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Effect of some biological supplementation on productive performance, physiological and immunological response of layer chicks

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ABSTRACT

The present study was carried out to evaluate the effects of biological (*Bacillus subtilis and Enterococcus faecium*) supplementation on productive performance, physiological and immunological response of Hy-line layer chicks. Total of 300 one-day old of Hy-line layer chicks, were randomly divided into three groups. The first group was fed a basal diet and served as a control. While the second and third groups were fed the basal diet that supplemented with the probiotic mixture at the rate of 1 and 2 gm/kg of diet, respectively, until 10 weeks of age.

Results indicated that treated groups with helpful bacteria (*B. subtilis* and *E. faecium*) showed significant effect on final body weight gain, feed conversion ratio and higher antibody levels against Newcastle disease virus as compared to the control one. Moreover, significant increase was recorded in the relative weight of carcass, liver, heart, kidney, proventiculus, small intestine, thymus, spleen, bursa of Fabricius and small intestine length (cm) in all supplemented groups as compared to the control group. On the other hand, there were no significant effects on serum total protein, albumin, globulin and creatinine concentrations, while, serum ALP, ALT, AST activities, uric acid, triglycerides and cholesterol concentrations in all treated groups were significantly lower than in control group. Furthermore, serum glucose, calcium, phosphorus concentrations and triiodothyronine hormone level were significantly higher in treated groups than the control. Red and white blood cell counts, hemoglobin level and hematocrit values were significantly increased in all treated groups as compared to control group.

In conclusion, biological (*B. subtilis* and *E. faecium*) supplementation can be used as one of important additive for enhancing the productive efficiency, and immunity of growing Hy-line chicks.

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1. Introduction

In Egypt, poultry farming is considered the most important way for solving the gap in meat production for human consumption. However, during intensive growth, this industry has always been confronted with challenges constraint to productivity that results in heavy economic loss to the poultry producers. Among these conditions, low growth performance and infectious diseases (Boirivant & Strober, 2007).

Biological supplementation to appropriate diets is highly helpful in the poultry industry for obtaining better productivity and health benefits (Hajati & Rezaei, 2010). The use of effective live microbes is recommended in the newly hatched chicks, to accelerate development of normal microflora and as safe alternatives to antibiotic growth promoters (Bansal, Singh, & Sachan, 2011). These could prevent diseases like early chick mortality, gastro-intestinal disturbances like scouring, loss of appetite, improper digestion, poor absorption of nutrients and infectious conditions by fighting against pathogenic microbes especially the enteric pathogens. Thus, these can alleviate reduced production performance and prevent heavy economic loss to the poultry producers (Dhama & Singh, 2010). In this regard, *Bacillus subtilis and Enterococcus faecium* are beneficial "live microbes", classified as probiotics (Mountzouris et al., 2007). A positive impact of probiotics supplementation in poultry has been well reported on production performance, (Awad, Ghareeb, Abdel-Raheem, & Bohm, 2009), feed intake, weight gain and feed conversion efficiency (Cavit, 2003), immune responses (Alkhalf, Alhaj, & Al-Homidan, 2010), and body's resistance to infectious diseases (Santos & Ferket, 2006) and help lowering of chick mortality (Dhama et al., 2008). The benefits of probiotics are based on improve the microbial environment of a bird's intestinal tract by displacing harmful bacteria. Thus, the use of defined probiotic cultures in the poultry industry has recently become more common for obtaining better digestion and absorption of carbohydrates, proteins and fats, which also increases the feed conversion efficiency and increases the body's resistance to infectious diseases by offering digestible proteins, vitamins, enzymes, various antibacterial substances and other important co-factors and by decreasing gut pH by production of lactic acids. As *'live enzyme factory'* (amylase, protease, lipase). Moreover, Probiotics help in metabolism of minerals and synthesis of vitamins (Biotin, Vitamin-B1, B2, B12 and K), which are responsible for proper growth and metabolism (Dhama & Singh, 2010). Unfortunately, little information is available concerning the effect of biological supplementation on layer chicks under Egyptian condition. Therefore, the present study was carried out to evaluate the effects of biological (*B. subtilis* and *E. faecium*) supplementation on productive performance, physiological and immunological response of Hy-line layer chicks from 1 to 10 weeks of age.

Table 1 – The ingredient composition and calculated chemical analysis of the basal diet.

Ingredients composition (kg)	Ingredient percentage
Yellow corn	54.25
soy bean meal (44%)	25.00
Glutin	6.00
Vegetable oil	3.50
Dicalcium. phosphate	2.00
Limestone	8.50
DL-methionine	0.10
Sodium chloride	0.30
Yeast	0.15
Amino vet.	0.05
Zinc pacitracin	0.015
Choline chloride	0.14
Lysine	0.20
Calcium carbonate	3.50
Vitamin and min. premix[a]	0.35
Calculated chemical analysis	
Crude protein, %	23
Metabolizable energy	3100 Kcal/kg

[a] Vitamin and mineral premix (contained per Kgm):- vit A, 1200 IU; vit D 1100 IU; vit E, 12 mg; vitB12, 0.02 mg; vit B1, 1 mg; choline chloride, 0.16 mg; copper, 3 mg; iron, 30 mg; manganese, 40 mg; zinc, 45 mg; and selenium, 3 mg.

2. Materials and methods

2.1. Experimental chicks and biological supplementation

A total number of 300 one-day old, Hy-line layer chicks with the average weight of 40 g, reared at the Poultry Experimental House, Nuclear Research Center, Egyptian Atomic Energy Authority were used in the present study. Hy-line layer chicks, with the average weight of 40 g were randomly divided into three equal groups. The first group was fed a basal diet and served as a control. While the second and third groups were fed the basal diet that supplemented with the probiotic mixture (*B. subtilis* and *E. faecium*) with 1 and 2 gm/kg, respectively, until 10 weeks of age. All groups were kept at the similar conditions of room temperature and under normal periods of light/dark. Feed and water supplemented were *ad libitum* throughout the experimental period. Body weight gain and feed consumption were recorded weekly during the experiment period. Feed conversion ratio (FCR) was calculated as the ratio between feed intake and body weight gain at the end of each week. The ingredients' composition and calculated chemical analysis of the basal diet are given in Table 1. The probiotics used in the experiment were white dried powders of double strain probiotic with the content of 3.0×10^{10} cfu/g. Probiotic containing (*B. subtilis* and *E. faecium*) was purchased from "Biopellet-S" and manufactured by Samu median Co., Ltd. (South Korea).

2.2. Carcass traits and blood analysis

At the end of experimental period (10 weeks of age), six chickens from each group, were randomly selected, weighed and slaughtered for carcass analysis. Head, feather, feet and viscera for each slaughter bird were handily removed. Carcass, liver, heart, kidney, proventiculus, small intestine, thymus, spleen, and bursa of Fabricius for each slaughter bird were calculated as a relative percentage of live body weight. In addition, small intestine length was determined. Blood samples were collected from slaughtered chicks and placed in two tubes, one with lithium heparin to determine hematological parameters and the other without anticoagulant and left to clot then centrifuged at 1600 × *g* for 15 min, and the resulting serum was stocked at −20 °C for hormonal and chemical analyses.

Serum total proteins, albumin, total calcium, inorganic phosphorus, alkaline phosphates (ALP), uric acid, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride, total cholesterol and glucose were determined colorimetrically using commercial kits produced by Stanbio Company, USA by computerized spectrophotometer model Milton Roy 1201. Serum Glob values were calculated by subtracting albumin values from their corresponding total proteins values of the same sample. Finally, triiodothyronin hormone (T3) was determined using radioimmunoassay (RIA) Commercial Kit produced by IZOTOP Company (INSTITUTE OF ISOTOPES Ltd.) (http://www.izotop.hu) and samples were counted on Pacard Gamma Counter. Concerning, blood hematological parameters, Red blood cells (RBCs) and white blood cells (WBCs) counts were determined according to Natt and Herrick (1952). Hemoglobin concentration (Hb) and packed cell volume (PCV %) were determined according to Dacie and Lewis (1991).

2.3. Immunological test

At the end of the experiment, six birds from each group were chosen at random and housed in multidisc batteries. Each bird was vaccinated against Newcastle disease with NDV clone 30 (Nobilis ND Clone 30; Intervet) by eye-drop. Blood samples were collected from wing vein using an insulin syringe at three times 3, 7 and 9 days of post-vaccination. Blood was allowed to clot then centrifuged immediately to separate serum to determine immune response (antibody titer) of the chickens derived from vaccination against Newcastle disease virus by performed Hemagglutination inhibition (HI) test on serum samples according to the method of (King & Seal, 1998).

2.4. Statistical analysis

One way, analysis of variance was done using the SAS General Liner Model procedure (SAS Institute, 2002). The main factor was the treatment (bacteria supplementation). Significance level was set at P < 0.05. Mean values were compared using Duncan's Multiple Range Test (Duncan, 1955) when significant differences existed. The model used was: $Y_{ij} = \mu + T_i + e_{ij}$Where:

Y_{ij} = any value from the overall population. μ = the overall mean.
T_i = the effect of the ith treatment (i = 1, control& 2, bacteria supplementation).
e_{ij} = the random error associated with the jth individual.

3. Results and discussion

3.1. Effect of biological supplementation on productive performance

The effects of biological "*B. subtilis* and *E. faecium*" supplementation on body weights, daily body weight gain, weekly feed consumption (g/bird) and feed conversion ratio are presented in Tables 2 and 3, respectively.

It was found that, weekly body weights (Table 2), daily weight gain and final body weight (Table 3) were significantly increased in the treated groups as compared to the control during overall experimental period. The third group (2 g/kg diet) was significantly higher than the second group (1 g/kg diet). Furthermore, weekly feed consumption (g/bird) and feed conversion ratio (Table 3), were significantly decreased in the third treated groups than the second group, and both of them were significantly decreased than the control group. These findings are in agreement with several reports demonstrating that probiotic supplemented to the birds improved the body weight gains of the broiler chickens (Benites, Gilharry, Gernat, & Murillo, 2008). Khaksefidi and Ghoorchi (2006) showed also that body weight gain of the birds fed diet supplemented with 50 mg/kg of probiotic (*B. subtilis)* were significantly higher than the control group and the feed conversion ratio was better. In addition, Mountzouris et al. (2007) and Bansal et al. (2011) found that broilers treated with probiotic containing *Pediococcus strain, Enterococcus strain, Lactobacillusstrains and Bifidobacterium strain* in feed and water had better feed conversion ratio.

In this study, biological "*B. subtilis* and *E. faecium*" supplementation is effective in promoting poultry growth and improving feed conversion ratio. This result may be due to "*B. subtilis* and *E. faecium*" supplementation enhancing the synthesis of certain vitamins, providing digestive enzymes and increasing the production of volatile fatty acids that finally are metabolized in favor of the host (Fuller, 2001). The treatment with biological supplementation may also increase the uptake of nutrients from gastrointestinal tract through their indirect effect on its permeability (Higgins et al., 2008). In this mention, Mountzouris et al. (2007) and Alkhalf et al. (2010) reported that probiotic's immunomodulatory activity and ability to fortify beneficial members of the intestinal microflora, improving efficiency of digestion and nutrient absorption processes of the host. Particularly, it was also worth noting that birds treated with "*B. subtilis* and *E. faecium*" displayed a great

Table 2 – Effects of biological (*Bacillus subtilis* and *Enterococcus faecium*) supplementation on live body weight of layer chicks.

Body weight (g), weekly	Experimental groups		
	0 g/kg diet	1 gm/kg diet	2 gm/kg diet
Initial body weight	40.9 ± 0.67^{a}	41.4 ± 0.93^{a}	40.4.0 ± 0.76^{a}
At 1st week	61.1 ± 1.14^{b}	65.0 ± 0.95^{a}	65.69 ± 0.71^{a}
At 2 nd week	91.3 ± 0.70^{c}	101 ± 1.60^{b}	109.8 ± 1.50^{a}
At 3rd week	139.9 ± 0.70^{c}	158.8 ± 1.17^{b}	179.4 ± 1.20^{a}
At 4th week	229.7 ± 1.26^{c}	253.9 ± 1.53^{b}	288.8 ± 1.24^{a}
At 5th week	334.85 ± 2.5^{c}	379.1 ± 1.91^{b}	417.14 ± 2.2^{a}
At 6 th week	480.2 ± 2.40^{c}	522.0 ± 2.14^{b}	586.4 ± 2.1^{a}
At 7th week	626.3 ± 1.70^{c}	693.0 ± 1.42^{b}	761.95 ± 1.8^{a}
At 8th week	790.6 ± 1.30^{c}	878.2 ± 1.85^{b}	969.1 ± 1.94^{a}
At 9th week	987.5 ± 2.50^{c}	1104 ± 1.18^{b}	1211 ± 2.04^{a}
Final body weight	1307.5 ± 2.30^{c}	1369.2 ± 1.74^{b}	1467.8 ± 2.3^{a}

a,b,c – Means in the same row with different superscripts are significantly different (P ≤ 0.05).

Table 3 – Effects of biological (*Bacillus subtilis* and *Enterococcus faecium*) supplementation on growth and feed performance of layer chicks.

Growth performance	Experimental groups		
	0 g/kg diet	1 gm/kg diet	2 gm/kg diet
Daily weight gain (g)	18.1 ± 1.5^c	18.97 ± 1.3^b	20.39 ± 1.5^a
Feed intake (g)/bird	2825.5 ± 2.3^a	2778.2 ± 2.3^b	2746.4 ± 2.3^c
Feed conversion ratio	2.13 ± 0.3^a	2.06 ± 0.3^b	1.87 ± 0.3^c

a,b,c – Means in the same row with different superscripts are significantly different ($P \leq 0.05$).

increase in body weight and feed conversion by 7.72, 12.3%, respectively, more than those of the control group.

Therefore, in our study, improvement in growth performance and feed conversion ratio of the chicks supplemented diet with "*B. subtilis* and *E. faecium*" may be attributed to the total effect of supplementation on the maintenance of beneficial microbial population that improving feed intake, digestion and the uptake of nutrients (fatty acids and glucose) and increasing digestive enzyme activity.

3.2. *Effect of biological supplementation on carcass traits and relative organ weights*

Data in Table 4 showed that, the relative weight of carcass, liver, heart, kidney, proventiculus, small intestine, thymus, spleen, bursa of Fabricius and small intestine length (cm) were significantly increased in the treated group as compared to the control during overall experimental period. And they highly significantly increased in the third group (2 g/kg diet) than the second group (1 g/kg diet). These results were totally coincided with the observations of Awad et al. (2009) who reported that carcass yield percentage was significantly increased in the probiotic fed broilers as compared with the control. Alkhalf et al. (2010) showed a significant increase in carcass yield percentage and immune organ weights in the probiotic supplemented broiler chicks as comparison with the control group. Zhang, Ma, and Doyle (2006) found that some probiotics or synbiotics increased body weight of the chickens. Also, these results are in similar to the results of Waldroup, Fritts, and Fenglan (2003). The significant increases in the absolute weight of the immune organs (thymus and bursa) were in harmony with the results of previous studies Wang, Du, Bai, and Li (2003). The increase in the relative weight of spleen is also in agreement with the findings of Willis, Isikhuemhen, and Ibrahim (2007) who found that the feeding broilers on probiotic caused increases in the relative weights of spleen of treatment group.

The significant increase in relative weight of bursa of Fabricius may be attributed to increase the number of immune cells. Findings encountered in this study is in agreement with that of Shoeib, Sayed, Sotohy, and Abdel Ghaffar (1997) who found that the bursa of Fabricious in probiotic treated group showed an increase in the number of follicles with high plasma cell reaction in the medulla. Meanwhile, Teo and Tan (2007) observed that birds provided feed supplemented with *B. subtilis* had a significantly heavier bursa

Table 4 – Effects of biological supplementation on relative weight of carcass and some organs of layer chicks.

Relative weight of carcass and some organs	Experimental groups		
	0 g/kg diet	1 gm/kg diet	2 gm/kg diet
Carcass	55.6 ± 0.580^c	64.8 ± 0.32^b	69.64 ± 0.62^a
Liver	1.82 ± 0.016^b	1.99 ± 0.023^a	2.04 ± 0.023^a
Heart	0.51 ± 0.012^c	0.53 ± 0.009^{ab}	0.56 ± 0.015^a
Proventiculus	0.34 ± 0.005^c	0.38 ± 0.004^b	0.42 ± 0.007^a
Kidney	0.53 ± 0.007^c	0.60 ± 0.003^b	0.64 ± 0.015^a
Small intestine	4.97 ± 0.040^b	6.13 ± 0.070^a	6.15 ± 0.07^a
thymus	0.54 ± 0.011^c	$0.62 \pm 0.0.013^b$	0.67 ± 0.006^a
Spleen	0.22 ± 0.005^c	0.26 ± 0.006^b	0.29 ± 0.002^a
Bursa	0.29 ± 0.003^c	0.33 ± 0.005^b	0.38 ± 0.007^a
Bursa	0.29 ± 0.003^c	0.33 ± 0.005^b	0.38 ± 0.007^a
Small intestine length(cm)	11 ± 0.0700^c	11.83 ± 0.048^b	12.2 ± 0.13^a

a,b,c – Means in the same row with different superscripts are significantly different ($P \leq 0.05$).

weight compared with control group. The effect of probiotic on the relative weight of thymus was also investigated in this study as shown in Table 4. Probiotic supplementation was significantly increased the relative weight of thymus in all probiotic treatment groups as compared to the control group. The significant increase in weight of thymus may be due to the effect of probiotic bacteria on the functional activities of the immune system responses which led to increase in the number of lymphocytes in the primary lymphoid organs.

Measurement of immune organ weight is a common method for evaluation of immune status in chickens (Heckert, Estevez, Russek-Cohen, & Pettit, 2002). Such related organs include thymus, bursa of Fabricius, liver and spleen. Good development of these organs is crucial for optimal Ig synthesis (Glick, 1977). Therefore, beneficial effects of "*B. subtilis* and *E. faecium*" supplementation in the gastrointestinal tract could result in an improvement of overall health, performance and immune response of layer chicks.

3.3. *Effect of biological supplementation on blood biochemical and hormonal*

Data in Table 5 clearly showed no significant difference was recorded among the three groups ($P \leq 0.05$) in serum total protein, albumin, globulin and creatinine concentrations. While serum alkaline phosphatase, ALT, AST, uric acid, triglycerides and total cholesterol concentrations showed a significant decrease in all treated groups than the control. Furthermore, serum concentration of glucose, calcium, inorganic phosphorus, and triiodothyronin hormone, showed a significant increase in the treated groups than the control group.

Our results were coincided with Al-Kassie, Al-Jumaa, and Jameel (2008) and Aluwong et al. (2012) who's showed no significant differences in total protein, albumin and globulin between treatments with probiotics and control group. In addition, Santoso, Tanaka, and Ohtania (1995) recorded that the probiotics had a lower levels of AST and ALT enzymes. While, Hussein (2014) reported that there were no effect on

Table 5 – The effect of biological (*Bacillus subtilis* and *Enterococcus faecium*) supplementation on some blood biochemistry of layer chicks.

Blood biochemistry	Experimental groups		
	0 g/kg diet	1 gm/kg diet	2 gm/kg diet
Total protein(g/dl)	3.04 ± 0.02^a	3.04 ± 0.02^a	3.02 ± 0.02^a
Albumin(g/dl)	1.29 ± 0.01^a	1.28 ± 0.01^a	1.30 ± 0.01^a
Globulin(g/dl)	1.74 ± 0.02^a	1.76 ± 0.02^a	1.72 ± 0.02^a
Uric Acid(mg/dl)	5.07 ± 0.05^a	4.88 ± 0.05^b	4.54 ± 0.05^c
Creatinine(mg/dl)	0.77 ± 0.01^a	0.78 ± 0.01^a	0.78 ± 0.01^a
Glucose(mg/dl)	69.74 ± 0.5^c	75.08 ± 0.5^b	78.54 ± 0.5^a
Calcium(mg/dl)	5.93 ± 0.05^b	6.18 ± 0.05^a	6.30 ± 0.05^a
Phosphorus(mg/dl)	2.95 ± 0.06^b	3.13 ± 0.06^b	3.39 ± 0.05^a
Alk. Phosphatase (IU/L)	41.16 ± 0.3^a	38.26 ± 0.3^b	35.68 ± 0.3^c
ALT (u/ml)	38.70 ± 0.02^a	38.40 ± 0.4^a	35.73 ± 0.4^b
AST (u/ml)	34.80 ± 0.3^a	33.00 ± 0.3^b	29.20 ± 0.3^c
Triglyceride(mg/dl)	189.2 ± 1.87^a	174.8 ± 1.87^b	159.7 ± 1.87^c
Cholesterol(mg/dl)	166.49 ± 1.3^a	156.08 ± 1.3^b	138.33 ± 1.3^c
T3(pg/ml)	1.70 ± 0.007^c	1.77 ± 0.007^b	1.85 ± 0.007^a

a,b,c – Means in the same row with different superscripts are significantly different ($P \leq 0.05$).

serum AST and ALT activities, after addition of probiotic (*Saccharomyces cerevisiae*) as compared with the control treatment. The significant decrease in blood ALT and AST activities within the normal range in treated groups suggested normal status of liver function as a result of biological supplementation with "*B. subtilis* and *E. faecium*". While, the significant increase in blood AST and ALT enzymes in control treatment (without supplementation) act as hepatocellular damage indicator (Yalcin, Yalcin, Uzunoglu, Duyum, & Eltan, 2012).

In addition, the decrease in serum alkaline phosphatase (ALP) activity obtained in the present study was agreement with Aluwong et al. (2012) who reported a significant decrease in the activities of serum alkaline phosphatase of the broiler chickens in all the probiotic supplemented groups, when compared with the control.

The significant decrease in serum total cholesterol and triglyceride levels ($P \leq 0.05$) in our treated groups in comparison to the control group are agreement with those finding by Hajjaj et al. (2005), Shareef and Al-Dabbagh (2009). They reported that there were a significant decrease in serum concentrations of cholesterol and triglyceride in broiler chicks by supplementation of *Saccharomyces Cerevisae* and *B. subtilis* to diets. Also, similar results were obtained by Jouybari, Malbobi, Irani, and Pour (2010), who reported significant reduction in cholesterol by 12% and triglycerides in broilers fed probiotic based diets. Our results indicated that *B. subtilis* and *E. faecium* might have anticholesterolaremic properties, influenced fatty acid synthesis in the liver of layer chicks as indicated by a decrease activity of acetyl –CoA carboxylase (Skorve et al., 1993). Other possible mechanism include assimilation of cholesterol by biological (*B. subtilis* and *E. faecium*) supplementation, has the ability to produce active bile salt hydrolase and maintain bile salt homeostasis, this may need more bile acids to be synthesized this in turn will reduce cholesterol levels in the body pool since cholesterol is the precursor for bile acids (Guo & Zhang, 2010). Also, Salarmoini and Fooladi (2011) explained that microorganisms such as *B. subtilis and Bacillus licheniformis* are able to synthesize estrase enzymes alongside with lipase enzymes, which converts free fatty acids to esterified form triglyceride in intestinal content and finally less chance for triglyceride absorption into the plasma. In contrast, Owosibo, Odetola, Odunsi, Adejinmi, and Lawrence (2013) reported that the serum cholesterol value was significantly increased by the probiotics supplementation in broiler chicks. While, Kawahara, Ueda, and Nomura (1991) did not find any effect of probiotics on serum cholesterol.

Concerning to kidney function, our results revealed that there was no significant change in creatinine level among all the groups. On the other hand, similar to our data, Kamgar, Pourgholam, Ghiasi, and Ghane (2013) reported that, there was a significant increase in uric acid level in the control group than the treated groups. While, Strompfová et al. (2006) reported that there was no effect on serum uric acid levels by the addition of probiotic (*Saccharomyces cerevisiae*) as compared with the control. The significant decrease in uric acid level in treated groups, indicating beneficial effect of the probiotic on the kidney function. On the other hand, certain probiotic microorganisms can utilize urea, uric acid and creatinine and other toxins as its nutrients for growth (Salim, Abd-Allah, & Fararh, 2011). Any abnormal increase in serum levels of uric acid and creatinine may imply kidney damage (Yalcin et al., 2012). Therefore, the relatively stable in serum levels of uric acid and creatinine may be associated with renal protective effects of the probiotic.

In our study, the significant increase ($P \leq 0.05$) in serum glucose concentration of the treated groups as compared with the control are agreement with Hussein (2014) who found a higher blood glucose concentration in broilers fed on diets supplemented with probiotics. This increase might be related to a temperate improvement in gluconeogenesis and increased lactose absorption (Das, Medhi, & Islam, 2005). While, our results are disagreement with Abd El-Baky (2007) who reported that there were no changes in blood glucose level in broiler treated with probiotic. Unlike, Al-Kassie et al. (2008) recorded reduction in serum glucose level in groups receiving probiotics as compared with the control.

In addition, the significant increase ($P \leq 0.05$) in serum calcium and inorganic phosphorous concentrations in the treated groups as compared with the control are agreement with a study by Nahashon, Nakaue, and MIrosh (1996) who indicated beneficial effects of probiotic supplementation on the damaged egg ratio through increased calcium retention in layers. Gilman and Gashman (2006) and Scholz et al. (2007) reported that probiotics can enhance the calcium absorption from intestinal tract. In addition, Strompfová et al. (2006) reported a significant increase in serum calcium level of treated groups with strain of *E. faecium* than the control. While, Hashemzadeh, Shaban, Mohammad, Karimi, and Ali Akbar (2013) reported no significant effect of probiotic on serum calcium and phosphorous levels in Broiler Chicks.

Finally, our results indicated that serum concentration of triiodothyronin (T_3) was significantly increased in the treated groups as comparison with the control group as shown in Table 5. Similar result was obtained by Chotinsky and Mihaylov (2013), who showed a significant increase in serum level of triiodothyronin with the supplementation of probiotics in the diet of broiler chickens. The present study reports for the first time that, the influence of biological supplementation on the level of thyroid hormone in the blood serum of layer chicks and provides new interesting data about a possible causal relationship between the growth promoting effect of probiotics and thyroid hormone. Depending on the previous results, it can be concluded that the observed significant increase in the triiodothyronine (T_3) in the treated groups as comparison with control group in this study is logic since it is necessary for most body functions because they directly affect a number of physiological and metabolic processes (McNabb, 2000). Dawson, McNaughton, Goldsmith, and Degen (1994) showed a significant positive correlation between thyroxin and body weight.

3.4. *Effect of biological supplementation on hematological responses*

Results in Table 6 indicated that, biological supplementation induced a high significant effect on the level of Hb concentration, PCV %, WBCs and RBC's counts in the treated groups when compared with the control group. And the highly significant increase was found in the third group (2 g/kg diet). These results are agreement with Paryad and Mahmoudi (2008) who found that WBC's count was higher in broiler chicks fed different levels of probiotics than those fed diets without probiotics. Also, Abdollahi, Kamyab, Bazzazzadekan, Nik-khah, and Shahneh (2003) reported that supplementation of broiler diets with *B. subtilis* probiotics increased leukocyte numbers. Cetin, Güçlü, and Cetin (2005) observed in turkey that the probiotic supplementation caused statistically significant increases in the erythrocyte count, hemoglobin concentration and hematocrit values. Also, Strompfová et al. (2006) reported a significant increase in the concentrations of hemoglobin, hematocrit value and red blood cell count after application of strain *E. faecium*. The previous study would be explained as the supplementation of dried *B. subtilis* and *E. faecium* to the basal diet resulted in better iron salt absorption from the small intestine and better produce of vitamins B that affecting positively blood-cell forming processes (Kander, 2004). Moreover, increased blood WBC's count might be related to the production of more immune cells (Gaggìa, Mattarelli, & Biavati, 2010) that play an important role in defending the biological system against different diseases (LaFleur & LaFleur, 2008).

Table 6 – Effects of biological (*Bacillus subtilis* and *Enterococcus faecium*) supplementation on some blood hematology levels of layer chicks.

Trails	Experimental groups		
	0 g/kg diet	1 gm/kg diet	2 gm/kg diet
RBCs Count × 10^6	5.0 ± 0.012^c	5.23 ± 0.027^b	5.44 ± 0.025^a
Hb (g/dl)	9.38 ± 0.43^b	10.75 ± 0.43^a	11.23 ± 0.13^a
PCV %	28.25 ± 0.47^b	32.5 ± 0.865^a	34.25 ± 0.48^a
WBCs Count × 1000	281.5 ± 7.53^c	339 ± 7.14^b	365.25 ± 6.89^a

a,b,c – Means in the same row with different superscripts are significantly different ($P \le 0.05$).

Table 7 – Effects of biological (*Bacillus subtilis* and *Enterococcus faecium*) supplementation on antibody titres to Newcastle disease virus of layer chicks.

Days of treatment	Antibody titres against newcastle disease virus		
	Experimental groups		
	0 g/kg diet	1 gm/kg diet	2 gm/kg diet
3rd day	3.75 ± 0.25^b	4.5 ± 0.29^{ab}	5.25 ± 0.25^a
7th day	5.25 ± 0.25^c	7.25 ± 0.25^b	8.25 ± 0.25^a
9th day	4.25 ± 0.25^b	5.75 ± 0.25^a	6.75 ± 0.475^a

a,b,c – Means in the same row with different superscripts are significantly different ($P \le 0.05$).

3.5. *Effect of biological supplementation on immune response*

There is increasing interest in evaluating non-medical alternatives for antimicrobials and antiviruses in terms of their ability to improve disease resistance, and enhance overall animal health and production in poultry. Therefore, in the present study, attempts were made to evaluate the use of biological (*B. subtilis* and *E. faecium*) and investigate the influence of such feed supplements on immune response.

Serum antibody titers against Newcastle disease virus based on HI test in chicken fed basal diet supplemented with "*B. subtilis* and *E. faecium*" was significantly higher ($P \le 0.05$) than those of chickens in the control group on days 3, 7 and 9 post vaccinations (Table 7). These findings are in agreement with several studies. Rowghani, Arab, and Akbarian (2007) reported that broiler chickens fed a diet supplemented with probiotic had a significant increase in the Newcastle antibody titers than the control group. Also, King and Seal (1998) and Rowghani et al. (2007) reported that the antibody titers against ND in broilers fed with diets supplemented with probiotics containing *B. subtilis* was significantly higher at 10 days post-immunization compared to the control birds. While, our results are disagree with Thongsong, Thongsong, and Chavananikul (2008) who found that there was no significant difference in the antibody titer responses to ND between treated and untreated groups. The significant increase in antibody titer production against Newcastle ND, compared to control group may be due to immune-stimulatory and immune-modulatory effect of using biological (*B. subtilis* and *E. faecium*) supplementation. Noverr and Huffnagle (2004) indicated that some probiotic could stimulate a protective immune response sufficiently to enhance resistance to microbial pathogens. At the end of experiment, serological data from the present study showed the effectiveness of "*B. subtilis* and *E. faecium* "supplementation on immune response of birds.

4. Conclusions

Biological (*B. subtilis and E. faecium*) supplementation can be used safely as immune-stimulatory, hypolipidemic, improve haematological parameters, improve the digestibility of feed, nutrient absorption processes and protein metabolism. Moreover, as regard to *B. subtilis* and *E. faecium* (2 gm/kg diet) group displayed a great increase in body weight and feed conversion by 7.72, 12.3%, respectively, more than those of the control group. Accordingly, we recommended add *B. subtilis* and *E. faecium* to the diet of growing Hy-line chicks as one of important additive for enhancing the productive efficiency, and immunity without side effect on blood biochemical level.

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