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Comparative effects of using prebiotic, probiotic, synbiotic and acidifier on growth performance, intestinal microbiology and histomorphology of broiler chicks

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Abstract

This study investigated the effects of dietary prebiotics, probiotic, synbiotic and organic acid salt supplementation on broiler growth performance, intestinal microflora, and histomorphology. A total of 300 one-day- old broiler chicks were randomly assigned to 5 different treatments with 3 replicates including 20 birds each. The birds received the same basal diet based on corn-soybean meal, and additives included in the diet at 0 control, prebiotic (1 g/kg), probiotic (1 g/ kg), synbiotic (1 g/ kg), and acidifier (5 g/ kg). The body weight, weight gain, feed conversion, intestinal morphology and microbiology of birds showed significant ($p<0.01$) improvement with dietary pre, pro, synbiotic and organic acid salt supplementation from 0 to 21d, 22-42 d and from 0-41 d in comparison with the control group. Synbiotic followed by probiotic supplemented groups revealed the highest final body weight, weight gain, better feed conversion and the highest antibody response to Newcastle disease vaccine (NDV) vaccine in comparison with prebiotic and organic acids. Moreover, synbiotic followed by probiotic supplementation significantly improved intestinal morphology and intestinal microbial ecology than prebiotic, organic acids and control groups. In conclusion, we suggest the use of synbiotic followed by probiotic is preferable as efficient growth and health promoters for broilers in comparison with prebiotic and organic acids.

Keywords: Prebiotic, Probiotic, Synbiotic, Organic acids, Broilers

Introduction

With increasing the risk of developing resistant bacteria to specific antibiotics and the presence of antibiotic residue in poultry feed led to a prohibition of using antibiotics as growth promoters in animal production in European Union since January 2006[9,14)]. In recent years,

particular concern has been paid on the use of probiotics, prebiotics, synbiotics, acidifiers, herbs and spices products as natural alternatives for AGP in animal and poultry diets to improve nutrients digestion, absorption, metabolism, performance and health. Prebiotics positively influence the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the intestine[23]. Probiotics defined as live microbial feed supplements that beneficially affect the host animal by improving its intestinal health[12]. When probiotics and prebiotics are used in combination, they are known as synbiotics[6,11]. This combination ork together in a synergistic way more efficiently promoting the probiotic and prebiotic benefits alone, and the coupling could also produce a synergistic effect in the reduction of pathogenic bacterial populations in the GIT[4,7]. Dietary organic acids can change the pH of the digestive tract of the animal. Also, the organic acids are capable of passing through the bacterial cell wall and making the microorganism unable to replicate efficiently and modify the intestinal microflora[18]. The results on the comparative efficacy of synbiotic, probiotics, prebiotics and organic acids products as feed additives in livestock and poultry needs further investigation to stand on the best combinations and to reach a good recommendations for poultry producers. Due to this need this study have been planned to compare the efficacy of prebiotics, probiotics, synbiotics and organic acids.

Materials and methods

Ethics Statement:

All live birds were conformed to protocols of national and international animal welfare, research regulations and Monitoring Committee of the Institute.

Birds and housing:

A total number of 300 one day old Cobb broiler chicks were randomly allocated into five treatment groups (60 birds/group) and housed in pens of identical size (2 m^2) in a deep litter system with a wood shaving floor. Each group included 3 replicates (20 birds/pen). The climatic conditions and lighting program will follow the commercial recommendations. The birds had free access to water and feed.

Dietary treatments:

The dietary treatments were; 1) a control or basal diet without supplementation; 2) a basal diet with a prebiotic Mannan oligosaccharides (BIO-MOS, Alltech, USA) at a level of 1 g/kg feed; 3) a basal diet with a probiotic 1g /kg (Primalac) 4) basal diet with Synbiotic Biomin® IMBO (a combination of *Enterococcus faecium and* oligosaccharides) at a level of 1 g/kg feed, and 5) a diet with organic acid salt calcium propionate at a level of 5 g/kg feed. Two basal starter and grower and finisher diets were formulated to contain the metabolizable energy (ME) density (3200 and 3220 kcal/kg) and crude protein (24 and 20%, respectively) and concentrations recommended by[22] (Table 1). All birds were subjected to a prophylactic vaccination against most common viral diseases and were kept under hygienic conditions.

Growth Performance:

Weekly body weight, feed consumption and mortality were recorded weekly in the course of the whole experiment per pen basis, and the cumulative feed intake were calculated at days 21 and 42 and the whole feed consumption were calculated and the feed conversion rates were calculated subsequently.

Microbiological examinations[5]

Sampling:

Five birds were randomly selected and slaughtered from each group and directly after dressing the intestinal tract was eliminated. Intestinal content from the duodenum and cecum was evacuated and mixed in sterile glass bottles. The sealed bottles were saved in the laboratory at 4°C till enumeration of microbial population (Table 3).

Analysis:

About 1 g of fresh samples was diluted 1:10 with

sterile 0.1% peptone water in sterile test tubes (PW, Oxoid CM9). Tenfold serial dilutions up to 10^7 of each sample were prepared using sterile peptone solution. Viable counts of total aerobes, total enterobacteria, fecal *E. coli*, and lactose fermenter were carried out. Enumeration of total aerobes were performed on standard plate count agar (PCA, Oxoid CM325). However enumeration of total enterobacteria were carried out on Violet Red Bile Glucose Agar (VRBG, Oxoid CM0485). E. coli counts was achieved on the eosin methylene blue (EMB) agar (Oxoid). lactose fermenter were counted on MacConkey agar as red colonies, respectively. Numbers of colony-forming units are stated as log colony-forming units per gram of digesta content.

Histopathological examination:

Fresh specimens from duodenum, jejunum and ileum were taken from five groups. Fixation of samples achieved by the use of 10% neutral buffer formalin, dehydrated in a graded alcohol series, cleared with methyl benzoate, embedded in paraffin wax, sectioned at 4 μ and stained with hematoxylin and eosin for histo-pathological examination by light microscopy (Olympus CX31) (*Lillie 1965*). Villus height and crypt depth was measured digitally using an Axiostar plus microscope (Carl Zeiss, Thornwood, NY, USA) interfaced with an Axiostar plus digital camera (Camera Olympus U-CMAD3made in Japan) and Axiovision 4.1 software (Carl Zeiss).

Blood Collection and Analysis:

At day 42, 5 birds were randomly selected from each group and blood samples were collected from the wing vein to estimate the post vaccination NDV antibody titers (3 weeks post vaccination). The collected blood samples were centrifuged at 4000 rpm for 15 min and the sera were transferred into aseptically vials and saved at – 20 °C until further analysis. The log10 NDV serum antibody titer were determined using commercial ELISA kits (IDEXX® Laboratories, B.V., The Netherlands). according to the recommendation of the supplier.

Statistical analysis:

Data were analyzed as a completely randomized block design, with 5 dietary treatments, using the ANOVA procedure of the SPSS[26] software program Version 16; (SPSS, USA). Significant differences among treatment means were separated by Duncan's test[10]. Statistical differences were considered significant at $P \leq 0.05$. Data are presented as means ± SE

Results

Growth Performance:

The effect of different additives on broiler performance is presented in Table 2. The initial BW of chicks did not differ ($P > 0.05$) between the dietary treatments. During the whole of experiment birds supplemented with dietary treatments had a greater ($P < 0.01$) BW, weight gain, and higher feed conversion into meat in comparison with birds of control group. The mortality percentage was lower numerically for the probiotic and synbiotic supplemented group (0 %) than prebiotic 3.33 %, organic acid 3.33 % and control 6.66 %.

Humoral antibody titre:

The effect of the feed additives on the humoral antibody titers post Newcastle disease virus (NDV) vaccination is shown in Table 2. As compared with the control group synbiotic, probiotics, prebiotics and organic acids increases the post vaccination $\log_{10}$ NDV antibodies significantly in serum at 3 weeks post vaccination, respectively.

Intestinal microbiology:

Prebiotic, probiotic, synbiotic and organic acids salts supplementation appeared to have a measurable effect on duodenal and cecal micro-flora of broilers Table 3. There were significant increase in total viable count in cecum, total entero-bacteriaceae in duodenum and cecum. Also, synbiotic and probiotic supplem-entation significantly decreased in fecal *E-coli* count in the duodenum and cecum. In addition, a numerical increases was observed in the lactose fermenter

colony count in the duodenum (tended to be higher ($p>0.05$), and a significant increase the cecum in syn-biotic supplemented broiler compared to pre, pro, organic acids and control groups.

Intestinal histomorphology

The means of duodenal, jejunal and ileal villus height, crypt depth, and villus height: crypt depth ratio are presented in Table 4. The addition of different additives in the current study increased the villus height, ($P < 0.01$) in duodenum, jejunum and ileum in comparison with the control diet. Synbiotic supplem-entation was superior in increasing villus height in duodenum and jejunum and ileum in comparison with pre, probiotic and organic acids. Synbiotic and Pro-biotic decreased crypt depth ($p<0.05$) in comparison with prebiotic and organic acids in duodenum, jejunum and cecum.

Table 1. Ingredient composition and chemical analysis of starter and grower diets for broiler chicks

Item	Starter d 1 to 21	7
Yellow corn	56	63.61
Corn gluten meal (62%)	4	3.5
Soybean meal (48)	32.41	25.5
Sunflower oil	4	3.5
Limestone	1.5	1.5
Dicalcium phosphate	1.2	1.5
Salt	0.4	0.4
L-Lysine	0.1	0.1
DL-Methionine	0.1	0.1
Premix[1]	0.25	0.25
Antioxidant	0.04	0.04
Total	100	100
ME (kcal/kg)	3200	3220
CP (%)	23	20
Methionine cystine (%)	0.76	0.77
Lysine (%)	1.24	1.06
Ca (%)	1.01	0.9
Av. P (%)	0.45	0.38
Na (%)	0.2	0.15
CF (%)	3.00	2.95

[1] Premix supplies per kg: 4, 8000000 IU Vit. A, 8, 00000 Vit D3, 4 g, Vit. E, 0.8 g Vit K3, 0.4 g Vit. B1, 2 g vit B2, 0.6 g Vit. B6, 4 mg Vit B12, 12 g nicotinic acid, 4 g pantothenic acid, 0.4 g folic acid, 20 g biotin, 100 g choline chloride 50 %, 12 g iron, 4 g copper, 20 g zinc, 24 g manganese, 0.4 g iodine, 0.04 g selenium, 0.04 g cobalt and carrier $Caco_3$ to 1 kg.

Table 2. Body weight gain, feed intake, feed conversion ratio (FCR), mortality and NDV antibody titre of different experimental groups[1].

Items	Control	Prebiotic	Probiotic	Synbiotic	Ca. propionate	Sig.
Body weigh						
d 1	42.33±0.66	54.23±13.48	43.87±0.61	45.00±0.75	50.44±0.88	ns
d 21	724.7±12.29^{b}	768.8±7.89^{a}	781.2±13.44^{a}	774.8±6.7^{a}	759.4±10.98^{a}	**
d 42	2335.0±23.16^{e}	2779.3±43.35^{c}	2834.5±44.2^{ab}	2937.7±44.4^{a}	2651.03±19.9^{d}	**
Weight gain (g)						
0-21 d	682.33±12.32^{e}	714.6±13.7^{c}	737.33±13.44^{a}	729.8±6.6^{b}	708.9±10.99^{d}	**
22-42 d	1610.3±25.66^{d}	2010.5±25.66^{b}	2053.3±42.5^{ab}	2162.9±42.5^{a}	1891.6±23.37^{c}	**
0-42 d	2292.67±23.3^{d}	2725.1±41.87^{b}	2748.65±44.4^{a}	2892.7±44.23^{a}	2600.6±19.87^{c}	**
Feed intake (g/bird)						
0-21 d	1121.00±9.92^{a}	1039 ±10.1^{bcd}	1012.67±11.1^{c}	1045.6±14.6^{bcd}	1068.0±13.65^{b}	**
22-42 d	3486±30.43^{a}	3193±62.00^{b}	3276.66±39.4^{b}	3229±18.7^{b}	3538.0±19.08^{a}	**
0-42 d	4607.00±37.6^{a}	4232±69.61^{b}	4289.3±30.85^{b}	4274.6±22.02^{b}	4606.0±12.98^{a}	**
FCR						
0-21 d	1.64±.01^{a}	1.45±0.01^{c}	1.37±0.01^{e}	1.44±0.02^{c}	1.51±0.02^{b}	**
22-42 d	2.16±0.01^{a}	1.59±0.03^{c}	1.58±0.02^{c}	1.53±0.04^{c}	1.87±0.01^{b}	**
0-42 d	2.01±0.01^{a}	1.55±0.02^{c}	1.52±0.01^{c}	1.51±0.03^{c}	1.77±0.01^{b}	**
Mortality, %	6.66	3.33	0	0	3.33	
ND Antibodies	2.86±0.14^{c}	3.7±0.03^{a}	3.82±0.01^{a}	3.87±0.03^{a}	3.2±0.12^{b}	**

^{abc}Different letters in the same row denote significant ($p<0.05$) differences among treatments, *P= <0.05; ** = P<0.01; ns = not significant, 1The results are reported as means ± SE, Prebiotic (1 g MOS /kg feed); probiotic (1 g / kg feed), synbiotic (1g /kg feed), calcium propionate (5 g/kg feed), FCR= Feed conversion ratio FCR= feed intake (g)/ weight gain (g), *ND = Newcastle disease antibody titer (log10 means ± SE).

Table 3. Effects of dietary treatments on intestinal microbiology (log10 CFU/g) at d 42.

Items	**Control**	**Prebiotic**	**Probiotic**	**synbiotic**	**Ca. propionate**	**Sig.**
Total viable count						
Duodenum	6.06±0.19	6.31±0.18	6.94±0.32	7.05±0.33	6.55±0.24	ns
Cecum	6.7±0.04^{b}	6.89±0.02^{b}	6.77±0.07^{b}	7.77±0.08^{a}	6.81±0.07^{b}	**
Enterobacteriaceae						
Duodenum	5.22±0.11^{b}	5.32±0.35^{b}	6.16±0.04^{a}	6.35±0.05^{a}	5.37±0.07^{b}	**
Cecum	6.76±0.03^{b}	6.73±0.11^{b}	6.61±0.07^{b}	7.48±0.09^{a}	6.6±0.06^{b}	**
Fecal E coli count						
Duodenum	6.46±0.12^{a}	5.51±0.34^{c}	4.33±0.33^{b}	4.43±0.43^{bc}	5.23±0.35^{bc}	*
Cecum	6.97±0.01^{a}	7.43±0.03^{a}	6.72±0.09^{ab}	6.21±0.06^{b}	6.85±0.38^{ab}	*
Lactose fermenter						
Duodenum	5.47±0.03	5.34±0.43	6.15±0.37	6.44±0.08	5.01±0.6	ns
Cecum	6.44±0.01^{b}	6.32±0.1^{c}	6.78±0.09^{b}	7.39±0.11^{a}	6.45±0.04^{c}	**

Data presented as logarithms of 5 samples per group (means ± SE)

^{abc}Different letters in the same row denote significant ($p<0.05$) differences among treatments

*P= <0.05; ** = P<0.01; ns = not significant, Prebiotic (1 g MOS /kg feed); probiotic (1 g /kg feed) and synbiotic (1g / kg feed), calcium propionate (5 g/kg feed).

Table 4. Effect of dietary treatments on histomorphological parameters of the duodenum, jejunum and ileum in broilers.

Items	Control	Prebiotic	Probiotic	synbiotic	Ca. propionate	Sig.
Villus height, μm						
Duodenum	967.6±7.8^{e}	1046±11.2^{c}	1255.6±14.6^{b}	1302.4±7.0^{a}	1078±8.3^{d}	**
Jejunum	776±12.08^{d}	1122±8.6^{c}	1170.0±3.5^{b}	1228±8.5^{a}	1117.8±281^{c}	**
Ileum	689.00±6.4^{e}	819.4±3.14^{c}	879±5.38^{b}	980±2.62^{a}	805±1.84^{d}	**
Crypt depth, μm						
Duodenum	241.6±6.04^{d}	185.2±1.5^{b}	167±2.09^{c}	153.8±2.15^{d}	173.2±2.67^{c}	**
Jejunum	213.2±2.08^{a}	185.8±1.46^{b}	173.6±1.02^{c}	150.3±1.77^{d}	175.4±3.26^{c}	**
Ileum	169.2±1.61^{a}	150.6±1.64^{b}	136.8±2.15^{c}	125.8±1.6^{d}	154.4±2.73^{b}	**
VH:CD ratio*						
Duodenum	4.01±0.13^{d}	6.05±0.05^{c}	6.19±0.1^{b}	8.47±0.15^{a}	6.23±0.12^{c}	**
Jejunum	3.64±0.06^{e}	6.05±0.05^{d}	6.74±0.02^{b}	8.17±0.13^{a}	6.38±0.13^{c}	**
Ileum	4.08±0.05^{d}	5.46±0.07^{c}	6.56±0.14^{b}	7.79±0.09^{a}	5.2±0.08^{c}	**

[1]The results are reported as means ±SE., n=5 samples, ^{abc} Different letters in the same row denote significant ($p<0.05$) differences among treatments, *P= <0.05; ** = P<0.01; ns = not significant, VH:CD =Villus height: crypt depth ratio, Prebiotic (1 g MOS /kg feed); probiotic (1 g /kg feed) and synbiotic (1g /kg feed), calcium propionate (5 g/kg feed).

Discussion

A lot of scientific research supports the role of probiotics, prebiotics synbiotics and organic acids as effective alternatives to the use of antibiotic growth promotors (AGP) in animal nutrition[3,4,6,20,24]. In agreement with the findings of other studies[2,28]. The results of the present study showed that, dietary supplem-entation with prepiotic, probiotic, syn-biotic or organic acids resulted in an improvement in broilers performance from d 0 to 42. A higher body weight and weight gain were observed in the chicks fed probiotic, synbiotic, 1 g/kg prebiotic and 5 g/ kg calcium propionate, respectively at d 21. Birds supplemented with probiotic and synbiotic exhibited the greater final body weight, final weight gain, the most efficient feed utilization and low mortality at day 42. The growth-promoting effect of these additives could be associated with a more efficient nutrient utilization (energy, protein, minerals and vitamins) from feed, which in turn results in an improved FCR. These additives improve feed efficiency by improving intestinal microflora population, intestinal integrity and stimulating appetite as well as stimulating the immune system[11,20]. Intestinal micro-flora plays an important role in the health status of host animals. Therefore, a common task to maintain host health is to increase the number of beneficial bacteria so as to inhibit colonization of pathogenic microorganisms[13]. In the current trail, there was statistical ($p<0.01$) decreases in the fecal *E-coli* colony count in the duodenum and the cecum as a response to dietary treatments and this decrease was more clear in synbiotic and probiotic supplemented broilers and this was in accordance with the previous results[1,4,8,11,25,28]. In addition, the significant increases in the lactose fermenter colony count in the cecum and the numerical increase in duodenum in synbiotic and probiotic supplemented broiler may due to alteration in the intestinal flora by the probiotic and synbiotic. Dietary prebiotics, probiotic, synbiotic change intestinal microbial community towards beneficial bacteria which play an important role in the prevention of colonization by pathogens in the gastrointestinal tract of chickens through a process known as competitive exclusion[16]. Maintaining good gut health is a key aspect of warranting the best bird performance and health. Supplementation of prebiotics or

probiotics or synbiotic and organic acids improve the antibody titer against NDV in the current work when compared with birds fed on none treated diets. Similar results reported by[15, 29,30]. It has been already established, morphological changes in the small intestine, such as increased villus height, villus width, and VH: CD ratio, can have beneficial effects on the performance of birds. Changing the intestinal tract integrity improve the absorptive surface area, which is important when alternative growth stimulators are applied. As an important finding of the present study, the addition of pre, pro, synbiotics and organic acids to broiler diets improved the morphological development of the intestine, as indicated by an increase in villus height and VH: CD ratio and by a decrease in villus crypt depth. These finding in accordance with the previous investigations regarding villus height[4,6,8, 11,28]. and the VH: CD ratio[27] and a decrease in the crypt depth[19], in different sections of the intestine.

Conclusion

From results of the current study, it is concluded that the use of synbiotic followed by probiotic is preferable as efficient growth and health promoters for optimization of feed conversion, growth performance, intestinal morphology and microbial community for broilers in comparison with prebiotic and organic acids.

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