

Influence of probiotic on serum biochemical profile, growth performance and immunostatus in broiler chickens

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A total of 90 one-day-old male broiler chicks were fed on balanced ration for 15 days and then divided into 3 equal groups. Control group fed ration probiotic free, group 2 and 3 fed ration supplemented with probiotic at concentration levels 0.5 and 1.0g/kg ration respectively for 4 weeks. It was found that probiotic improve body weight gain and feed conversion ratio. Relative organs weights were not altered in groups of birds fed ration mixed with probiotic. Serum values of biochemical parameter (AST, ALT, ALP, total protein, A/G ratio, Ph) were not significantly changed in comparison to control group. Probiotic enhance the immune response of broiler chickens in a dose dependent relation as documented by increasing the serum NDHI antibody geometric mean titres to ND vaccine, phagocyte percentage and phagocytic index of peripheral blood monocyte of broiler chickens. We concluded that probiotic composed of 3 strains of bacteria (*Pediococcus acidilactici*, *Pediococaoccus pentosaceus* and *Bacillus Amylolique- faciens*) in addition to 2 strains of yeast (*Pichia farinose* and *Dekera anomala*) enhance body weight gain, feed conversion, growth performance and Newcastle disease antibody titres in broiler chickens. Moreover, probiotic was safe as proved by serum biochemical profile and relative organ weight of male broiler chickens.

Antimicrobials have been supplemented to poultry feed for more than 50 years to enhance growth performance and to prevent diseases in poultry. However, in recent years great concern has been arisen about the addition of antibiotics at sub-therapeutic level to poultry feed. Due to emergence of multiple drug resistant bacteria (Wray and Davies, 2000) antibiotics as a feed additive was recommended to be replaced by alternatives such as probiotic.

Probiotic are live non pathogenic microorganism that when administered in adequate amount exert beneficial to host health and immune system, through out improvement in intestinal microbial equilibrium (Fuller, 1989; Reid *et al.*, 2003; Gunal, *et al.*, 2006; Vinderola *et al.*, 2006). Therefore, they are called friendly bacteria. They cooperatively maintain a delicate balance between the gastro-intestinal tract and immune system, when this balance is disrupted, diseases and inflammation results. Inflammation and over stimulation of the immune system by pathogenic bacteria are competitively inhibited by mucosal adherence of normal beneficial microflora (Wysocka, 2001) however, the role of different types of probiotic on health performance, serum biochemical changes and

immunostatus of male broiler chicks still not fully clear.

Therefore, this study purposed the evaluation of the effect of a new probiotic (3 strains of bacteria and 2 strains of yeast) in serum biochemical profile, performance (body weight gain and feed conversation), and immunostatus of broiler chickens.

Materials and methods

Probiotic. Probiotic (BIOWISH)^R was obtained from OCTA Egypt Company in the form of feed additives powder. It was composed of 3 strains of bacteria (*Pediococcus acidilactici*, *Pediococaoccus pentosaceus* and *Bacillus Amylolique- faciens*) in addition to 2 strains of yeast (*Pichia farinose* and *Dekera anomala* and vehicles). It was added to the ration in two concentration levels 0.5 or 1 g/kg ration for 4 successive weeks.

Experimental design. A total of 90 one-day-old male Hubbard broiler chickens were fed on balanced ration and *water ad libitum* for 15 days, then divided randomly into 3 equal groups of 30 birds each. Group 1. fed ration probiotic free (control group). Group 2. fed ration supplemented with probiotic 0.5 gram /kg ration. Group 3. fed ration supplemented with probiotic 1 g /kg ration.

Vaccination. Broilers were vaccinated through

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drinking water by La Sota strain at 17th day then repeated every 10 days intervals.

Ration. A basal ration was formulated and considered as control according to recommendation of NRC (1994) for starter (0-21 days, crude protein 21%) and grower (22-42 days, crude protein 18.5%).

All birds were reared on floor pens for the end of the experiment (42 days).

Body weight gain and feed conversion ratio were recorded weekly. Birds were slaughtered at the end of the experiment for weighing of carcasses, feather and skin, liver, gizzard, heart and spleen.

Blood sampling. Blood samples were collected for:

Biochemical studies. At 42nd days of the experimental period 5ml of blood/bird was collected from wing vein from birds in each group. Blood samples were centrifuged (at 3000 x rpm for 10 min) and serum was separated and stored at -20°C until assayed for the following, using appropriate laboratory kits for AST, ALT and ALP (Tietz, 1999); total protein and albumin (Young, 1995). The serum globulin was calculated by subtracting serum albumin from serum total protein levels and A/G ratio. Creatinine was determined according to Bartels, (1961) while, calcium and phosphorus was detected according to Tietz, (1995).

Immunological status studies. Blood samples were collected from all groups of birds every ten days post vaccination for serum separation to determine antibody against Newcastle diseases virus (NDV) vaccine by haemagglutination inhibition (HI). Hepranized blood samples were collected at 1st day post the booster dose to determine the phagocytic activities.

Haemagglutination inhibition test (HI). The serum samples were utilized for HI test to find out the level of antibody titres developed against NDV vaccine in different groups of birds. The test was carried out according to King and seal, (1998).

Phagocytosis. Mononuclear phagocytic activity: Peripheral blood monocytes activity was carried out using *Candidia albicans* by seperation of peripheral blood mononuclear cells as described by **Boyum, (1968)**. Mononuclear cell layer was collected, washed and resuspended in RPMI-1640 supplemented with 10% foetal calf serum and viability was done after Hanks and Wallace, (1985). The test was performed according to procedure described by Chu and Dietert, (1989).

Stastistical analysis. Data were an analyzed using the one-way ANOVA procedure according

to Petrie and Watson, (1999) and t-test (Snedecor, 1969).

Results and discussion

The obtained result revealed that ration supplemented with probiotic at concentration 0.5 or 1 g/kg ration improve body weight gain in comparison to control group of birds (Tables, 1, 2, Fig.1). Feed conversion rate was improved in group of chicks fed ration mixed with probiotic 0.5 g/kg ration (2.5) than either birds fed 1g probiotic /kg ration or control group of birds fed ration free probiotic (3.3 and 3.4 respectively) (Table 3, Fig. 2). The improvement of body weight gain and feed conversion in group of birds fed ration supplemented with probiotic might be due to improved intestinal microbial balance, reduced pathogenic flora which accelerated food absorption (Swain *et al.*, 1996). Probiotic may stimulate appetite and improve nutrition by the production of digestible enzymes, vitamins and detoxification of injurious compounds in the diet (Pelicano *et al.*, 2003).

Our result substantiated the findings of Thitaram *et al.*, (2005); Nayebpor *et al.* (2007); Ashayerizadeh *et al.* (2009) who reported that probiotics can improve the weight of birds. Moreover, adding probiotic to the ration was effective in improving the feed conversion ratio (Zulkifli *et al.*, 2000; Cavit, 2004), Which is contrary to the report of Gunal *et al.*, (2006); Willis *et al.*, (2007); Yalcinkayal *et al.*, (2008) who reported that using this additives shed in the broiler ration had no significant effects on body weight gain and feed conversion ratio after 42 days (the experimental period).

For safety evaluation of probiotic as a fed additive, it was found that relative organs weights of liver, gizzard, heart and spleen were within the normal weight without any significant changes as comparied with the control group of birds (Table, 4). This indicates that probiotic not affect organ weight in birds in group 2 or 3. This finding was in agreement with Mohan *et al.*, (1996); Islam *et al.*, (2004) who found that supplementation of chicken with probiotics had no significant effect on relative organs weights. While the relative carcass weight of chicken groups fed ration mixed wih probiotic (0.5g/kg ration) was increased significantly (P<0.01).

Regarding, the recorded serum biochemical parameter including total protein, albumin, ALT, AST, ALP, creatinine and phosphorus, there was no significant alteration by the probiotic addition to the ration of chickens (Table, 5). Similarly,

Table (1): Body weight (g) of male broiler chickens fed ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1 g/kg balanced ration) (mean \pm S.E.) n=30.

Group	Dose (g/kg ration)	Age/weeks				
		2 nd week	3 rd week	4 th week	5 th week	6 th week
Control group	0.0	333.8 \pm 7.03	753 \pm 21.67	1225.17 \pm 26.27	1792.67 \pm 41.75	1917.17 \pm 47.63
2 nd group	0.5	362.73 \pm 6.67**	787.667 \pm 14.97	1309.5 \pm 22.11*	1902.5 \pm 31.25*	2270 \pm 40.11***
3 rd group	1	362.83 \pm 5.83**	771.5 \pm 15.78	1267.5 \pm 22.20	1798 \pm 36.84	2137 \pm 53.02**

Significant at * P < 0.05 ** P < 0.01 *** P < 0.001

Table (2): Body weight gain of male broiler chickens fed ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1 g/kg balanced ration) for 4 successive weeks (mean \pm S.E.) n=30.

Group	Dose(g/kg ration)	3 rd week	4 th week	5 th week	6 th week
Control group	0.0	419.2 \pm 17.1	472.17 \pm 9.61	570.83 \pm 15.99	251.72 \pm 10.22
2 nd group	0.5	424.93 \pm 11.62	521.83 \pm 11.99**	593.62 \pm 17.72	387.14 \pm 14.38***
3 rd group	1	408.67 \pm 11.84	496.0 \pm 9.895	528.1 \pm 17.51	353.45 \pm 26.19**

Significant at ** P < 0.01 *** P < 0.001

Table (3): Mean of body weight gain, mean of ration consumed and mean of feed conversion ratio of male broiler chickens fed ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1 g /kg ration) for 4 successive weeks (Mean \pm S.E.) n=30.

Groups	Dose (g/kg ration)	Mean of body wt. gain	Feed conversion	Mean of total ration consumed (kg)
Control group	0.0	1604.24 \pm 53.41	3.4	5416
2 nd group	0.5	1947.83 \pm 68.87***	2.5	4833
3 rd group	1	1697.41 \pm 80.76	3.2	5416

Significant at *** P < 0.001.

Midilli *et al.*, (2004) observed that feeding of probiotic did not affect the serum total protein in the treated groups of broilers. Also Rangasamy and Kaliappan, (2007) reported that the serum ALP, AST, ALT and total protein levels in probiotic broiler fed groups were not significantly differ from control group.

Globulin and A/G ratio were increased significantly in group of birds fed ration mixed with probiotic 1g/kg ration. This may indicate that probiotic at this concentration enhance globulin formation which indicate higher defensive mechanism of the birds. These results were assured by immunological studies as it was reported that probiotics stimulate production of natural antibodies in chickens (Hamid *et al.*, 2006). However, serum calcium concentration was decreased significantly (p<0.01) in group of birds fed ration mixed with probiotic at 1g/kg ration in comparison to control group but still within normal serum calcium levels of broiler (Table 5) without any adverse effect of

hypocalcemia. This result was in agreement with Antunovic *et al.*, (2005). They found that serum calcium level decreased significantly (P<0.01) in lamb fed ration mixed with probiotic for 35 days.

Geometric mean HI titres against NDV are shown in (Table 6). The HI titre against NDV were higher in groups 2 and 3 of broiler fed 0.5g and 1g probiotics/kg ration respectively, while the control group which fed basal diet (Group1) recorded the lower titre. The level of NDHI titre was increased continuously up to 30 days post vaccination in probiotics treated groups (2 and 3) as compared with the control one. Rangasamy and Kaliappan, (2007) indicating immunomodulatory effect of probiotics in broilers, and a higher HI titre against NDV in probiotic treated groups while, the control showed low titre, additionally Kabir *et al.*, (2004) found that supplementation of probiotics significantly increased the antibody production in broilers.

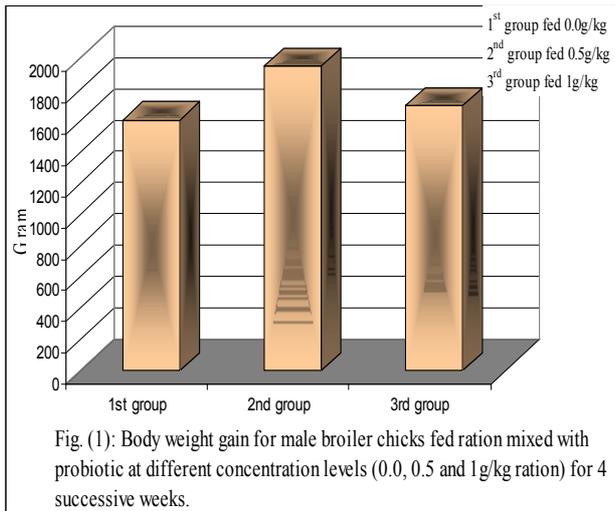


Fig. (1): Body weight gain for male broiler chicks fed ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1g/kg ration) for 4 successive weeks.

*1st group is the control group.

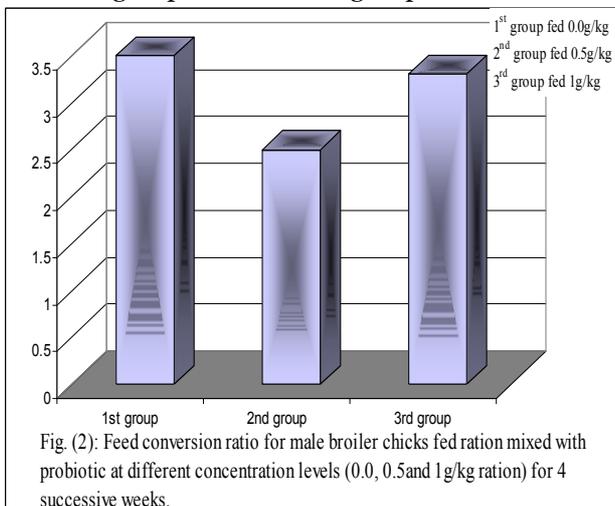


Fig. (2): Feed conversion ratio for male broiler chicks fed ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1g/kg ration) for 4 successive weeks.

*1st group is the control group

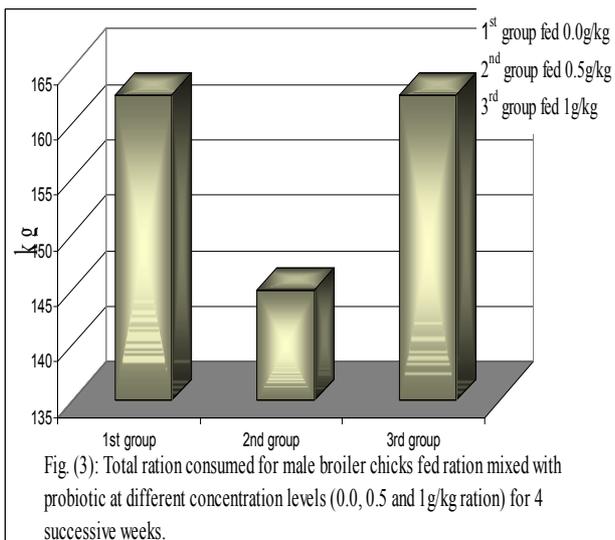


Fig. (3): Total ration consumed for male broiler chicks fed ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1g/kg ration) for 4 successive weeks.

Referring to phagocytic activity of monocytes (Table 7), a significant increase was recorded in both phagocytic percentage and index in all treated group as compared to control

group. Probiotic bacteria contact as mucosal and systemic adjuvant, this induced an interaction of probiotic bacteria with the immune cells of Peyer's patches from the innate immune system, and therefore cytokines production is enhanced. These cytokines are the biological messengers of signals that activate the systemic immune response (Galdeano *et al.*, 2007). Also, Schiffrin *et al.*, (1997) stated that a bacterial signal could also act directly on epithelial cells or the intraepithelial lymphoid compartment of the proximal small intestine. This lymphoid population, on activation, can produce cytokines, such as interferon γ and interleukin 2 (Ebert, 1989 and 1990). Cytokines released from these lymphoid populations may then act locally or alter the systemic reactivity of the host. It is tempting to speculate that for some probiotic activities such as immunomodulation. This may explain that probiotics confer immune adjuvant properties to the host when administered in appropriate dose. The ability of probiotics to prevent several pathological conditions was by stimulating the immune system to generate an immune response to be able to interact or send signals to immune cells associated with the gut (Isolauri *et al.*, 2001).

In conclusion, dietary supplementation of probiotic for male broiler chickens significantly increased body weight gain, feed conversion ratio and improve immunostatus. Moreover, probiotic was safe as proved by serum biochemical profile and relative organ weight of male broiler chickens. In veterinary medicine probiotics may be effectively used to optimize digestive processes, stimulate growth and prevent diseases of the digestive tract.

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Table (4): Means of relative organ weights of male broiler chickens fed balanced ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1.0 g/kg ration)at time of slaughter (42 days) (mean ± S.E.) n=30.

Groups	Dose g/kg ration	Feather and skin	Carcass	Liver	Gizzard	Heart	Spleen
Control group	0.0	12.51± 0.34	63.23± 0.76	3.006± 0.14	1.819± 0.06	0.472± 0.02	0.19± 0.04
2 nd group	0.5	13.17± 0.31	67.98± 0.36***	2.744± 0.09	2.044± 0.08	0.532± 0.02	0.176± 0.01
3 rd group	1	13.297± 0.41	65.08± 0.93	2.756± 0.18	1.829± 0.05	0.512± 0.03	0.177± 0.04

Significant at *** P < 0.001

Table (5): Serum biochemical parameters of male broiler chickens fed ration mixed with probiotic at different concentration levels (0.0, 0.5 and 1g /kg ration) for 4 successive weeks. (Mean ± S.E.) n=30

Groups	Dose (g/kg ration)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	Creatinine (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	ALT (u/L)	AST (u/L)	ALP (u/L)
Control group	0.0	3.95±0.119	1.166±0.077	2.784±0.133	0.426±0.042	0.311±0.016	11.24±0.33	9.296±0.31	42.5±3.44	149.0±11.23	164.56±11.1
2 nd group	0.5	3.7±0.23	1.27±0.19	2.55±0.175	0.458±0.042	0.366±0.029	10.88±0.08	8.176±0.4	39.25±3.136	146.0±15.16	160.45±11.7
3 rd group	1	4.4±0.22	1.02±0.029	3.382±0.2*	0.306±0.016*	0.317±0.015	9.92±0.04**	8.477±0.19	38.25±4.27	138.3±15.49	152.98±9.9

Concentration levels (0.0, 0.5 and 1g /kg ration) for 4 successive weeks. (Mean ± S.E.) n=30

*Significantly different from control at P < 0.05

** Significantly different from control at P < 0.01

Table (6): Serum antibody Geometric mean titres of ND vaccinated chickens by Haemagglutination inhibition (HI) test in male broiler chicks fed ration mixed with probiotic at different concentration levels(0.0,0.5 and 0.1 g/kg ration) .

Groups	Dose (g/kg ration)	Time		
		10 days	20 days	30 days
Control group	0.0	49.324	89.808	123.286
2nd group	0.5	54.975	93.845	155.965
3rd group	1	52.293	90.166	146.979

Table (7): Average phagocytosis of peripheral blood monocyte of broiler chickens fed ration mixed with probiotic at different concentration levels(0.0, 0.5 and 1.0g/kg ration).(m \pm s.e).

Groups	Dose (g/kg ration)	Parameter	
		1 day p. booster dose of vaccine	
		Phagocytic percentage	Phagocytic percentage
Control group	0.0	54.00 \pm 0.577 ^c	54.00 \pm 0.577 ^c
2nd group	0.5	62.00 \pm 1.154 ^a	62.00 \pm 1.154 ^a
3rd group	1	73.00 \pm 1.732 ^b	73.00 \pm 1.732 ^b

Means with different superscripts in the same column are significantly different at P < 0.05.

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تأثير البروبيوتك على التغيرات البيوكيميائية ومعدلات النمو والاستجابة المناعية لبدارى التسمين

أجريت التجربة على عدد ٩٠ من ذكور بدارى التسمين عمر يوم واحد . غذيت على عليقة متزنة لمدة خمسة عشر يوما ثم قسمت إلى ثلاثة مجموعات متساوية المجموعة الأولى ضابطة (غذيت على عليقة خالية من البروبيوتيك) المجموعة الثانية والثالثة غذيتا على عليقة مضاف إليها ٠,٥ جرام أو جرام لكل كيلو جرام عليقة بالترتيب من عمر ١٥ يوم حتى عم ٤٢ يوم . وجد أن اضافة البروبيوتيك للعليقة حسن من معدل النمو والتحويل الغذائي ولم يلاحظ أي تغيرات أو فروق معنوية في الوزن النسبي للأعضاء الداخلية . كما أنه لم توجد أي تغيرات معنوية في قياسات نشاط انزيمات (AST,ALT, ALP) أو تغيرات معنوية في قياسات (Ph ,A/G ratio, Total protein في المصل بالمقارنة بالمجموعة الضابطة . كما وجد أن البروبيوتيك ينشط المستوى المناعي للطيور أمام التحصين باللقاح بفيروس النيوكاسل بعلاقة طردية مع جرعة البروبيوتيك في العليقة وكذلك رفع البروبيوتيك معدل نشاط الخلايا الأكلة ونسبتها في بدارى التسمين مما يوضح الأثر التنشيطي المناعي للبروبيوتك. ويمكن استخلاص ان البروبيوتيك حسن من معدل النمو والتحويل الغذائي وكفاءة الصحة العامة للطيور كم حسن من الاستجابة المناعية لبدارى التسمين.