significant decrease (P <0.05) in the total count of bacteria in the cecal of birds in Antibiotic, Probiotic, Organic acids treatments. The results showed also a significant increase (P <0.05) in the *Lactobacillus* counts in cecal of birds of Probiotic treatment compared with other groups and this is in agreement with the study done by [12], while [13] did not find significant difference between the prebiotic treatment bird and the birds of the control group in this indicator despite the increase in *Lactobacillus* count in in cecal of birds in this age. While adding growth promoters had no effect on the *Bifidobacter* count in cecal, no significant differences (P> 0.05) in this indicator were noted between the different groups and a control group at (21) d of birds age, this is in agreement with the results of [14,15] while [16] found significant increase (P <0.05) in the *Bifidobacter* count in cecal of birds when Organic acid (2% Glaconic Acid) was added to the diet, while the

number of *Bifidobacter* significantly decreased in cecum of birds in the group that antibiotic (Zinc Bacitracin) was added to the diet .

As for the *Coliformes* number it was significantly decreased (P <0.05) in birds cecum of the antibiotic and probiotic treatments in this age and this is agreement with [14] results, which noted a significant decrease in the *Coliformes* count in birds cecum, especially in the end of the second week of age when probiotic was added to the diet .

Results showed a significant decrease (P <0.05) in the *E.Coli* count in birds cecum of the antibiotic, probiotic and organic acids treatments in this age compared with control, and this is in agreement with [16 ,12 and 17] results.

Table (3): Effect of different growth promoters on the PH and cecal microflora at the (21) d of birds age.

Component	Dietary treatments					L.S.D 5%
	Control	Antibiotic	Probiotic	Prebiotic	Organic acid	
pH of caecal digesta	7.45 [a]	7.43 [a]	7.48 [a]	7.83[a]	7.25 [a]	–
Total count log 10 cfu/g	12.36 [a]	8.34 [b]	11.41 [c]	12.25 [a]	11.36 [c]	0.80
***Lactobacillus* spp** log 10 cfu/g	4.60 [a]	4.39 [a]	5.41 [b]	4.65 [a]	4.50 [a]	0.67
Bifidobacterium log 10 cfu/g	5.66 [a]	6.35 [a]	6.46 [a]	5.45 [a]	5.76 [a]	–
Coliforms log 10 cfu/g	8.31 [a]	6.61 [b]	7.04 [c]	8.28 [a]	8.32 [a]	0.41
E.Coli log 10 cfu/g	6.53 [a]	4.32 [b]	4.46 [b]	6.18 [a]	5.39 [c]	0.69

Means in each row with different superscripts are significantly different (P<0.05).

2-In (42) d of age:

A significant decrease (P <0.05) was noted (table 4) in the cecal PH in the intestines of birds at (42) d of birds age in the probiotic, prebiotic and organic acids treatments compared with other treatments, and this is in agreement with the study done by [18], which indicates to the effect of probiotics in reducing the cecal PH that leads to inhibition the *Coliformes* bacteria even partially in cecum.

Also a significant decrease (P <0.05) was noted in the total count of bacteria in the cecum of birds in Antibiotic, Prebiotic and Organic acids treatments in this age compared with other treatments and these results agreed with [8] study, While [19] and [20] attributed this decrease in the total count of bacteria to the curb and inhibitory effect of antibiotic and organic acids on colonies of microbial harmful pathogens in the gut. The results agreement with [21] study where they did not find any significant differences in this indicator between the various probiotics treatments for different groups of birds and the control of age (42) d.

As for *Lactobacillus* count, a significant increase (P <0.05) was observed in the number of *Lactobacillus* in birds cecum for Prebiotic and Organic acids treatments compared with control birds while a significant decrease (P <0.05) was noted in count in birds cecum of antibiotic treatment compared with birds of other groups. That is in agreement with results of [22, 23] while [24] indicated a significant decrease (P <0.05) in the *Lactobacillus* count in cecum of birds which fed on organic acids. Adding different growth promoters led to significant increase (P <0.05) in the *Bifidobacter* count in cecal of all treatments birds compared with control birds. [25,14] have a significant increase in the Bifidobacter count in the cecum of birds that gradual levels of probiotic were added to their diets, while [15] did not find any effect of adding yeast to the diets of broiler in *Bifidobacter* count, [26] showed the effect of adding prebiotic (inulin) on the increase of the *Bifidobacter* count.

Table (4): Effect of different growth promoters on the PH and cecal microflora at the (42) d of birds age.

Component	Dietary treatments					L.S.D 5%
	Control	Antibiotic	Probiotic	Prebiotic	Organic acid	
pH of caecal digesta	7.55 [a]	7.48 [ac]	7.10 [b]	7.30 [c]	7.05 [b]	0.22
Total count log 10 cfu/g	9.85 [a]	6.38 [b]	9.54 [a]	7.38 [c]	7.41 [c]	0.45
***Lactobacillus* spp** log 10 cfu/g	4.73 [a]	3.53 [b]	4.75 [a]	6.39 [c]	6.30 [c]	0.36
Bifidobacterium log 10 cfu/g	4.92 [a]	7.76 [b]	9.78 [c]	5.71 [d]	8.95 [e]	0.47
Coliforms log 10 cfu/g	6.42 [a]	5.23 [b]	5.57 [c]	6.51 [a]	6.31 [a]	0.34
E.Coli log 10 cfu/g	4.75 [a]	3.45 [b]	3.32 [b]	4.60 [a]	4.30 [c]	0.28

Results indicated a significant decrease ($P < 0.05$) in the *Coliformes* count in cecum of antibiotic and probiotic treatments birds compared with other treatment, and this is in agreement with [27] results.

As for the cecal content of *E.Coli* in (42) d of age, a significant decrease ($P < 0.05$) was noted in the birds cecum of antibiotic, probiotic, organic acids treatments compared with control birds and this is in agreement with[28,29] results, [30] indicated that organic acids form the acidic environment in the gut and lead to the development and increase in the *Lactobacillus* count which impedes the increase in the number of *E.Coli* or *Salmonella* and other bacteria, in addition to their ability to exclude the *coliform* of the intestine.

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