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Effect of Two Probiotics and Bioflavonoids Supplementation to the Broilers Diet and Drinking Water on the Growth Performance and Hepatic Antioxidant Parameters

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Abstract: The objectives of the current study were to investigate the effects of two probiotics (Super-CyC and Antapro-EF) and/or *bioflavonoids* mix (Bio-Guard) to the diet and water of broiler chickens on the growth performance and hepatic anti-oxidants parameters under practical conditions. Three hundreds, one–day–old, broiler chicks (Cobb) were assigned into 6 groups. On days 21 and 42, blood, liver tissue and carcass ready to cocked weight were obtained from six slaughtered birds of each group. The present results indicated that there were significant differences in live body weights, body weight gain, feed conversion ratio and carcass ready to cocked weight by using probiotics and/or bioflavonoids supplemented groups (G3 : G6). Bio-Guard treated group (G2) were recorded the highest values of the superoxide dismutase (SOD) activity and reduced glutathione levels in relationship to all groups of the current experiment. Concurrently, the level of malondialdehyde (MDA) was significantly decreased in G2 and G4, G6 relative to untreated controls group. The high efficacy of the bioflavonoids allows their use at the recommended doses and making their use in broiler nutrition acceptable, whether alone or in combination with other products as probiotics. Overall, the current study recommends use combination of bioflavonoid (Bio-Guard- in drinking water) and probiotic (Super-CyC mixed in ration) as in group 6. This synergistic effect of Bio-Guard and Super-CyC were reflected on the highest significant data of the live body weight, body weight gain, feed conversion ration and carcass ready to cocked weight and antioxidant status of broiler chicks.

Key words: Probiotic · Bioflavonoids · Broilers · Growth Performance · Hepatic Antioxidants

INTRODUCTION

The presence of undesired antibiotic residues in poultry products and environmental contamination has largely added to the public concern regarding the use of antibiotics in the feed [1]. The current European Commission's ban on the use of subtherapeutic levels of in-feed antibiotics in animal production is likely to affect the trade of meat products between the EU and the countries continuing to use antibiotics. Trade regulations and consumer demand may eventually force the worldwide elimination of prophylactic in-feed antibiotics in livestock production [2]. Poultry meat is the second largest global food commodity [3] and to sustain poultry production to meet global demand, antibiotic replacements are needed [4].

Mannanoligosaccharide (MOS) is a prebiotic, which increased beneficial organisms in gastrointestinal tract [5], improved the immunity of the birds [6] and improved body weight gain and feed conversion [7]. Similarly, probiotics also improved the production performance of poultry [8]. Both can be used as alternative to antibiotic growth promoters.

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Bioflavonoids (bioavailable flavonoids, vitamin P) are a class of water-soluble plant pigments with antioxidant, anti-inflammatory, antiallergenic, antiviral and anti-carcinogenic properties. They are essential for the absorption of vitamin C and the two should be taken together [9, 10]. The term bioflavonoids refers to many different ingredients and include hesperin, hesperidin, eriodictyol, quercetin, quercetin, rutin etc. This nutrient cannot be manufactured by the body and must be supplied in the diet [11, 12]. Antioxidants are the natural defense against free radicals. Antioxidants are enzymes that roam around body's cells looking for free radicals. When they find one, they take hold of it and neutralize it without being damaged themselves. The antioxidant enzymes stop the invasion and remove the free radical from our circulation [13].

Therefore, the objectives of the present study were to investigate the effects of two probiotics (Super-CyC and Antapro-EF) and/or polyherbal *bioflavonoids* mix (Bio-Guard) to the diet and water of broiler chickens on the growth performance and hepatic anti-oxidants parameters under practical conditions.

MATERIALS AND METHODS

The experiment was conducted at the experimental house of the Faculty of Veterinary Medicine, Sadat City University, Sadat City- Egypt.

Birds and Housing: Three hundreds, one–day–old, unsexed commercial broiler chicks (Cobb) were assigned into 6 groups of 50 chicks in a chamber. Each chamber was provided with feeder and drinker. Broiler chicks were kept on a deep litter floor system (with fresh wood shavings from day of hatch). All chamber partitions, drinkers, feeders and heaters were cleaned and disinfected prior to the beginning of the trial. Environmental temperature was set at 33°C for the first week and 30°C for the second week, which was further decreased to 26°C until the end of the experiment. Relative humidity was set at 50-60% throughout the study. During the first week, the light regimen was 22L:2D, which was reduced to 20 h of light afterward. Water and feed were provided *ad–libitum* and diets contained no antimicrobial growth promoters. All experimental procedures used in the present study were approved by the Animal Care and Use Committee at the National Institute of Agricultural Science and Technology and conform to the US National Institutes of Health guidelines for the care and use of laboratory animals. Corn and soybean meal-based ration was formulated according to the requirements suggested by NRC [14]. Diets were formulated as starter and finisher diets (Table 1). Routine and occasional management, vaccination and medication were carried out as and when due. Chicks were vaccinated against Infectious Bronchitis and Newcastle Disease.

Table1: The ingredients and composition of basal diet.

Ingredients and composition (%)	Starter	Finisher
Corn	55.59	61.07
Soy bean meal	37.32	31.83
Soy oil	2.98	3.41
Lime stone	1.21	1.42
Dicalcium Phosphate	1.60	1.16
DL. Methionine	0.20	0.10
*Vitamin and Minerals	0.60	0.60
Sodium chloride	0.23	0.18
Sodium bicarbonate	0.27	0.23
Chemical Analysis (%)		
ME (kcal/kg)	2950	3050
Crude Protein (%)	21.20	19.16
Lysine (%)	1.14	1.01
Methionine (%)	0.50	0.39
Methionine and Cysteine (%)	1.03	0.84
Available Methionine + Cysteine (%)	0.85	0.71
Calcium (%)	0.93	0.90
Available Phosphate (%)	0.44	0.35

*Supplied per kilogram of diet: vitamin A, 1,500 IU; cholecalciferol, 200 IU; vitamin E, 10 IU; riboflavin, 3.5 mg; pantothenic acid, 10 mg; niacin, 30 mg; cobalamin, 10 ìg; choline chloride, 1,000 mg; biotin, 0.15 mg; folic acid, 0.5 mg; thiamine 1.5 mg; pyridoxine 3.0 mg; iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, 0.18 mg; copper, 8 mg; selenium, 0.15 mg.

Table 2: Bioflavonoid and probiotics used in the current study

1-Bio-Guard [Egyptian Veterinary Products Company (EVEPCO)- Egypt]
*Acts as antioxidant
Bio-Guard is a comprehensive well balanced antioxidant formula of 100% plant origin. Each liter contains vitamin C [3.348 g], vitamin A [476.619 IU], zinc sulfate [14.035 g], sodium selenite [3.240 g] and total bioflavonoid content (Vitamin P ~5%) in a soluble active form.
*Dose: 2.5ml/L of drinking water.
2-Anta ® Pro EF [© Dr. Eckel GmbH, Niederzissen, Germany, www.dr-eckel.de]
* Acts as probiotics
* Anta®ProEF contains viable lactic acid producing bacteria of the strain Enterococcus faecium DSM 10663 NCIMB 10415. The strain DSM 10663 NCIMB 10415 has been approved by the European Union as a microbial additive for use in feeds for various animal species.
*Dose: 100g per 5000 birds per day in drinking water.
3-Super-CyC [Choong Ang Biotech Co. Ltd. South Korea "www.choongangbio.com"]
* Acts as probiotics
*Each kilogram contains:
-*Bacillus subtilis* (CA #20, 1.0X10^9 CFU)……. 100.0g
-*DL-Methionine* ……..…….... 8.6g
-CYC-100 (*Saccharomyces cerevisiae*) …….. 891.4g
[CYC-100 Strain : KCTC No.7193 (*Saccharomyces cerevisiae*)]
*Dose: 2.5 Kg/ 1 Tone of ration.

Experimental Design: Chicks were randomly assigned to the following experimental groups:

Group 1 (Control Negative): Chicks fed only basic diet (Table 1) without supplementation of probiotics and/or bioflavonoid (Bio-Guard).

Group 2: Chicks fed basic diet with supplementation of Bio-Guard (as antioxidant in drinking water, Table 2).

Group 3: Chicks fed basic diet with Anta®Pro EF (as probiotics in drinking water, Table 2).

Group 4: Chicks fed basic diet with Anta®Pro EF and Bio-Guard (in drinking water).

Group 5: Chicks fed basic diet mixed with Super-CyC (as probiotic - Table 2).

Group 6: Chicks fed basic diet mixed with Super-CyC and Bio-Guard (in drinking water).

Growth Performance Parameters: Feed intake (FI), body weight (BW), body weight gain (BWG), feed conversion ratio (FCR, g feed/ g gain) were recorded weekly for each group. At the end of the experiment, six birds per group were randomly picked up for data sampling.

On days 21 and 42, the birds were weighed to obtain live body weight, then slaughtered by a sharp knife for complete bleeding (collect the drainage blood on heparinized and non heparinized tubes). The birds were reweight after removal of skin, head and shanks. After that, viscera were removed, then reweight to obtain the all viscera weight and ready to cook (RTC) carcass weight.

Measurement of Hepatic Antioxidative Status: On days 21 and 42, liver tissue were dissected out as soon as possible from the slaughtered birds of each group. Tissue homogenate was prepared from the liver according to Combs *et al*. [15]. Cell debris was removed by centrifugation at 12,000×g (4°C) for 20 min. to collect supernatants for determination of malondialdehyde (MDA- as a marker of radical-induced damage-[16], glutathione (GSH-[17]) concentrations and superoxide dismutase (SOD- [18]) activity. Total protein concentrations were measured in homogenate according to the Gornall *et al*. [19] method to measure antioxidant parameters per tissue protein.

Statistical Analysis: Results are expressed as mean ± standard error (SE). Differences between means in different groups were tested for significance using a one-way analysis of variance (ANOVA) followed by Duncan's test and *P* value of 0.05 or less was considered significant (using the statistical analysis system, SPSS, [20].

RESULTS

Effect of Probiotics and Bioflavonoids on Broiler Growth Performance Parameters and Carcass Quality: Results obtained in Table 3 showed that probiotics and/or bioflavonoids supplemented groups (G3 : G6) improved extensively ($p<0.05$) the live body weights at 42 days of age relative to control groups (G1and G2). The body weight gain were recorded the highest values ($p<0.05$) in G3, G4, G5 and G6 groups at 22-42 days and the overall of the experimental period (0-42days). The feed conversion ratio were declined significantly ($p<0.05$) in probiotics

and/or bioflavonoids supplemented groups (G3 : G6) during the both periods "22-42 and 0-42 days" of age (Table -3) in comparison to control groups (G1& G2).

The carcass ready to cocked weight were recorded the highest values in groups supplemented with probiotics and/or bioflavonoids (G3, G4, G5 and G6) especially birds in group 6 at 42 days of age (Table 4).

Generally, the growth performance and carcass quality parameters of the tested birds were not recorded significant differences during 0-21 days of the experiment period (Tables 3 & 4).

Antioxidant Enzymes and Lipid Peroxidation

Malondialdehyde (MDA) Concentration: The hepatic MDA levels of broiler treated with bioflavonoids and/or probiotics were illustrated in Table 5. The obtained result revealed that the level of MDA was significantly decreased *(P<0.05)* in G2 and G4, G6 (Bio-Guard and/or probiotics combination) in comparison to untreated normal controls after 21 day, while the level of MDA was significantly decreased in all treated group (groups 2, 3, 4, 5& 6) in comparison to normal control after 42 day. The more significantly decreased in the level of MDA was shown in Bio-Guard supplemented group (G2) after 42 days.

Superoxide Dismutase Activity (SOD): The levels of superoxide were significantly increased *(P<0.05)* in all treated group (group 2, 3, 4, 5 and 6) in comparison to normal control at 21st and 42nd day of age. The more prominent significantly increased *(P<0.05)* were shown in G4, G6 (combination of Bio-Guard and probiotics) in Table 5 at 21 and 42 days old.

Table 3: Effect of probiotics and bioflavonoids supplementation on broiler growth performance parameters

	Group					
Times	*G1*	*G2*	*G3*	*G4*	*G5*	*G6*
Live body weight (g)						
0=w_1	44±1.16 ^{a}	45±0.93 ^{a}	46±0.93 ^{a}	46±1.70 ^{a}	44±1.16 ^{a}	46±0.93 ^{a}
21=w_2	738.5±12.75 ^{a}	734.33±6.28 ^{a}	758.33±3.94 ^{a}	696.67±34.38 ^{a}	754.17±30.70 ^{a}	764.33±43.81 ^{a}
42=w_3	2131.70±64.05 ^{bc}	2110.00±40.66 ^{c}	2391.70±78.55 ^{a}	2316.70±49.31 ^{ab}	2240.00±70.90 ^{ac}	2388.30±102.48 ^{a}
Body weight gain (g)						
0-21	694.5±12.32 ^{a}	689.33±6.99 ^{a}	712.33±4.46 ^{a}	650.67±35.43 ^{a}	710.17±31.53 ^{a}	718.33±43.22 ^{a}
22-42	1393.20±57.73 ^{b}	1375.00±41.75 ^{b}	1633.30±80.92 ^{a}	1620.00±53.74 ^{a}	1485.83±73.42 ^{ab}	1624.0±112.57 ^{a}
0-42	2087.00±63.58 ^{cb}	2065.00±40.22 ^{c}	2345.70±77.73 ^{a}	2270.70±49.39 ^{ab}	2196.00±71.51 ^{ac}	2342.30±103.03 ^{a}
Feed conversion ratio						
0-21	1.68±0.03 ^{b}	1.70±0.02 ^{b}	1.69±0.01 ^{b}	1.91±0.10 ^{a}	1.73±0.08 ^{ab}	1.72±0.09 ^{ab}
22-42	2.17±0.09 ^{ab}	2.27±0.06 ^{a}	1.96±0.09 ^{b}	1.90±0.06 ^{b}	2.08±0.10 ^{ab}	1.95±0.15 ^{b}
0-42	2.00±0.06 ^{ab}	2.08±0.04 ^{a}	1.87±0.06 ^{b}	1.90±0.04 ^{b}	1.96±0.07 ^{ab}	1.86±0.08 ^{b}

Mean ± SE (n=6)

$^{a,b,c.}$ Means (in the same raw) having different superscripts are significantly different at $P<0.05$

G1= Control group, G2= Bioflavonoids (Bio-Guard), G3= Probiotic I (Anta ® Pro EF), G4= Anta ® Pro EF + Bio-Guard, G5= Probiotic I I (Super-CyC), G6= Super-CyC + Bio-Guard.

Table 4: Effect of probiotics and bioflavonoids supplementation on broiler carcass quality and mortality rate

	Group					
Times	*G1*	*G2*	*G3*	*G4*	*G5*	*G6*
	Carcass weight without " blood, skin, head and shank" (g)					
0-21	553.00±8.98 ^{ab}	558.00±7.00 ^{ab}	572±4.92 ^{ab}	527.67±24.34 ^{b}	626.5±35.57 ^{a}	586.17±35.50 ^{ab}
22-42	1671.70±56.30 ^{c}	1683.30±44.77 ^{cb}	1888.30±88.75 ^{a}	1791.70±34.87 ^{ac}	1740.00±58.14 ^{ac}	1866.70 ±83.65 ^{ab}
	Carcass ready to cocked weight (g)					
0-21	453.67±4.62 ^{a}	445.17±7.41 ^{a}	458.50±6.31 ^{a}	424.67±21.88 ^{a}	451.00±25.59 ^{a}	465.50±29.22 ^{a}
22-42	1398.30±63.06 ^{b}	1478.30±42.07 ^{ab}	1571.67±34.78 ^{a}	1536.67±24.04 ^{ab}	1540.00±57.45 ^{ab}	1626.70±81.43 ^{a}
	Mortality Numbers					
0-21	1 Ascites	1 Tracheitis& Enteritis	0	0	0	0
22-42	0	0	0	0	0	0

Mean ± SE (n=6)

$^{a,b,c.}$ Means (in the same raw) having different superscripts are significantly different at $P<0.05$

G1= Control group, G2= Bioflavonoids (Bio-Guard), G3= Probiotic I (Anta ® Pro EF), G4= Anta® Pro EF + Bio-Guard, G5= Probiotic I I (Super-CyC), G6= Super-CyC + Bio-Guard.

Table 5: Effect of probiotics and bioflavonoids supplementation on hepatic broiler antioxidant enzymes and lipid peroxidation

	Groups					
Time (days)	G1	G2	G3	G4	G5	G6
	Malondialdehyde (MDA) concentration (nm/mg tissue protein)					
21	0.80 ± 0.09[a]	0.63 ± 0.04[bc]	0.71±0.03 [ab]	0.53± 0.03[c]	0.85± 0.05[a]	0.62± 0.05[bc]
42	0.71±0.04 [a]	0.20±0.04 [c]	0.53± 0.06 [b]	0.25± 0.02[c]	0.52±0.03[b]	0.60±0.04 [b]
	Superoxide dismutase activity (IU/mg tissue protein)					
21	0.099±0.005[c]	0.137±0.010 [ab]	0.128±0.010 [b]	0.153±0.007[ab]	0.144±0.010 [ab]	0.160±0.006[a]
42	0.098±0.006[c]	0.140±0.007[ab]	0.126±0.008 [bc]	0.147±0.006[ab]	0.149±0.010[ab]	0.168±0.020 [a]
	Reduced glutathione concentration (um/mg tissue protein)					
21	8.24±0.32[c]	13.01±0.44 [a]	9.48±0.35 [bc]	10.05±0.40[b]	9.33±0.79 [bc]	10.08±0.43 [b]
42	9.52±0.46[bc]	12.56±0.65[a]	7.93±1.63 [b]	12.24±0.96[a]	10.46±0.72 [ab]	11.12±0.61[ac]

Mean ± SE (n=6)

[a,b,c.] Means (in the same raw) having different superscripts are significantly different at $P<0.05$

G1= Control group, G2= Bioflavonoids (Bio-Guard), G3= Probiotic I (Anta ® Pro EF), G4= Anta® Pro EF + Bio-Guard, G5= Probiotic I I (Super-CyC), G6= Super-CyC + Bio-Guard.

Reduced Glutathione Levels: The effect of Bio-Guard and probiotic on the reduced glutathione levels were illustrated in table 5. The reduced glutathione levels were significantly (P<0.05) increased in G2 (Bio-Guard) and G4, G6 (combination of Bio-Guard and probiotics) in comparison to untreated normal controls (Table 5). Moreover, Bio-Guard treated group (G2) were recorded the highest values (P<0.05) of the reduced glutathione levels in relationship to all groups of the experiment at 21 and 42 days old.

DISCUSSION

Growth Performance Parameters and Carcass Quality: The present results indicated that there were significant differences ($p<0.05$) in live body weights, body weight gain and feed conversion ratio by using probiotics and/or bioflavonoids supplemented groups (G3 : G6). Baidya *et al*. [21], were stated that probiotics were the most effective growth promoter. Probiotics fed chickens had more weight than other groups [22-26]. Recently, it has been reported that poultry growth is promoted with the increasing doses of probiotic (Protexin, Hilton Pharma, Karachi Pakistan) from 0.5 to 1.5 grams per 10 kg feed. Moreover, many studies show that probiotics supplementation in feed of chickens improve the feed conversion ratio [27-30].

The increased body weight gain in chicks fed probiotic may be due to improvement in digestibility and availability of many nutrients such as proteins, fats and carbohydrates, as well as, some mineral elements and vitamins [31, 32]. Probiotic provides nutrients, effectively stimulates the growth of beneficial microflora in the small and large intestines resulting in the better balance of bacterium population [33-36]. Furthermore, the dietary probiotic supplementations resulted in an increase in the villus height and crypt depth of intestinal mucosa of broilers. The increase in the villus height and villus height:crypt depth ratio was associated with improvement of growth performance for probiotic- fed broilers compared with the control group [37, 38].

In contrast, the present results were dissimilar with previous studies in laying hens [39- 41] and broilers [42, 43] that observed feed intake was not affected by yeast inclusion of diet. Yousefi and Karkoodi [41] also reported that feed conversion ratio was not affected by the dietary probiotic and yeast supplementation. In addition, Ahmad [26] and Chumpawadee *et al*. [43] could not detect any difference in the feed conversion ratio of the broilers as compared to the control.

This contradiction, may be due to different probiotics types and doses used by different authors or may be due to absence of the synergistic bioflavonoid effect with probiotic as in current study. Flavonoids are polyphenolic compounds with antioxidant properties, widely distributed in foods of plant origin. In animals, they are thought to be essential for absorption and metabolism of vitamin C and to have a protective function for it. The two compounds in fact exhibit powerful synergy at quite low concentrations [44]. The third suggestion, the bioflavonoid (Bio-Guard) supplementation in the existing study, enhance regulation of the intestinal microflora, by protecting the beneficial microorganisms and inhibiting the pathogenic ones which were reflected on mortality rate percent of the current study [36].

Antioxidant Enzymes and Lipid Peroxidation: Bioflavonoid (Bio-Guard) treated group (G2) were recorded the highest values (P<0.05) of the SOD activity and reduced glutathione levels in relationship to all

groups of the current experiment. Concurrently, the level of MDA was significantly decreased *(P<0.05)* in G2 and G4, G6 (Bio-Guard and/or probiotics combination) relative to untreated controls group. The present data were matching the conclusion of Kamboh and Zhu [45], who stated that using bioflavonoids genistein and hesperidin could positively improve the fatty acid and lipid metabolite profile of broiler breast meat. Additionally, they were recorded a dose-dependent increase in plasma antioxidant parameters (including total antioxidant capacity, malondialdehyde production and total superoxide dismutase activity). Bio-Guard contains various polyphenolic compounds (Vitamin P~5%- according to manufacturing Co.) as antioxidants due to their ability to scavenge free radicals by single-electron transfer [46].

There are important interactions among the activities of several antioxidant enzymes and various reactive oxygen species (ROS) and cellular reactions. Superoxide dismutase catalyzes the conversion of superoxide anions to hydrogen peroxide and is one of the primary enzymatic defenses against ROS [47]. Non-enzymatic decomposition of hydrogen peroxide involving transition metals, such as iron, in a Fenton-type reaction can be more damaging to the cell than the production of the hydroxyl radical species [48]. Furthermore, increased levels of hydrogen peroxide within the cells reduce SOD activity [49], thereby increasing superoxide levels within the cell. Generally, antioxidant status of chickens after consumption of probiotic strains was significantly increased in blood in probiotic treated groups [36].

At the systemic level, the antioxidant effects have different beneficial effects. One of the most important is the protection of the immune system against free radical damage, giving rise to better protection against the damage caused by stresses such as infection, pollution, high temperature and high density. Furthermore, antibody production has been found to improve by addition of citrus extract to the feed [44]. This fact were reflected on the minimal mortality rate (0.67%[2/300]) throughout 42days of the of the present study.

CONCLUSION

The high efficacy of the bioflavonoids allows their use at the recommended doses and making their use in broiler nutrition acceptable, whether alone or in combination with other products as probiotics. Overall, the current study recommends use combination of bioflavonoid (Bio-Guard- in drinking water) and probiotic (Super-CyC mixed in ration) as in group 6. This synergistic effect of Bio-Guard and Super-CyC were reflected on the highest significant data of the live body weight, body weight gain, feed conversion ration and carcass ready to cocked weight, mortality numbers and antioxidant status of broiler chickens.

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