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Effect of Dietary Additives of OTC, Probiotic and Citric Acid on Growth Rate, Blood Parameters and Intestinal Morphology of Broiler Chickens

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Abstract: This study was amid to evaluate the effect of supplementation of oxytetracylin (OTC), probiotic (Pro.), and citric acid (CA) separately on growth rate hematological, intestinal microflora and morphology of broiler chickens. A total of 270 one- day old male broiler chickens (Ross 308), were randomly kept into six groups floor pens consisting 3 replicates of 15 birds each. Treatment were as following: T_1 (control diet without supplement), T2 supplementing with OTC (0.5 g/ kg); T_3 and T_4 supplementing with CA (1.0 or 1.5 g/ kg diet respectively), T_5 and T_6 supplementing with CA (1.0 or 1.5 g/ kg diet respectively). The results showed, there were significant increase in weight gain in broilers fed T_3 and T_4 dietary treatments, and no significant difference between the other treatments. Significant decrease in RBC, PCV and Hb, while increased in WBC, Lymphocytes, ALT and AST in birds supplemented with T_2 dietary treatment. The diet containing OTC T_2 and CA T_6 caused reduced in micoflora count in jejunum area. There was significant increase in villi height and crypt depth for T_6 while there was significant decrease in T_2. In conclusion, probiotic and citric acid are good alternative to antibiotics in promoting growth, and beneficial modulation of intestinal micoflora and improve intestinal morphology.

Keyword: Broiler, Oxytetracylin, Probiotic and citric acid, Hematology, Morphology

Introduction

There is an increasing demand for animal protein in many developing country. Although broiler production cannot be over emphasize in face of rising demand for animal protein. Nowadays livestock producers raise genetically animal yields and faster growing and maximized production, animals must be free from disease and fed appropriate diets. Antibiotics are used in animal feed for about fifty years as feed additive in poultry industry to promote animal growth and protect animals against infection of pathogenic microorganisms (Ferket *et al*., 2002). Tetracyclines oxytetracycline (OTC) are the most commonly used therapeutic antibiotics in food animal production (Alam, 2000; Zulkifli *et al*., 2000; Fairchild *et al*., 2005). Talabi *et al*., (2013) also documented the use of OTC powder in feed of broiler chicks at 0.05 g/ kg as a growth promoter. However, with increasing problems brought by abuse of antibiotics, such as antibiotic resistance in human pathogenic bacteria which could result in bans on subtherapeutic antibiotic usage in the poultry industry in European Union (Castanon, 2007).

Probiotics serve as alternatives to antibiotics are growth promoter in animal production and the use of alternatives additives non-antibiotic have been increased (Higgins *et al*., 2008; Markovic *et al*., 2009; Huyghebart *et al*., 2011; Zhang and Kim, 2013; Mohammadi *et al*., 2016). Furthermore may reports have indicated that addition probiotics to the diets have improved performance, intestinal microflora, development of intestinal mucosa morphology, modulated immunity, and are able to benefit the host animals basing on different mechanism (Mountzouris *et al*., 2010; Keyi *et al*., 2011; Sen *et al*., 2012; Liu *et al*., 2012; Fallah *et al*., 2013; Lee *et al*., 2014; Zhang and Kim, 2014; Patel *et al*., 2015). Whereas no significant effect were reported of dietary supplemented with probiotic on growth performance (Rhimi *et al*., 2011; Wolfenden *et al*., 2011; de Souza *et al*., 2018).

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In addition probiotics could inhibit excess of oxidative free radicals that can cause cell damage and finally effects on performance (Li *et al*., 2012). Although probiotics can be composed of one or many strains of microbial species with the more common one belonging to the genera lactobacillus, Bifidobacterium, Enterococcus, Bacillus and Pediococcus (Gaggia *et al*., 2010). Kotowska *et al*., (2005) found that saccharomyces, boilardii is considered as useful probiotics and its oral administration could improve enzymatic activity and antioxidation functions and protect intestinal mocosa during transitional time in the intestine. Rajput *et al*., (2013) also found that supplementation of saccharomyces boulardii and bacillus subtitles could be applied to enhance digestive enzyme activities, antioxidation and blood profile of broilers. Where's lactobacillus ssp. showed no favorable effects on broiler performance (Olnood *et al*., 2015). Yeast probiotics have been found more effective than other probiotics to enhanced performance of birds this kind of effect is due the bacteriocin producing effects of yeast probiotics (Reisinger *et al*., 2012; Yasar and Akincl, 2014; Yasar *et al*., 2016; Chen *et al*., 2016).

The efficacy of organic acid in improving growth performance, enhancing mineral absorption and enhancing protein digestion (Syed *et al*., 1994; Abdo, 2004; Afsharmanesh and Porreza, 2005; Wolfenden *et al*., 2011). Citric acid has been reported to improves the retention of phosphrous, calcium and protein (Vargs-Rodring *et al*., 2002; Rafacz-Living-Ston *et al*., 2005). Citric acid reduces the excretion levels and increases the digestibility of protein, nitrogen and calcium and has effect on the response of the phytase affect the excretions of protein and nitrogen (Vargas-Rodrigues *et al*., 2015). Therefor the present study to evaluate growth rate, blood traits, and intestinal micoflora and morphology of broiler chickens fed supplemented diet with OTC probiotic and citric acid.

Material and Methods

All experimental work with animal was done at the Animal Production Department/ College of Agriculture/ University of Anbar in the alternative site (Abu- Ghraib- Baghdad).

Birds and Diet

In this experiment 270 broiler male chicks one-day old, of the commercial Ross 308 strain were randomly assigned to six treatments with 3 replicate pen of 15 chicks per pen, and reared in pens (1.5 × 1.2 m) on wood shavings with gas heating and ventilation with a temperature- controlled fan. The initial house temperature was set at 32C° and thereafter, was gradually reduced based on normal management practice to 21 C° by 35 days of age. A lightening schedule of 22 h of light followed by 2 h darkness was maintained during the experiment. Chicks were vaccinated at hatch for Marek's Newcastle and Infectious Bronchitis Disease. Birds were fed one of the six dietary treatment as follows: 1) Control, 2) Control + 5 mg OTC/ kg, 3) control + 1.0 g probiotics/ kg, 4) Control + 1.5 g probiotics/ kg, 5) Control + 1.0 g citric acid, 6) Control+ 1.5 g citric acid/ kg. The birds were fed a mash, starter diet until 11 days of age, a mash grower diet from 12 to 21 day of age and finisher diet from 22 to 35 day of age (Table 1). Feed and water were provided *ad libitum*.

The prebiotic were obtained commercially (Biosb-Gold), each kg contains live Saccharomyces cerevisiae more than 3.0×10^{11} CFU, and Bacillus subtilis, more than 4.0×10^{9} CFU. The chicks were subjected to standard brooding in deep litter system, and were fed with formulated broiler starter, grower and finisher. The composition of the experimental basal diet is presented in (Table 1). Fresh water and feed were supplied *ad libitum*. Routine vaccination and medication were administered the birds accordingly.

Table 1. Composition of basal diets (%)

Ingredients	Diets treatments		
	Starter	Grower	Finisher
Yellow corn	53	55	57
Wheat grain	10	10	10
Barley	30	27	24
Soybean meal[1]	5	5	5
Protein meal (40%)	1	2	3
Dicalcium phosphate	0.7	0.7	0.7
NaCl	0.3	0.3	0.3
Total	100%	100%	100%
Calculated analyses[2]			
Metabolism Energy (kcal/ kg)	3182	3099	3015
Crude protein (%)	21.8%	20.8%	19.5
Methionine (%)	0.52	0.47	0.50
Methionine + cysteine (%)	0.94	0.90	0.86
Lysine (%)	1.27	1.06	1.08
Arginine (%)	1.21	1	1
Calcium (%)	0.75	0.80	0.78
Available phosphorus (%)	0.55	0.45	0.57

[1] Supplied per kg of diet: 40% crud protein, 5% crud fat, 2% crud fiber, 6.5% calcium, 4% available p, 3.85% lysine, 3.7% methionine, 4% methionine + cystine, 2.3% Na, 2100 ME.
[2] Based on National Research Council (NRC, 1994).

Measurements

Body weight gain (BWG) were recorded during the starter 0-21, 22- 35 and 0- 35 days of days of age. Mortality was also recorded. At end of the experiment 3 birds per pen (9 birds per treatment) were randomly chosen from each treatment for, blood collecting, and were slaughter, for and the small intestine were removed for determined intestinal microflora and histological traits.

Intestinal Microflora

At 35 days of age, one bird were randomly chosen from each replicate (3 birds/ treatment). The small intestine were removed, one gram of the pooled digest was taken from duodenum and jejunum, in sterile rubes and podded by replicate, and diluted to 10 ml with sterilized deionized water. The sample were then serially diluted to final of 10^{-5}. Total bacteria population was enumerated on Nutrient agar after incubation at 37 C° in an aerobic chamber for 24 h. according to (Samanta *et al*., 2010).

The villi high and crypt depth were measured at 35 days old, one birds from each pen (3 birds/ treatment) were slaughter, the small intestine was removed. The contents of the ceca and small intestine. A 2 cm segment of jejunum (2 cm from the end of duodenum) was excised, washed in physiological saline solution and fixed in 10% buffered formalin, embedded in paraffin, and 2 mm section placed on glass slide and stained with hematoxylin and eosin. Ten measurement were taken per bird for each variable were examined as described by Baurhoo *et al*., (2007).

Blood Characteristic

At 35 days of age 54 broiler chickens, (3 from each replicate pen) were randomly chosen from each treatment, for blood collecting whereas amount of 5 ml of blood sample were pulled of the brachial vein (Campbell, 1995). The collected blood sample 1 ml were transferred to Ethylen Diamin Tetra Acid (EDTA) tube in order to determine White and Red blood cells. Lymphocytes, hemoglobin and cell volume (Gross and Siegel, 1983). The rest amount 4 ml of blood samples were centrifuged at 3000 ×g for 15 min to isolate the blood serum to determine the blood biochemical traits involving glucose, total protein, albumin, cholesterol, triglyceride, High Density Lipoprotein (HDL), Low density Lipoprotein (LDL) were spectrophotometrically determined by using commercial kit (Stanbio Laboratory Boerne TX). The difference between total protein and albumen represents the calculated globulin. Liver enzymatic activities of Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP) were analyzed by microplate, using commercial kit (BioMerieux-France) according to the instructions of the manufacturer.

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) in a completely Randomized Design using (SAS, 2012). Significant means among variable were separated using Duncan Multiple Range Test (Duncan, 1955).

Results and Dissection

Body weight gain (BWG)

The effect of dietary addition of OTC, probiotic, and citric acid on broiler BWG are shown in Table 2. No mortality occurred during the entire feeding period in all the groups. There were no significant ($p>0.05$) differences between BWG of treatments from day to 21. A significant effect of treatment was observed from day 22 to 35, with the T_4 (probiotic 1.5 g/ kg) group showing a greater BWG compared with all other treatments ($p<0.05$). No significant was observed with dietary supplementation of OTC on BWG compared with control and those supplemented with citric acid. From day 22 to 35 a significant effect of treatment was found ($p<0.05$), where the T_4 and T_3 (probiotic) groups treatment had greater BWG than all other groups. When comparing for over period (1 to 35 day), the BWG of broilers in probiotics groups was the highest compared with other groups ($p<0.05$).

This finding is in agreement with several reports demonstrating that probiotic (Bacillus subtilis 4.0×10^9 and Saccharomycem cervisae 3.0×10^{11}) were found to be enhanced BWG of the experimental birds, probably by their beneficial effect on gut microflora in testinal of broiler chickens (Apata, 2008; Zhou *et al*., 2010). Several researchers have reported that supplementing diets with probiotic may improve growth performance of broiler chickens (Falaki *et al*., 2011; Li *et al*., 2014; Murshed and Abudabos, 2015; Yang *et al*., 2016). Also according to Sen *et al*., (2012) reported that probiotic based of Bacillus subtilis on different levels of inclusion in diet increase BWG, On other hand other researchers have not verified the effect of probiotic supplementation on performance (Shams Shargh *et al*., 2012; Nosrati *et al*., 2017). Our results are in agreement with these previous studies, and indicate that supplementing broiler diet with 1.5 g/ kg probiotic improve BWG. However these results contradict those reported by Pelicano *et al*., (2005) and Bitterncourt *et al*., (2011) who observed that BWG was not affected by probiotic supplemented. No significant effect was observed with dietary supplementation of probiotic on BWG (Rahimi *et al*., 2011; Wolfenden *et al*., 2011; de Souza *et al*., 2018). This inconsistency might be attributable to the strain of probiotics, administration dosage or to the form of probiotics (Zhang *et al*., 2012). Many mechanisms have been proposed to explain the positive effect of probiotic on growth performance. Probiotic have been reported to prevent gut colonization by pathogic bacteria through the mechanism of competitive exclusion (Abudabos *et al*., 2013). Wilson *et al*., (2005) proposed that growth suppressing effect on pathogenic bacteria was due to the production of toxic metabolites that irate the gut mucosa, thereby inhibiting nutrient absorption. The probiotic used in this study also improved BWG to similar level as that obtained as the antibiotic group suggesting that probiotic may replace in feed antibiotics without any negative effect on broiler performance from 1 to 21 days of age.

Table 2. The effects of dietary feed additive on live body weight gain (g) of chickens

Treatments	Body Weight Gain (BWG) g		
	0-21 days	22-35 days	0- 35 days
T_1 control (basal diet)	904 ± 17.5	1130 ± 27.3 b	2034 ± 34.1 b
T_2 OTC (5 mg/ kg)	867 ± 13.7	1142± 20.6 b	2010 ± 34.1 b
T_3 probiotic (1.0 g/ kg)	890 ± 14.6	1183 ± 29.8 ab	2073 ± 58.6 ab
T_4 probiotic (1.5 g/ kg)	886 ± 15.8	1207 ± 22.3 a	2093 ± 37.9 a
T_5 citric acid (1.0 g/ kg)	878 ± 15.0	1134 ± 20.5 b	2012 ± 37.0 b
T_6 citric acid (1.5 g/ kg)	878 ± 15.6	1161 ± 24.5 ab	2047 ± 40.2 ab

Value are means n=45
a, b= Means within column having different superscripts are significantly different (p<0.05)

Effect of OTC, Probiotic and Citric Acid on İntestinal Micoflora

The ceca microflora of broilers at 35 days of age is shown in Table 3. Total number of bacteria in duodenum of birds supplemented with OTC, and probiotic (1.0 g/ kg) groups had lowest in the count of bacteria, while there was significant (p<0.05) increase in other treatment groups. Table 5 also show the total number of bacteria in jejunum of birds supplemented with citric acid (1.5 g/ kg) diet had lowest bacteria count while the birds received probiotic (1.5 g/ kg) diet had the highest bacteria count (p<0.05). However, no significant were observed in other treatment groups.

Also our results show that probiotics supplemented diets displayed a greater increase in micoflora count in intestinal of T_3 and T_4 broiler groups. Similar results were also reported by other Chen *et al*., (2013) found that feed supplemented with Bacillus subitilis and Saccharomyces cerevisiae increase bacteria population in small and large intestine corroborating our finding. Seifi *et al*., (2015) found that broiler provided organic acid to the diet cause a significantly decrease micoflora count in small intestine. However, Mountzouris *et al*., (2007) and Yakhkeshi *et al*., (2011) found, no significant different in bacteria count and *E. coli* in birds supplemented with antibiotic or probiotic feed additive.

Other studies found that probiotic can moderate the gut environment by increasing the number of benifical microbes and hindering rapid multiplication of intestinal pathogens (Patterson and Burkholder, 2003; Higgins *et al*., 2008).

The diets with organic acid had a positive effect by reducing the intracellular pH of gut microflora which in turn may improved broiler performance (Kopecky *et al*., 2012). Although the addition of citric acid increases the digestibility and reduced the excretion of calcium and nitrogen and has effect on the response of the phytase to affect the excretions of P and N (Vargas-Rodriguez *et al*., 2015). On the other hand supplementation of probiotic augmented growth of specific bacteria which produce organic acids and this, in turn might increase the villi height (Pelicano *et al*., 2005).

Table 3. Effect of dietary feed additives of OTC, probiotic and citric acid on viable count of bacteria in ceca digest of broilers

Treatments	Microbial count in different segment of small intestine ($\times 10^5$)	
	Duodenum	Jejunum
T_1 Control	6.49 ± 0.018 a	7.47 ± 0.035 ab
T_2 OTC (5 mg/ kg)	5.90 ± 0.247 b	7.43 ± 0.013 ab
T_3 Probiotic (1.0 g/ kg)	6.53 ± 0.029 a	7.44 ± 0.041 ab
T_4 Probiotic (1.5 g/ kg)	6.11 ± 0.386 ab	8.05 ± 0.512 a
T_5 Citric acid (1.0 g/ kg)	6.43 ± 0.015 a	7.52 ± 0.488 ab
T_6 Citric acid (1.5 g/ kg)	6.49 ± 0.006 a	6.71 ± 0.012 b

Means with different superscripts a, b, c within the raw differ significantly (p<0.05)

Effect of OTC, Probiotic and Citric Acid on İntestinal Morphological

Supplementation of dietary additive of OTC, probiotic and citric acid had variable effect on the villus height and crypt depth in jejunum with no relation villous: crypt of the small intestine (Table 4). Villus height in jejunum were significantly ($p<0.05$) improved on birds supplemented with citric acid 1.5 g/ kg diet. However, there were no differences in villus height among birds in OTC, probiotic and citric acid (1.0 g/ kg) diet. Birds fed control diet exhibited the smallest villus high compared with others treatments. Results also indicated that crypt depth in the birds fed citric acid (1.5 g/ kg) and probiotic (1.0 g/ kg) diets was higher ($p<0.05$) than those fed other treatments diets. The birds fed control diet exhibited the lower ($p<0.05$) crypt depth among all treatments. However, estimates of villi height: crypt depth were not affected by dietary treatments (Table 4).

The addition of citric acid increased the villi height and crypt depth were found to be superior in the birds fed (citric acid 1.5 g/kg) diet. These finding are similar to those reported by other researchers (Adil *et al*., 2010; Ghazalah *et al*., 2011) they found that a significant villi height in the bird supplemented with organic acid in broiler diets. Other authors, however reported that villi height and crypt depth did not infected by addion citric acid to broilers diets (Levy *et al*., 2015; Tossenberger *et al*., 2016). Birds fed diets containing yeast probiotics had an increase in the villi height in the duodenum compared with antibiotic free diet (Solis de los Santos *et al*., 2007). To us our finding suggest that probiotic and citric acid have benefical effect to villi height and crypt depth, because long villi are correlated with improved gut health. Similar results were also reported by (Gunal *et al*., 2006; Sen *et al*., 2012) show that the use of the Bacillus subtilis caused increased in height of intestinal villi. However, Mountzouris *et al*., (2012) did not found any changes in villi height and crypt depth in small intestine when using combined of many bacteria.

Table 4. Effect of dietary feed additive of OTC, probiotic and citric acid on villi height and crypt depth of broilers chickens

Treatments	Item		
	Villi height (mm)	Crypt depth (mm)	Villi height/ crypt depth ratio
T_1 control (basal diet)	293 ± 20 b	185 ± 28 c	2.21 ± 0.28
T_2 OTC (5 mg/ kg)	453 ± 9 b	275 ± 66 b	1.82 ± 0.39
T_3 probiotic (1.0 g/ kg)	486 ± 33 b	308 ± 22 a	1.59 ± 0.10
T_4 probiotic (1.5 g/ kg)	450 ± 32 b	241 ± 8 b	1.87 ± 0.19
T_5 citric acid (1.0 g/ kg)	460 ± 40 b	266 ± 16 b	1.74 ± 0.21
T_6 citric acid (1.5 g/ kg)	536 ± 9 a	316 ± 8 a	1.69 ± 0.20

Value are means n=3

Means with different superscripts a, b, c within the raw differ significantly ($p<0.05$)

Liver Enzymatic Activities

Dietary additive, OTC, probiotic and citric acid had significantly affect liver enzymatic activities in broiler chickens. Birds in T_2 had the highest ALT and AST concentration compared with other treatment ($p<0.05$). However, birds fed with T_4 have higher ALP concentration while T_1 and T_2 had the lowest concentration ($p<0.05$) Table 5.

Supplemented birds diet with antibiotic led to increased ALT and AST. These results showed that adding antibiotic to broiler diets did not had benefical effect on liver enzyme activities in this study. In agreement with our results (Miles *et al*., 2006). However, ALP concentration found to be higher in T3 and T4 probiotic groups which had greater BWG. Moreover, the birds supplemented with antibiotic had least BWG compared with other groups.

On the other hand, several studies haves reported a positive effect of probiotic on growth performance of broilers (Zhou *et al*., 2010; Zhang and Kim, 2014). In this study there was a positive correlation between ALP concentration and BWG (Table 5).

Table 5. Effect of dietary feed additive of OTC, Probiotic and Citric acid on liver enzymatic activities of broilers chickens

Treatments	Liver enzymatic		
	ALT U/ L	ALS U/ L	ALP U/ L
T_1 control (basal diet)	8 ± 0.57 b	7.66 ± 0.88 b	41.76 ± 1.56 c
T_2 OTC (5 mg/ kg)	14.33 ± 1.45 a	13.33 ± 1.45 a	41.66 ± 2.68 c
T_3 probiotic (1.0 g/ kg)	8.66 ± 0.88 b	7.66 ± 0.88 b	51.83 ± 1.84 b
T_4 probiotic (1.5 g/ kg)	9.33 ± 0.88 b	7.66 ± 1.20 b	80.20 ± 2.57 a
T_5 citric acid (1.0 g/ kg)	9 ± 1.73 b	7.66 ± 2.02 b	39.40 ± 2.49 a
T_6 citric acid (1.5 g/ kg)	9.23 ± 0.88 b	8.66 ± 0.88 b	37.60 ± 4.26 c

Value are means n=3
Means with different superscripts a, b, c within the raw differ significantly ($p<0.05$)

Haematological Parameters

Analysis of haematological parameters showed that the effects of dietary feed additives in some blood indices of broiler were observed (Table 6 and 7). Table 6 counts of blood cells were affected by dietary treatments, PCV%, Hb mg/ dL and RBC count were decreased ($P<0.05$), while WBC count and H/L% was increased ($P<0.05$) in OTC group compared with control and other treated groups. Perhaps it was caused by the major toxic effects mechanism of antibiotics which included their potential to cause lipid peroxidation, which is primarily responsible for toxication. The same results were described when broilers treated with OTC (Farombi, 2000; Donkova, 2004; Ridell, 2011; Britannica, 2013).

Data presented in Table 7, indicate there was significant ($p<0.05$) differences in the blood biochemical parameters value in OTC dietary group, as compared with other treatment groups, with lowest in OTC groups. However, there was an in significant ($p>0.05$) differences between treatments in glucose concentration. Birds fed citric acid (1.0 g/ kg) group had highest, total protein and globulin concentration ($p<0.05$). Also birds fed citric acid (1.0 g/ kg) groups had highest LDL and lowest HDL concentrations than other treatments groups, while the lowest concentration of LDL was found in the birds fed OTC (300 mg/ kg) diet groups. This may suggest poor immune response and insufficient antibody production in the birds fed OTC diet, which is in line with the finding of (Rosa *et al*., 2001; Slyamova *et al*., 2016), who found that antibiotics influence the dynamic's of haematopoiesis and biochemical indices of broilers. In addition, Alonge *et al*., (2017) observed that inclusion of probiotics and prebiotics in the diets of broilers chickens elicited no adverse effect on haematological and serum biochemical parameters.

Conclusion

We have shown that dietary supplementation with antibiotic reduced the microbial count, villi high and crypt depth, and did not effect BWG compared with control groups. The addition of probiotic and citric acid increased microbial count, villi height, crypt depth and BWG. Addition probiotic and citric acid may assist to alternative to antibiotic use in broilers diet.

Recommendation

The use of growth stimulating OTC (0.5 g/ kg diet) in feeding of broiler chickens promoters change in BWG, microbe, villi height, crypt depth of small intestion. The addition of probiotic o citric acid increased BWG, microbial count, villi height, crypt depth, and improve blood parameters, thus, they can be used as alternative for antibiotic.

Table 6. Effects of dietary feed additives of OTC blood constituents in broilers

Item	Treatments					
	T_1 Control	T_2 OTC	Probiotic/ kg		Citric acid/ kg	
			T_3 1.0 g	T_4 1.5 g	T_5 1.0 g	T_6 1.5 g
PCV (%)	32.00 ± 0.57 a	24.33 ± 1.76 b	31.66 ± 1.45 a	32.66 ± 1.45 a	31.66 ± 3.28 a	29.33 ± 0.88 ab
Hb (mg/ dL)	9.63 ± 0.14 a	6.93 ± 0.27 b	9.56 ± 0.35 a	9.80 ± 0.30 a	9.63 ± 0.82 a	9.13 ± 0.20 a
RBC (cell/ mm^6)	2.96 ± 0.03 a	2.33 ± 0.08 b	3.02 ± 0.13 a	3.12 ± 0.15 a	3.09 ± 0.38 a	2.77 ± 0.07 ab
WBC (cell/ mm^3)	18.13 ± 0.66 c	28.30 ± 0.77 a	19.73 ± 1.01 bc	21.80 ± 0.80 b	17.10 ± 1.37 c	22.16 ± 1.28 b
H/L (%)	0.51 ± 0.12 b	0.88 ± 0.9 a	0.45 ± 0.7 b	0.52 ± 0.5 b	0.52 ± 0.5 b	0.57 ± 0.4 b

Means with different superscripts a, b, c within the raw differ significantly ($p<0.05$)
PCV%= Paced cells volume, Hb (mgl dL)= Haptoglobin, RBC= Red blood cells, WBC= White blood cell, H/L(%)= Lymphocyte.

Table 7. Effects of dietary feed additives of OTC, probiotic and citric acid on blood biochemical analysis of broilers

Item	Treatments					
	T_1 Control	T_2 OTC	Probiotic/ kg		Citric acid/ kg	
			T_3 1.0 g	T_4 1.5 g	T_5 1.0 g	T_6 1.5 g
Glucose (mg/ 100 ml)	187 ± 2.64	164 ± 15.5	190 ± 14.1	198 ± 22.5	164 ± 9.9	151 ± 0.2
Total protein (g/ 100 ml)	2.65 ± 0.26 b	3.49 ± 0.40 b	3.63 ± 0.40 b	3.69 ± 0.20 b	4.39 ± 0.14 a	3.59 ± 0.58 b
Albumin (g/ 100 ml)	2.33 ± 0.29 a	1.96 ± 0.49 b	2.23 ± 0.14 a	2.33 ± 0.27 a	2.53 ± 0.23 a	2.33 ± 0.31 a
Globulin (g/ 100 ml)	1.33 ± 0.57 b	1.53 ± 0.24 b	1.40 ± 0.35 b	1.36 ± 0.26 b	1.86 ± 0.16 a	1.26 ± 0.28 b
Cholesterol (mg/ 100 ml)	139 ± 3.48 a	109 ± 4.48 b	142 ± 2.30 a	159 ± 11.5 a	155 ± 22.3 a	149 ± 7.50 a
Triglycerides (mg/ 100 ml)	79.67 ± 4.48 ab	62.33 ± 7.85 b	83.67 ± 4.25 ab	92 ± 14.50 a	76.67 ± 8.66 ab	76 ± 4.35 ab
HDL (mg/ 100 ml)	61.33 ± 2.60 ab	45.66 ± 4.91 c	66.33 ± 2.18 a	70.33 ± 4.40 a	43.66 ± 1.76 ac	61 ± 4.58 ab
LDL (mg/ 100 ml)	62.76 ± 6.38 ab	44 ± 4.35 b	60 ± 4.58 ab	70.33 ± 12.9 ab	76.66 ± 1.76 a	61 ± 4.58 ab

Means with different superscripts a, b, c within the raw differ significantly ($p<0.05$)

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