IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 10, Issue 9 Ver. I (September 2017), PP 50-57
www.iosrjournals.org

Application of Probiotic with Different levels of Citric Acid Supplementation in the Diet for Promotes the Production Efficiency of Broiler Chickens

[1]Mahdi S. Jasim,[*2]Ali A. Fehan
Animal Production DepartmentCollege of Agriculture University ofDiyala,Iraq
*Corresponding Author:*Ali A. Fehan*

Abstract:*This study was conducted to evaluate the effects of addition probiotic andcitric acid separately and in a combination on performance parameters, intestinal microflora and morphology and immune systemof Ross 308 broiler chicks. The treatment were as the following :T1 (control diet) without supplement ,T2 supplementing with probiotic (0.05%), T3 and T4 supplementing with citric acid (0.15 and 0.3 %respectively), T5 supplementing with probiotic and citric acid (0.05 and 0.15 % respectively) and T6 supplementing with probiotic and citric acid (0.05and 0.3% respectively)performance parameters was massured weekly. At 21 and 42 d of age four chicks from each treatment were butcheredfor evaluation intestinal microfloraand morphologyand humeral immunity against newcastle and infectious bronchitis diseases. The results recorded asignificant increase in final live body weight and cumulative feed consumption for the treatment T5. The results showed significant increase in feed conversion ratio for treatments T5 and T6.In 21d,the results showed there were no significant differences in total bacteria count while there was significant increase in lactobacillus forT6 and significant decrease in E.coli for T5 and T6 . In 42 d, the results showed there was significant decrease in total bacteria count for T5 while there was significant increase in lactobacillus for T4 ,T5 andT6 and significant decrease in E.coli for T2 and T6 . Acording to intestinal morphology , the results showed there was significant increase in villi height for T3 and T5 in 21 d broilars while there were no significant differences in crypt depth in this age. In 42 d there was significant increase in villi height for T5 and T6 and there was significant increase in crypt depth for T5. In immunological evalulation ,the results showed there was significant increase inimmunity against Newcastle (ND) and infectious bronchitis (IB) for T5 in both age, while there was significant increase in immunity against ND for T2 in 21 d of age.*

Keywords:*Broiler; probiotic; citric acid; performance parameters;intestinalmicroflora;morphology;Immune system.*

Date of Submission: 30-08-2017 Date of acceptance: 17-09-2017

I. Introduction

The use of antibiotics as feed additive isunder discussion in regard to human foodsafety because of the potential development of antibiotic resistant bacteria. Antibioticresistant strains of *C. jejuni* and *C. coli* from broilers have also been discovered[1]. Therefore, there isan urgent demand to search for alternativestrategies to control *Campylobacter* both inhumans and chickens. Because of the vastnumber of pathogens in feces, leaking intestinal content during the slaughter process frequently contaminates poultry carcasses with *Campylobacter* spp[2]. Using alternativemethods to prevent colonization of these bacteria in the intestinal tract of flocks may help control the transmission of these bacteria from food to humans. Among the candidates for replacement of antibiotics are organic acids, enzymes, probiotics, prebiotics and plant extracts,which have been suggested to control intestinal microbial growth[3].probioticsis a live microbial feed that is beneficial to health[4], [5]and [6]. and stimulate the immune system[7].They may contain only one, or several (a consortium) different bacterial species.The mode of action of different bacterial strains in a probiotic consortium may differ[8]. Organicacids work in poultry, not only as a growth promoter but also as a meaningful tool of controlling all entrinticbacteria, both pathogenic and non-pathogenic[9],[10].Moreover,organic acids feedingis believed to have several beneficial effects such as improving feed conversion ratio, growth performance,enhancing mineral absorption and speeding recovery from fatigue[11],[12],[13],[14],[15],[16]and[17]. Contrary to antibiotics, organic acids have other properties like; lowering of the chime pH consequently, enhancing of protein digestion[18],[19] suggested that the reduction in gastric pH which occurs following organicacid feeding may increase pepsin activity. Moreover, peptides arising from pepsin proteolysis trigger the release of hormones, including gastrin and cholecystokinin which regulate the digestion and absorption of protein[20]. Therefore, the acid anion has been shown to complex with Ca, P, Mg, and Zn, which improved digestibility of these minerals[21].There are

little information about effect of combined probiotic and organic acid supplementation in broiler diet onperformance parameters , intestinal microflora and morphology and immune system. the present experiment was carried out on Ross 308 broiler chicks.

II.Materials And Methods

2.1Experimental design,birds, and diets

This study was carried out at poultry feild of animal production department\collage of agriculture\university of Diyala in order to study the effects of adding probiotic and different levels of citric acid to the diets of broiler and the impact on the performance parameters, microflora ,intestinal morphology and immune system .This study used 450 broiler (Ross 308), allocated randomly into 6 equal treatment groups with 3replicates per treatment (25 broiler\replicate). The treatments as follow T1: control was free from addition ,the T2: was feed diet with 0.05% of probiotic , T3and T4 were feed diets with 0.15 % and 0.3 % of citric acid respectivelywhile T5 and T6 were feed diets with probiotic 0.05% and different levels of citric acid 0.15% and 0.3% respectively. Experimental diets were feed in starter (1-10) grower (11-24) and finisher (25-42) d. Composition of basal diets are shown in **table 1**.Above mentioned feed additions were added to basal diets in recommended levels at different phases of the experiment.

2.2Performance parameters

Body weight and body weight gain of each bird were determinedweekly according to[22]. The feed consumption(g/ week) was calculated per group by obtainingsum difference between the weight of offered feed and theremained portion for 7 days. Feed conversion ratio (FCR)was calculated weekly.

$$FCR = \frac{(Feed\ consumption\ (g)\ /\ bird\ /\ week)}{(Body\ weight\ gain\ (g)\ /\ bird\ /\ week)}$$

2.3 Intestinal Microflora

At 21 and 42 d of age, four chickens were randomly selected from each treatment and slaughtered by exsanguinations (these chickens were the same used for slaughter test).10gram of jejunum was taken and put in salt solution 90 ml and then diluted unitle 10^{-5} for enumeration of bacteria.Using these diluted subsamples,Total bacteria population was enumerated on Nutrient agar,*lactobacillus* bacteria population was enumerated on De Man-Rogosa-Sharpe (MRS) agar and *E. coli* was counted on Mac Conkey (MC) agar after incubation at 37°C in an anaerobic chamber for 48 hrs and in an aerobic chamber for 24 hrs, respectively [23].

Table 1: Composition of basal diets (%)

Ingredient (%)	Starter	Grower	Finisher
Corn	52	55.2	59
Soybean meal	42	38	33.7
Premix[1]	2.5	2.5	2.5
Sunflower oil	3	4	4.5
DicalciumPhosphate	0.5	0.3	0.3
Total	100	100	100
Calculated analyses[2]			
ME (kcal/kg)	2983	3092	3168
Protein (%)	23.16	21.67	20.1
Methionine%	0.56	0.53	0.52
Methionine+Cystine%	0.94	0.90	0.86
Lysine%	1.39	1.29	1.19
Ca%	0.87	0.87	0.8
Available P%	0.44	0.39	0.38

[1]Supplied per kilogram of diet: vitamin A, 400.000 IU/ kg vitamin D3,100.000 IU/ kg vitamin E, 1600IU/ kg vitamin K3,80 ppmvitamin B1, 80 ppm vitamin B2, 300 ppm vitamin B12 ,1000 ppb Niacin 1400 ppm Folic acid, 40 ppm D- Biotin,2000 ppb and 4000 ppm Betain.[2]Based on National Research Council recommendations[24].

2.4Intestinal mucosal morphology

After slaughter, the small intestine was removed, and a 5- cm long segment was dissected from the middle of the jejunum, fixed in 10% buffered formalin for 48 h, de-hydrated in increasing concentrations of ethanol, and placed into paraffin. Sections (5 µm thick) from paraffin-embedded samples were then stained with hematoxylin-eosin for observation with a light microscope. Villus height was measured as the length between

the tip of the villus and the villous-crypt axis. Measurements for crypt depth were taken from the valley between individual villi to the baso- lateral membrane as described by[25].

2.5Estimation of humeral immunity

All birds were vaccinated against infectious Newcastle disease (ND) and infectious bronchitis (IB) at fourth and twenty-five day of age through intraocular route and at eighteenth day of age vaccinated against in Newcastle disease through intraocular route, respectively. In order to measure the primary and secondary humoral immune response at 21and 42 d of age, blood was obtained from collected when slaughtering of four birds from each treatment. The serum samples were collected by centrifugation at 3000 × g for 10 minutes and stored at -20◦C for further analysis. Antibody titer against ND and IB were determined using ELISA kits (SYNBIOTICS, ME, USA) as described by[26].

2.6 Statistical Analysis

Data obtained from this experimentwere subjected to one-way analysis of variance by using SPSS software and general linear model (GLM) procedureSPSS[27]. InstituteSignificant differences among treatments were identified at P<0.05 by Duncan's new multiple rang test[28].

III. Results And Discussion

3.1BroilerPerformance

3.1.1 Live body weight(g/bird)

Result of this section presented in **table 2**. As shown in this table,there was no significant differences in first and second week ,while in third week there was significant(P<0.05) increase forT3,T6 in compare with othertreatments. In fourth week there was no significant difference among treatment while in fifth and sixth week there was significant (P<0.01) increase forT5 ,T6 in compare with other treatments.

Table 2. Effects of probiotic and citric acid supplementation onLivebody weight (g)of broiler chickens (Means±SE).

Treatments	Age (d)					
	7	14	21	28	35	42
T1 Control	121.40 ±6.80	278.53 ±8.22	715.00^{ab} ± 17.32	1406.33 ±8.81	2003.00^{c} ± 11.54	2581.66^{d} ± 17.32
T20.05% Pro	126.06 ±5.40	265.73 ±10.56	699.66^{ab} ±17.32	1409.0 ±20.98	2011.90^{c} ±11.54	2675.33 ^{abc} ±14.43
T3 CA 0.15%	118.93 ±11.19	284.46 ±11.54	742.6^{a} ± 17.32	1425.00 ±14.43	2026.0^{bc} ±11.54	2659.33 ^{bc} ±21.16
T4 CA 0.3%	126.53 ±11.04	283.00± 11.54	675.86 ^{b} ±14.14	1398.60 ±18.47	2009.56^{c} ±6.31	2617.00 ^{cd} ± 15.58
T5 Pro 0.05 % + CA 0.15%	127.06 ±4.6 3	269.66 ±13.32	698.00^{ab} ±17.32	1425.40 ±17.32	2072.0^{a} ±17.32	2738.66^{a} ±29.79
T6 Pro 0.05% +CA 0.3%	126.46 ±6.56	284.20 ±11.66	749.00^{a} ±17.32	1420.93 ±12.70	2062.5^{ab} ±17.30	2709.66 ^{ab} ±19.91
Significantly	N.S	N.S	*	N.S	**	**

Pro, probiotic; CA,citric acid:Means with different letters in the same column are significantly different * (p<0.05),** (p<0.01), NS: Non significant

3.1.2Feed intake (g/day)

Result of this section presented in **table 3**. As shown in this table,there was no significant differences in first and second week ,while in third week there was significant(P<0.05) increase for T3,T6 in compare with other treatments . In fourth week there was no significant difference among treatment While in fifth week there was significant(P<0.05) increase forT5. In sixth week there was significant(P<0.01) increase forT2, T5.while cumulative feed consumption shown significant(P<0.05) increase forT5**.**

Table 3. Effects of probiotic and citric acid supplementation onaverage Feed intake (g/day)of broiler chickens(Means±SE).

Treatments	Age (d)						
	1-7	7-14	14-21	21-28	28-35	35-42	Over all
T1 Control	15.29 ±1.20	35.57 ±0.65	76.46 ^{b} ±1.51	149.46 ±3.70	143.92 ^{b} ±2.82	169.53^{b} ±1.64	4131.63^{b} ±13.43
T20.05% Pro	15.85 ±0.38	32.26 ±2.94	76.52 ^{b} ±2.30	143.75 ±3.45	145.00 ^{b} ±1.73	183.14^{a} ±2.88	4173.93^{ab} ±84.32
T3 CA 0.15%	15.75 ±1.44	37.04 ±7.41	83.53 ^{a} ±1.73	145.20 ±4.76	143.00 ^{b} ±1.73	168.56^{b} ± 1.73	4148.70^{ab} ±121.15
T4 CA 0.3%	15.78 ±0.69	34.27 ±4.77	77.21 ^{ab} ±1.73	146.81 ± 5.68	147.4 ^{ab} ±4.21	168.74^{b} ±2.31	4131.35^{b} ±149.4
T5 Pro 0.05 % + CA 0.15%	16.40 ±0.84	32.03 ±1.88	75.81 ^{b} ±2.55	147.05 ±6.17	155.67 ^{a} ±2.60	177.24^{a} ± 2.30	4230.43^{a} ±43.36
T6 Pro 0.05% +CA 0.3%	16.25 ±0.827	35.68 ±6.00	81.91 ^{ab} ±1.82	145.96 ±4.93	150.0^{ab} ±2.88	167.99^{b} ±1.73	4185.63^{ab} ±108.6
Significantly	N.S	N.S	*	N.S	*	**	*

Pro, probiotic; CA,citric acid:Means with different letters in the same column are significantly different
* ($p<0.05$),** ($p<0.01$),NS: not significant

3.1.3 Feed conversion ratio

Result of this section presented in **table 4**. As shown in this table,in first week there was significant ($P<0.05$) improve forT2,T4 in compare with others. In second week there was no significant differences while in third week there was significant ($P<0.05$) decrease forT4. In fourth week there was significant ($P<0.05$) improve forT2, T5. There was no significant difference in fifth week. In sixth week there was significant ($P<0.05$) improve forT6 Cumulative feed conversion ratio was significantly($P<0.05$) improvedforT5,T6.

Table 4. Effects of probiotic and citric acid supplementation onaverageFeed conversion ratioof broiler chickens(Means±SE).

Treatments	Age (d)						
	1-7	7-14	14-21	21-28	28-35	35-42	Over all
T1 Control	1.345 ^{ab} ±0.02	1.584 ±0.02	1.230^{a} ± 0.04	1.516^{b} ±0.03	1.688 ±0.05	2.062 ^{b} ±0.09	1.626^{b} ±0.01
T20.05% Pro	1.314 ^{a} ± 0.07	1.610 ±0.01	1.242^{a} ±0.03	1.430^{a} ±0.01	1.675 ±0.05	1.937^{ab} ±0.03	1.580^{ab} ±0.01
T3 CA 0.15%	1.458 ^{b} ±0.02	1.567 ±0.03	1.279^{ab} ± 0.04	1.541^{b} ±0.02	1.676 ±0.08	1.908 ^{ab} ±0.01	1.580^{ab} ±0.01
T4 CA 0.3%	1.320 ^{a} ±0.10	1.559 ±0.01	1.368^{b} ± 0.02	1.470^{ab} ±0.01	1.688 ±0.02	1.954 ^{ab} ±0.07	1.600^{ab} ±0.01
T5 Pro 0.05 % + CA 0.15%	1.344 ^{ab} ±0.02	1.579 ±0.01	1.245^{a} ±0.04	1.418^{a} ± 0.02	1.695 ±0.06	1.876^{ab} ± 0.09	1.567^{a} ±0.01
T6 Pro 0.05% +CA 0.3%	1.348 ^{ab} ±0.02	1.569 ±0.01	1.231^{a} ±0.003	1.539 ^{b} ±0.01	1.682 ±0.16	1.817^{a} ±0.07	1.568^{a} ±0.01
Significantly	*	N.S	*	*	N.S	*	*

Pro, probiotic; CA,citric acid:Means with different letters in the same column are significantly different
*($p<0.05$), NS: Non significant

The results shown that addition of probiotic or citric acid to feed of broiler had morally effect of imporovement, this improvement in production criteria may be due to probiotic's content of beneficial microorganisms which may lead to morphological alternations in intestinal tract in addition to presence of beneficial bacteria on intestinal villi which improve the height of villi [29],[30].these improvement of villi play important role in increasing gut capacity and it's ability in digestion thus decrease speed of diet passage in intestinal tract so this will increase it's biological activities and improve the nutritional value of nutritial elements of feed and also through secretion of digestive enzymes for carbohydrates, proteins and fats [31]. the addition of organic acids has positive effect in protein digestion, stimulate secretion of pancreatic enzymes,it work as moderator to speed metabolism , negative portion of acid join the many of minerals such as phosphorus ,calcium, magnesium and others thus improve their absorption and also organic acid increase secretion of amylase enzyme from lactobacillus for starch breakdown [32].increasing numbers of beneficial bacteria and their distribution on mucus layer on mucin fibers which cover intestinal cells will provide suitable enveroument for their growth and reproduction. production of short chain organic acids in intestine which improve villi motality and increase intestinal cells multiplication and blood passage in mucus layer of intestinal tract, when they absorped by intestinal barrier will enter blood stream and become source of energy in the body and regulate some of metabolic processes[33].

3.2Jujenummicroflora

The results of this section are presented in **table 5**. As shown in this table,In 21d ,the results showed there was no significant differences in total bacteria count while there was significant($P<0.01$) increase in *lactobacillus* for T6 and significant decrease in *E.coli* for T5 and T6. In 42 d, the results showed there was significant($P<0.05$) decrease in total bacteria count for T5. while there was significant ($P<0.01$)increase in *lactobacillus* for T4 ,T5 andT6and significant($P<0.01$) decrease in *E.coli* for T2 and T6.

Table 5. Effects of probiotic and citric acid supplementation on Jujenum microflora (log_{10} cfu/g) of broilerchickens at 21 and 42 d of age(Means±SE).

Treatments	**21 days**			**42days**		
	Total count	***Lactobacillus***	***E.coli***	***Total count***	***Lactobacillus***	***E.coli***
T1 Control	**7.33 ±0.08**	**6.71 ^{c} ± 0.11**	**6.69^{c} ± 0.2**	**7.48 ^{a} ±0.05**	**6.82 ^{c} ± 0.2**	**6.91^{c} ± 0.03**
T20.05% Pro	**7.30 ± 0.02**	**7.12 ^{ab} ±0.15**	**6.54 ^{bc} ±0.2**	**7.44 ^{ab} ± 0.004**	**7.24 ^{ab} ±0.04**	**5.93 ^{a} ±0.23**
T3 CA 0.15%	**7.34 ±0.05**	**7.23 ^{ab} ±0.19**	**6.51 ^{bc} ± 0.1**	**7.42 ^{abc} ± 0.01**	**6.94 ^{bc} ±0.1**	**6.59 ^{bc} ±0.05**
T4 CA 0.3%	**7.29 ±0.1**	**6.93 ^{bc} ±0.17**	**6.10 ^{ab} ± 0.07**	**7.45 ^{ab} ±0.006**	**7.31 ^{a} ±0.02**	**6.33^{ab} ±0.27**
T5 Pro 0.05 % + CA 0.15%	**7.45 ±0.01**	**7.28 ^{ab} ±0.06**	**5.88 ^{a} ±0.06**	**7.33 ^{c} ±0.01**	**7.37 ^{a} ±0.1**	**6.18^{ab} ±0.23**
T6 Pro 0.05% +CA 0.3%	**7.20 ± 0.01**	**7.38 ^{a} ±0.06**	**5.73 ^{a} ±0.07**	**7.38 ^{bc} ± 0.04**	**7.36 ^{a} ±0.02**	**5.86^{a} ±0.15**
Significantly	**N.S**	**	**	*	**	**

Pro, probiotic; CA,citric acid:Means with different letters in the same column are significantly different
* ($p<0.05$),** ($p<0.01$),NS: Non significant

The improvement in microbial balance in broiler intestine in treatment of probiotic and citric acid may be due to probiotic ability for encouragement beneficial bacteria on reproduction and growth especially lactobacillus and inerease their numbers by competitive exclusion or by close micro- organismes receptors on epithelial cells by bacteria of probiotic and prevent adhesion of pathaogenic bacteria and thus reinforce the presence of beneficial progeny of bacteria [34], [35].probiotic also stimulate intestinal epithelial cells to produce mucin on mucin network fibers that cover intestinal villi to provide suitable environment for beneficial types of bacteria and increase their numbers[36]. the addition of citric acid has important role in lowering pH of intestinal tract thus lowering numbers of pathogenic bacteria especially *E.coli* , because *E.Coli* is very sensitive to acidity , so organic acids penetrate walls of pathogenic cells and that lead to suppress their growth and reproduction[37],[38],and [39].while beneficial bacteria will

increase in number because they not influence by acidity and this due to their content of high level of potassium[40]. which activate cytoplasmic enzymes of bacterial cell and transport systems which make cell more resistence for osmotic pressure[41].

3.3Intestinal Morphology

The results of this section are presented in **table 6**. As shown in this table,there was significant($P<0.01$) increase in villi height for T3 and T5 in 21 d, while there was no significant differences in crypt depth in this age. In 42 d there was significant($P<0.01$) increase in villi height for T5 and T6and there was significant ($P<0.05$)increase in crypt depth for T5.

Table 6. Effects of probiotic and citric acid supplementation on mucosal morphology(μm) of the jejunum of broilerchickensat 21 and 42 d of age(Means±SE)**.**

Treatments	21 days		42days	
	Villus height	Crypt depth	Villus height	Crypt depth
T1 Control	783.87 ^{b} ± 14.6	175.75 ±9.01	1109.25 ^{b} ±4.2	204.31 ^{c} ±5.9
T20.05% Pro	845.10^{ab} ±8.16	171.00 ±20.5	1165.66^{ab} ±38.3	237.87 ^{ab} ±4.7
T3 CA 0.15%	881.00^{a} ±30.79	161.50 ±9.50	1112.61 ^{b} ±13.4	218.62^{abc} ±13.2
T4 CA 0.3%	798.00^{b} ±24.49	166.25 ±11.95	1151.60^{ab} ±27.4	209.0^{bc} ±3.6
T5 Pro 0.05 % + CA 0.15%	868.75^{a} ±14.94	163.87 ±10.53	1213.0 ^{a} ±27.03	242.50 ^{a} ±14.7
T6 Pro 0.05% +CA 0.3%	832.25^{ab} ±13.06	156.75 ±24.96	1225.12 ^{a} ±17.5	232.18 ^{abc} ±12.3
Significantly	**	N.S	**	*

Pro,probiotic;CA,citric acid:Means with different letters in the same column are significantly different* ($p<0.05$),** ($p<0.01$),NS: Non significant

The positive of effects diet supplementation of probiotic and citric acid or mixture of probiotic and citric acid in increasing length of intestinal villi and crypts in broiler is due to role of probiotic in increasing intestinal normal flora such as lactobacillus, this bacteria able to produce group of vitamins ,minerals and amino acids which decrease intestinal PH that increase lenght of intestinal villi and obsorpion in addition to it role in increasing production of ammonia in intestine[42]and [43]. this effect may be due to role of short chain fatty acids in increasing number of goblet cells inintestinal villi which secrete mucin in intestinal tract, mucin form gelatinous substance in mucus layer to improve repair of epithelial cells of small intestine[44] .

3.4Antibody Response

The results of this section are presented in **table 7**. As shown in this table,In 21 d, of age there was significant ($P<0.01$)increase for T2against Newcastle disease (ND) whilethere was ($P<0.05$)significantincrease for T5againstinfectious bronchitis (IB). In 42 d, of age there wassignificant($P<0.01$)increase for T5against Newcastle disease (ND) whilethere was significant($P<0.05$)increase for T5 and T6againstinfectious bronchitis (IB).

Table 7. Effects of probiotic and citric acid supplementation onantibody titer against Newcastle disease andinfectiousbronchitisof broilerchickensat 21 and 42 d of age(Means±SE)

Treatments	21 days		42days	
	Anti-ND titre	Anti-IB titre	Anti-ND titre	Anti-IB titre
T1 Control	1687.50^{b} ±60.19	393.66^{b} ±26.01	2101.25 ^{d} ±232	2427.75^{ab} ±206.78

T20.05% Pro	**1849.12^{a} ±20.49**	**389.33^{b} ±28.80**	**4901.25^{ab} ± 334**	**2727.25^{ab} ±50.96**
T3 CA 0.15%	**1662.50^{b} ±24.62**	**412.65^{ab} ±9.29**	**3321.50^{cd} ±876**	**2251.65^{b} ±247.2**
T4 CA 0.3%	**1656.75^{b} ±54.10**	**384.33^{b} ±42.06**	**2885.50^{cd} ±206**	**2460.25^{ab} ±268.9**
T5 Pro 0.05 % + CA 0.15%	**1778.00^{ab} ±26.15**	**498.00^{a} ±50.91**	**5906.50^{a} ±476**	**3078.0^{a} ±297.5**
T6 Pro 0.05% +CA 0.3%	**1740.81 ^{ab} ±32.64**	**431.00^{ab} ±5.00**	**3626.7^{bc} ±883**	**3111.01 ^{a} ±251.82**
Significantly	**	*	**	*

Pro,probiotic;CA,citric acid:Means with different letters in the same column are significantly different * ($p<0.05$),** ($p<0.01$).

The reason of elevating immune respone by using probiotic is due to role of intestinal normal flora in increasing phagocytosis of macrophages and other type of white blood cells which engulf antigens (bacteria or viruses) also these cell able to destroy virus infected cells or cancerous cell, that lead to improve immune system by elevate level of antibodies against pathogenic agents [45].Theeffect of these diet supplemention on immune system activity may be due to increase differentiation of lymphatic system by increasing activity of Hexose Mono phosphate pathway thusincreasingantibodies [46].

IV.Conclusion

It has been suggested that antibioticalternatives cause reduce pathogenic bacteriain digestive tract of broiler chickens, which canhelp to improve intestinal health of these birds. The addition of the probiotic and citric acidin thebroilersdiethas increased the length of the villiand the depth of the crypts. It also increase the antibody titter against Newcastle and infections bronchitis compared to control treatment thus improve performance parameter of these birds.The effect of these Addition on traits of birds reinforced byAddition combination of probiotic or citricacid in compare to effect of singleAdditionof probiotic or citric acid, which means a synergistic effect between the probiotic and citric acid.

Reference

[1]. Jorgensen, F., Bailey, R. and Williams, S. 2002. Prevalence and Numbers of Salmonella and Campylobacter spp. On Raw, Whole Chickens in Relation to Sampling Methods. Int. J. Food Microbiol., 76: 151–164.

[2]. Rosenquist, H., Sommer, H. M., Nielsen, N. L. and Christensen, B. B. 2006. The Effect of Slaughter Operations on the Contamination of Chicken Carcasses with Thermotolerant Campylobacter. Int. J. Food Microbiol., 108: 226–232.

[3]. Higgins, S. E., Higgins, J. P., Wolfenden, A. D., Henderson,S. N., Torres-Rodriguez, A., Tellez, G. and Hargis, B. 2008. Evaluation of a Lactobacillus-Based Probiotic Culture for the Reduction of Salmonella Enteritidis in Neonatal Broiler Chicks. Poult. Sci., 87: 27–31.

[4]. Fook, L. and GIBSON, G. 2002. Probiotics as modulators of the gut flora. British.Journal. Nutr 88: S39-S49.

[5]. Nethewood, T., Gilbert, H.J., Parker, D.S. and O'Donnell, A.G. 1999. Probiotics shown to change bacterialcommunity structure in the avian gastrointestinal tract. Appl. Environ. Microbiol 65: 5134-5138

[6]. Patterson, J.A. and Burkholder, K. 2003. Application of prebiotics and probiotic in poultry production. Poultry.Science 82: 627-631.

[7]. Havenaar, R. and Spanhaak, S. 1994. Probiotics from an immunological point of view. Curr. Opin. Biotechnol 5: 320-325.

[8]. Bomba, A., Nemcova, R., Gancarcikova, S., Herich, R., Guba, P. and Mudronova, D. 2002 Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructooligosaccharides and polyunsaturated fatty acid. British.Journal. Nutr., Suppl S95-99.

[9]. Vogt, H., Matthes, S. and Harnisch, S. 1981. Der einfluss organischer sauren auf die leistungen von broiler undlegehennen. Archiv fur geflugelkunde 45: 221-232

[10]. Vogt, H., Matthes, S. and Harnisch, S.1982. Der einfluss organischer sauren auf die leistugen von broiler. 2. Mitteilung. Archiv fur geflugelkunde 46: 223-227

[11]. Wolfenden, A.D., Vicente, J.L., Higgins, J.P., Andreatti Filho, R.L., Higgins, S.E., Hargis, B.M. andTellez, G. 2007. Effect of Organic Acids and Probiotics on Salmonella enteritidis Infection in Broiler Chickens. International journal of poultry science 6: 403-405.

[12]. Syed, M., Mashook, A. and Rehman, S. 1994. The effect of dietary vinegar on the performance of broiler chicks in hot weather. Sarhad J. Agric 10: 31-34

[13]. bdel-azeem, F., El-hommosany, Y.M. and Nematallah, G.M. ALI. 2000. Effect of citric acid in diet with different starch and fiber levels on productive performance and some physiological traits of growing rabbits. Egypt. journal. Rabbit Science 10: 121-145.

[14]. Gornowicz, E. and Dziadek, K. 2002. The effects of acidifying preparation added to compound feeds on management condition of broiler chickens. Ann. Anim. Sci., Suppl 1: 93-96.

[15]. FushimI, T., TAYAMA, K., FukaYA, M. , Kitakoshi, K., Nakai, N. Tsukamoto, Y. and Sato, Y., 2001. Acetic acid feeding enhances glycogen repletion in liver and skeletal muscle of rats. Journal. Nutr 131: 1973-1977.

[16]. Gornowicz, E. and Dziadek, K. 2002. The effects of acidifying preparation added to compound feeds on managementcondition of broiler chickens. Ann. Anim. Sci., Suppl 1: 93-96.

[17]. Abdo, M.A. Zeinb. 2004 .Efficacy of acetic acid in improving the utilization of low protein-low energy broiler diets.Egypt. Poultry Science 24: 123-141.

[18]. Gauthier, R. 2002. Intestinal health, the key to productivity (The case of organic acids) xxvll Convencion ANECAWPDSA Puerto Vallarta, Jal. Mexico. 30 April 2002.
[19]. Afsharmanesh, M. and Porreza, J. 2005. Effects of calcium, citric acid, ascorbic acid, vitamin D on the efficacyof microbial phytase in broiler startrs fed wheat-based diets: perfprmance, bon mineralization and ileal digestibility.International journal of poultry science 4: 418-424.
[20]. Hersey, S.J. 1987. Pepsin secretion. In Physiology of the Gastrointestinal tract, (L.R. Johnson, Ed.). New York: Raven Press 2: 947-957.
[21]. Kishi, M., Fukaya, M., Tsukamoto, Y., Nagasawa, T., Kakehana, K. andMohan, B., Kadirvel, R., Natarajan, A. and Bbhaskaran, M. 1996. Effect of probiotic supplementation ongrowth, nitrogen utilization and serum cholesterol in broilers. British. Poultry. Science 37: 395-401.
[22]. Brady W (1968). Measurements of some poultry performance parameters. Vet. Rec. 88: 245-260.
[23]. Samanta, S., S. Haldar and T. K. Ghosh. 2010. Comparative efficacy of an organic acid blend and Bacitracin Methylene Disalicylate as growth promoters in broiler chickens : Small intestinal milieu . SAGE - Hindawi Access to Research Veterinary Medicine International . 1-8.
[24]. N . R . C . , National Research Council . 1994. Nutrient Requirements of Poultry , 9th ed . , National Acad . Press , Washington , D . C NAS , Pp . 155. ‘
[25]. Baurhoo B, Phillip L & Ruiz-Feria CA. 2007. Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. Poultry Science 86: 1070–1078 Al-Mayah,.A. A. S. 2009. Effect of fich oil immune response in broiler chicks vaccinated against IBD. Internation al gournal of poultry scince. 8:1156-1161.
[26]. SPSS Inc. 2011. Statistical Package for Social Science version 20 forwindowLEAD Technologies. Inc. USA.DUNCAN, D. B. 1955. Multiple rang and multiple F tests. Biometrics 11:1.
[27]. Mahdavi,A.H., H.R. Rahmani, and J. Pourreza . 2005.Effect of Probiotic Supplements on Egg Quality and Laying Hen's Performance.International Journal of Poultry Science, 4: 488-492
[28]. Beski, S.S.M. and S.Y.T. Al-Sardary. 2015. Effects of Dietary Supplementation of Probiotic and Synbiotic on Broiler Chickens Hematology and Intestinal IntegrityInternational Journal of Poultry Science. 14: 31-36.
[29]. Haddadin, M . S . Y . , S . M . Abdulrahim , E . A . R. Hashlamoun , and R . K . Robinson. 1997. A proposed protocol for checking the suitability of Lactobacillus acidophilus for use during feeding trials with chickens . Trop . Sci . 37 : 16 - 20 .
[30]. Gondwe , T. N. and C. B. A. Wollny. 2005. Evaluation of the growth potential of local chickens in Malawi .Int. J. of Poult. Sci. 4:64-70.
[31]. Scheppch, W. 1998. Butyrate and the epithelium of the large intestine. Proc. Of the proyibre Cons-functional properties of Non – diges tible Carbohydrates, Gullon et all teds, Lisbon, Portugal.
[32]. Cavazzoni, V . and A . Adami . 1998. Ceacal microflora in chickens fed with Bacillus coagulans as probiotic . Ann. Di. Microbiologia-de- Enzimologia . 48:13-18 .
[33]. Mack, D.R., S. Ahrne, L. Hyde, S. Wei and M.A. Holling_Swarth. 2003. Exteacellular MVC3 nucin secretion follows adherence of lactobacillus strain to intestinal epithelial cell in vitro.Gut. 52 :827-833.
[34]. Lee, Y.K., K.Y. Puong, K.K. Ouweh and S. Salminin. 2003. Displacement of bacterial pathogens from mucus and caco cell surface by lactobacilli. J.Med.Microbiol. 52(10):925-930.
[35]. Cherrington, C. A., M. Hinton, G. C. Mead and I. Chopra .1991. Organicacids: Chemistry Antibacterial activity and practical applications. Advances in Microbial Physiology. 32:87-107.
[36]. Roe, A.J., D.Mclaggan, I.Davidson, C.O.B yre, I.R. Both. 1998.perturbation of anion balance during in hibition of growth of Escherichia coli by weak acids. J.of Bacteriology.180:767-772.
[37]. Abdul Jalil, raghad Ali. 2006. impact plus organic acid Galic acid and Probiotic Iraq in productive traits and some health indicators in broiler. master – College of veterinary medicine-University of Baghdad
[38]. Russel, J. B. and F.Dies-Gonzales. 1998.The effect of fermentation acids on bacterial growth . Advances in Microbial physiology. 39:205-234.
[39]. Epstein,W. 2003. The roles and regulation of potassium in bacteria. Progress in Nucleic Acid Research and Molecular Biology .75:293-320.(Abst).
[40]. Gunal,M.,G.Y,O.Kaya,N.Karahan and O.Sulak.2006. The effect of anti- biotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers.International Journal of Poultry Sci. 5(2):149-155.
[41]. Cho, S. S. and E. T. Finocchiaro. 2010. Handbook of prebiotics and probiotics ingredients. CRC Press Taylor X Francis Group. Printed in U. S. A.
[42]. Smirnov, A., E. Tako, P.R. Ferket and Z. Uni. 2005. Mucin gene expression and mucin content in the chicken inestental goblet cells are affected by in ovo feeding of carbohydrates. Poultry Sci., 84: 669-673.
[43]. Naji, Saad Abdul Hussein, Bushra Saadi Rasool, Mohammed Farouk Abdul Hamid, Hammoud Khalaf Al Janabi and Ghaleb Alwan Al-Qaisi. 2011. Iraqi Bioenergy. First Edition. Ababeel Printing Office. BaghdadLohakare, JD., Ryu MH, Hahn T-W, Lee JK, BJ. Chae. 2005. Effects of supplemental ascorbic acid on the performance andimmunity of commercial broilers. J Appl Poultry Res. 14:10-19.

Mahdi S. Jasim . “Application of Probiotic with Different levels of Citric Acid Supplementation in the Diet for Promotes the Production Efficiency of Broiler Chickens.” IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS), vol. 10, no. 9, 2017, pp. 50–57.