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EFFECT OF FEEDING PROBIOTIC ON HEMATOLOGICAL, BIOCHEMICAL PROPERTIES AND IMMUNE RESPONSE IN BROILER

Salim, H.A.[a], **Abd-Allah, O. A.**[b], **and Fararh, K.M.**[a]

[a] *Clinical pathology department, Faculty of Veterinary Medicine, Benha University,* [b]*Clinical pathology department, Faculty of Veterinary Medicine, Suez Canal University, Egypt.*

A B S T R A C T

The research work was conducted on "Hubbard breeds" broilers to evaluate the effect of probiotics on leukocytes, biochemical parameters and immune response. One day old broiler chicks were randomly divided into four groups as follow: control group, probiotic fed group (NPRO), infected group with salmonella typhimurium (INT, non-treated with probiotic) and infected treated group (IPRO). Bacillus subtilis was the main constituent of the Probiotic. Results of probiotic supplementation revealed significant leukocytosis and lymphocytosis, hyperprotinemia, hyperglobinemia, and significant decrease in triglycerides, cholesterol, and glucose without significant change in AST, ALT, uric acid and creatinine. Significant increase in HI titer, phagocytic activity and phagocytic index was observed. Infection with Salmonella typhimurium showed leukocytosis, heterophilia and lymphopenia. hypoproteinemia, hypoalbuminemia, elevation of liver enzymes (AST and ALT), uric acid, and creatinine which indicate damage of liver and kidney . Immunological parameters revealed increase in serum alpha and beta globulins, and significant decrease in Phagocytic activity and Phagocytic index. In IPRO group, Bacillus subtilis decreased elevated liver enzymes, uric acid and creatinine. Probiotic also reduce the percentage of serum triglycerides and total cholesterol. From the results of this study we can concluded that probiotic Samu Biogen (bacillus subtilis) had clear impact in increasing leukocyte and immune response which appeared to reduce the damaging effect of Salmonella typhimurium infection on liver and kidney.

KEY WORDS: Bacillus subtilis, Broiler, Probiotics, Salmonella

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1. I N T R O D U C T I O N

Many probiotics raise a particular interest as products of substitution to antibiotics in order to improve performances and the health of animals [5]. Majority of the probiotic products are based mainly on *Lactobacillus acidophilus*, although other organisms such as *Streptococcus faecium*, *Bacillus subtilis* and yeast are also used [7].

The probiotics act through competitive exclusion, sticking to specific sites located in the intestinal epithelium thus decreasing colonization by pathogenic micro-organisms [8]. Supplementation with probiotics caused significant reduction in the concentrations of total lipid, total cholesterol and significant increase in globulin concentration [3].

The present study was undertaken to determine the effect of feeding probiotic (*bacillus subtilis*) on leukocytes, biochemical parameters and immune response in normal and salmonella infected broilers.

2. MATERIAL AND METHODS

2.1. *Birds*:

80 broiler chicks (Hubbred breed) one day old were used in this study. Birds were randomly divided into four equal groups, each group contain 20 birds. All birds were subjected to the ordinary vaccination program for broilers against New castle, Gumboro diseases. All birds were fed balanced commercial starter and growing rations (21% and 18% protein respectively) and water *ad-libtium*. The birds were housed in floor-pen ($0.1m^2$/bird) and clean well ventilated separate experimental rooms

2.2. Probiotic:

2.2.1. *Samu Biogens*:
Bacillus Subtilis (natto) not less than 1×10^6 CFU
2.2.2. *Dosage (per 1000 birds)*: 10-20g for one week old chicks, 30-70g for 2-18 weeks old chicks and 70-150g for chicks over 19 weeks old.

2.3. *Experimental design*:
Eighty broiler chicks of one day old were divided into 4 groups: Control Group: non-infected, non-treated. NPRO Group: non-infected, treated with *bacillus subtilis*. INT Group: infected but non-treated. IPRO Group: infected but treated with *bacillus subtilis*. Birds of INT and IPRO groups were experimentally infected at the 10^{th} day of age orally with 0.1 ml saline containing (9×10^8 CFU) *S. typhimurium*.

2.4. *Hematological examination*:
Total and differential leukocyte counts were determined according to the methods described by Bernard et al. [6].

2.5. *Biochemical parameters*:
Serum total protein, albumin, globulin, Aspartate amino transferase and Alanine amino transferase activities (AST and ALT) triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL), glucose, uric acid and creatinine were determined using commercial diagnostic kits (Stanbio, USA).

2.6. *Immunological study*:
2.6.1. Determination of different serum protein fractions by electrophoresis according to the procedure of Mahdavi et al. [17].

2.6.2. *Estimation of humeral immunity* by using HI test against ND using the standard microplate system as described by Laemmli [16].
2.6.2. Determination of phagocytic activity and phagocytic index according to Khaksefidi and Ghoorchi [14].

2.7. *Statistical analysis*:
The obtained data were compared across groups using analysis of variance (ANOVA). Data was expressed as mean (±S.E.). Level of significance of $P<0.05$ was chosen to identify the significant differences [22].

3. RESULTS

Leukogram:
There was a significant increase in total leukocyte and lymphocytes count (leukocytosis and lymphocytosis) in NPRO group without change in heterophils count compared to the control group. While there was a significant increase in total leukocyte count and heterophils count (heterophilia) and decrease in lymphocytes count (lymphopenia) in INT group compared to the control group. On the other hand, there was significant increase in lymphocyte count and significant decrease in heterophils count in IPRO group compared to INT group (Table 1).

Serum total protein, albumin, globulins and A/G ratio:
There was significant increase in Serum total protein and globulins and significant decrease in A/G ratio without change in albumin in NPRO group compared to control group. There was significant decrease in serum total protein, albumin and A/G ratio and significant increase in globulins of INT group compared to

control group. Significant increase in Serum total protein of IPRO group compared to INT group was observed (Table 2).

*Serum AST, ALT, Serum uric acid and Creatinine***:**
There was significant increase in serum AST, ALT, Serum uric acid and Creatinine in infected non-treated group compared to the control group. While at the end of experiment, there was significant decrease in serum AST and ALT in IPRO group compared to INT group (Table 3).

Serum glucose:
There was significant decrease in serum glucose in NPRO group compared to the control group. Also, there was significant decrease in serum glucose in IPRO group compared to INT group (Table 3).

*Serum Lipogram***:**
There was significant decrease in serum triglycerides, total cholesterol, low density lipoproteins cholesterol (LDL) and very Low density lipoproteins cholesterol (LDL) and significant increase in high density lipoprotein cholesterol (HDL) in NPRO group compared to the control group.

Table 1 Leukogram different groups

Group	Age	WBCS (10^3/µl)	Lymphocytes (10^3/µl)	Neutrophils (10^3/µl)	Monocytes (10^3/µl)	Eosinophil (10^3/µl)	Basophils (10^3/µl)
control	14	22.2 ± 0.58[a]	11.67 ± 0.32 [a]	7.63 ± 0.20 [a]	1.90 ± 0.16 [a]	0.17 ± 0.08 [a]	0.81 ± 0.17 [a]
	28	22.2 ± 0.86 [a]	11.82 ± 0.27 [a]	7.34 ± 0.76 [a]	1.80 ± 0.10 [a]	0.52 ± 0.05 [a]	0.70 ± 0.08 [a]
	42	22.2 ± 0.37 [a]	1157 ± 0.24 [a]	7.58 ± 0.52 [a]	2.02 ± 0.10 [a]	0.13 ± 0.06 [a]	0.88 ± 0.18 [a]
NPRO	14	24.6 ± 0.68[b]	13.44 ± 0.34 [b]	8.04 ± 1.05 [a]	1.97 ± 0.17 [a]	0.23 ± 0.07 [a]	0.90 ± 0.08 [a]
	28	25 ± 0.55 [b]	14.37 ± 0.29 [b]	6.96 ± 0.70 [a]	2.30 ± 013[b]	0.54 ± 0.07 [a]	0.81 ± 0.07 [a]
	42	24.4 ± 0.51 [b]	13.82 ± 0.26 [b]	8.61 ± 0.90 [a]	2.01 ± 0.18 [a]	0.18 ± 0.09 [a]	0.99 ± 0.12 [a]
INT	14	24.4 ± 0.51[c]	8.78 ± 0.20 [c]	13.45 ± 0.36[b]	1.37 ± 0.10 [b]	0.18 ± 0.09 [a]	0.61 ± 0.07 [a]
	28	24.6 ± 0.93 [c]	10.17 ± 0.16 [c]	11.40 ± 0.88[b]	1.84 ± 0.05 [a]	0.46 ± 0.04 [a]	0.71 ± 0.05 [a]
	42	25.6 ± 0.81 [c]	9.52 ± 0.19 [c]	13.28 ± 0.7 [b]	1.88 ± 0.08 [a]	0.21 ± 0.07 [a]	0.71 ± 0.1 [a]
IPRO	14	24.6 ± 0.75 [c]	9.87 ± 0.36 [d]	11.81 ± 0.97[b]	1.72 ± 0.06 [b]	0.19 ± 0.06 [a]	1.00 ± 0.11 [b]
	28	25.54 ± 0.98 [c]	11.48 ± 0.21 [d]	10.75 ± 0.91[b]	1.84 ± 0.07 [a]	0.59 ± 0.01 [a]	0.72 ± 0.06[a]
	42	25.4 ± 0.81 [c]	10.30 ± 0.09 [d]	11.83 ± 1.08[c]	1.98 ± 0.13 [a]	0.22 ± 0.07 [a]	1.06 ± 0.18[b]

Means (±S.E.) with different superscript (a, b, c, d) within the same column are significantly different at $p<0.05$.

Table 2 Serum biochemical parameters in different groups

Group	Age	T. Protein (g/dl)	Albumin (g/dl)	globulins (g/dl)	A/G ratio
control	14	4.7 ± 0.3[a]	2.22 ± 0.06[a]	2.48 ± 0.02[a]	0.90 ± 0.05[a]
	28	4.62 ± 0.06[a]	2.18 ± 0.06[a]	2.44 ± 0.07[a]	0.89 ± 0.05[a]
	42	4.66 ± 0.12[a]	2.04 ± 0.02[a]	2.62 ± 0.12[a]	0.78 ± 0.04[a]
NPRO	14	4.95 ± 0.05[b]	2.16 ± 0.05[a]	2.79 ± 0.09[b]	0.77 ± 0.04[b]
	28	4.94 ± 0.07[b]	2 ± 0.07[a]	2.94 ± 0.11[b]	0.68 ± 0.05[b]
	42	5.06 ± 0.07[b]	1.96 ± 0.04[a]	3.10 ± 0.09[b]	0.63 ± 0.03[b]
INT	14	4.16 ± 0.05[c]	1.36 ± 0.04[b]	2.80 ± 0.04[b]	0.48 ± 0.02[b]
	28	4.18 ± 0.06[c]	1.32 ± 0.04[b]	2.86 ± 0.07[b]	0.46 ± 0.02[b]
	42	4.22 ± 0.09[c]	1.34 ± 0.05[b]	2.88 ± 0.11[b]	0.47 ± 0.04[b]
IPRO	14	4.52 ± 0.06[d]	1.48 ± 0.09[c]	3.04 ± 0.05[b]	0.49 ± 0.05[b]
	28	4.56 ± 0.05[d]	1.76 ± 0.09[c]	2.80 ± 0.13[b]	0.64 ± 0.06[b]
	42	4.6 ± 0.08[d]	1.62 ± 0.09[c]	2.98 ± 0.14[b]	0.55 ± 0.05[b]

Means (±S.E.) with different superscript (a, b, c, d) within the same column are significantly different at $p<0.05$.

Table 3 Serum biochemical parameters in different groups

Group	Age	AST (U/L)	ALT (U/L)	Uric Acid mg/dl)	Creatinine (mg/dl)	Glucose (mg/dl)
Control	14	63.00 ± 0.71[a]	68.00 ± 0.23[a]	2.0 ± 0.23[a]	1.22 ± 0.04a	83.00 ± 0.63[a]
	28	64.20 ± 0.58[a]	69.60 ± 0.68[a]	1.80 ± 0.2[a]	1.12 ± 0.4[a]	84.0 ± 0.45[a]
	42	65.0 ± 0.32[a]	68.8 ± 0.51[a]	1.8 ± 0.24[a]	1.14 ± 0.04[a]	80.8 ± 0.58[a]
NPRO	14	62.60 ± 0.68[a]	67.6 ± 0.51[a]	1.5 ± 0.16[a]	1.24 ± 0.04[a]	79.4 ± 0.24[b]
	28	63.6 ± 0.68[a]	69.40 ± 0.81[a]	2.4 ± 0.24[a]	1.14 ± 0.05[a]	81.0 ± 0.32[b]
	42	65.4 ± 0.51[a]	68.2 ± 0.37[a]	2.2 ± 0.24[a]	1.22 ± 0.04[a]	72.6 ± 0.51[b]
INT	14	66.0 ± 0.32[b]	74.0 ± 0.32[b]	3.4 ± 0.24[b]	1.46 ± 0.05[b]	81.6 ± 0.68[a]
	28	68.8 ± 0.51[b]	74.8 ± 0.58[b]	3.4 ± 0.24[b]	1.38 ± 0.04[b]	82.0 ± 0.45[c]
	42	68.2 ± 0.37[b]	71.2 ± 0.73[b]	2.8 ± 0.37[b]	1.46 ± 0.05[b]	80.6 ± 0.24[a]
IPRO	14	64.4 ± 0.51[b]	72.6 ± 1.29[b]	3.0 ± 0.32[b]	1.38 ± 0.06[b]	76.4 ± 0.68[d]
	28	67.6 ± 0.75[b]	73.6 ± 0.75[b]	2.8 ± 0.37[b]	1.38 ± 0.04[b]	79.4 ± 0.68[d]
	42	66.2 ± o.73[a]	69.2 ± 0.37[a]	2.6 ± 0.24[b]	1.3 ± 0.05[c]	71.0 ± 1.14[d]

Means (±S.E.) with different superscript (a,b,c,d) within the same column are significantly different at $p<0.05$.

On the other hand, there was significant decrease in serum triglycerides, total cholesterol, low density lipoproteins cholesterol (LDL) and very Low density lipoproteins cholesterol (LDL) in INT, IPRO groups compared to control group (Table 4).

Gel electrophoresis:
There was significant increase in serum alpha, beta and gamma globulins of NPRO group compared to the control group. Also there was significant increase in serum alpha and beta globulins of INT and IPRO groups compared to control group (Table 5).

Haemagglutination inhibition (HI) test: There was significant increase in antibody titer in NPRO group compared to the control group. Also there was significant increase in antibody titer in IPRO group compared to the INT group (Table 6).

Phagocytic activity and Phagocytic index:
There was significant increase in Phagocytic activity and Phagocytic index of NPRO compared to the control group. While there was significant decrease in Phagocytic activity and Phagocytic index of INT compared to the control group. Significant increase in Phagocytic activity and Phagocytic index of IPRO compared to INT group was observed (Table 7).

Table 4 Lipogram in different groups

Group	Age	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL(mg/dl)	VLDL (mg/dl)
Control	14	188.4 ± 1.69[a]	214.6 ± 1.03[a]	38 ± 0.45[a]	138 92± 0.88[a]	37.68 ± 0.34[a]
	28	182 ± 0.71[a]	208.8 ± 2.06[a]	93.2 ± 0.58[a]	135.2 ± 1.04[a]	36.6 ± 0.14[a]
	42	185 ± 0.32[a]	208 ± 0.32[a]	33.5 ± 0.79[a]	137.5 ± 1.17[a]	37 ± 0.06[a]
NPRO	14	180.6 ± 0.4[b]	207 ± 0.84[b]	41.6 ± 0.4[b]	129.28 ± 0.74[b]	36.12 ± 0.08[b]
	28	178.6 ± 0.24 [b]	202.2 ± 0.37[b]	42.8 ± 0.37[b]	127.64 ± 0.87[b]	35.76 ± 0.07[b]
	42	181.4 ± 0.6[b]	204 ± 0.71[b]	39 ± 0.71[b]	128.72 ± 0.87[b]	36.28± 0.12[b]
INT	14	185.4 ± 0.81[c]	208 ± 0.32[c]	37.2 ± 0.58[a]	133.72 ± 0.76[c]	37.08± 0.16[c]
	28	185.6 ± 0.51[c]	202.8 ± 1.16[c]	39 ± 0.32[a]	127.8 ± 1.26[c]	37 ± 0.14[c]
	42	184.6 ± 1.03[a]	211± 0.95[c]	34.4 ± 1.03[a]	139.68 ± 1.75[a]	36.92 ± 0.21[a]
IPRO	14	186 ± 0.95[c]	206.4 ± 0.75[c]	38.6 ± 0.51[c]	130.4 ± 1.3[d]	37.4 ± 0.14[c]
	28	183.2 ± 0.58[d]	202.6 ± 0.68[c]	44.6 ± 0.51[d]	115.72 ± 0.29[d]	37.08 ± 0.1[c]
	42	181 ± 0.45[d]	203.8 ± 0.86[d]	34.8 ± 0.37[c]	129.52 ± 1.15[d]	36.48± 0.21[c]

Means (±S.E.) with different superscript (a,b,c,d) within the same column are significantly different at $p<0.05$.

Table 5 Serum electrophoretic pattern in different groups

Group	Alpha (g/dl)	Beta (g/dl)	Gamma (g/dl)
Control	0.393 ± 0.017^{a}	0.655 ± 0.03^{a}	1.572 ± 0.07^{a}
NPRO	$o.465 \pm 0.014^{a}$	0.775 ± 0.02^{b}	1.86 ± 0.06^{b}
INT	0.532 ± 0.016^{c}	0.82 ± 0.028^{c}	$1.528 + 0.067^{a}$
IPRO	0.547 ± 0.021^{c}	0.745 ± 0.035^{c}	1.688 ± 0.084^{a}

Means (±S.E.) with different superscript (a,b,c,d) within the same column are significantly different at $p<0.05$.

Table 6 Haemagglutination inhibition test in different groups

Group	2 weeks	4 weeks	6 weeks
Control	3.2 ± 0.12^{a}	3.4 ± 0.19^{a}	3.5 ± 0.22^{a}
NPRO	4.5 ± 0.16^{b}	4.4 ± 0.51^{b}	4.9 ± 0.33^{b}
INT	3.3 ± 0.24^{a}	3.5 ± 0.22^{a}	$3.4 \pm 0.2b^{a}$
IPRO	4 ± 0.32^{c}	4.1 ± 0.19^{c}	4.1 ± 0.33^{c}

Means (±S.E.) with different superscript (a,b,c,d) within the same column are significantly different at $p<0.05$.

Table 7 Phagocytic activity (PA) and phagocytic index (PI) in different groups

Group	PA	PI
Control	17.0 ± 0.32^{a}	1.52 ± 0.04^{a}
NPRO	20.0 ± 0.55^{b}	2.04 ± 0.09^{b}
INT	15.4 ± 0.51^{c}	1.38 ± 0.07^{c}
IPRO	17.6 ± 0.51^{d}	1.50 ± 0.04^{a}

Means (±S.E.) with different superscript (a,b,c,d) within the same column are significantly different at $p<0.05$.

4. DISCUSSION

There is increasing interest in evaluating non-medical alternatives for antimicrobials in terms of their ability to improve disease resistance, and enhance overall animal health and production in poultry. In the present study, attempts were made to evaluate the use of probiotic *(Bacillus subtilis)* and investigate the influence of such feed supplements on Salmonella infection. Concerning to leukogram, our result revealed that there was leukocytosis and lymphocytosis when probiotic used, thus could be due to immune-stimulatory and immune-modulatory effect of probiotic. While after salmonella challenge there was a significant increase in total leukocyte count and heterophils count compared to control group. There was improvement of theses result by using probiotics. These results agree with Abdollah et al. [2] who recorded that supplementation of broiler diets with bacillus probiotics caused increased leukocyte numbers. Also agree with Anderson and Stephens [4] who reported that infection with Salmonella species resulted in the development of a severe heterophilia. Our results disagree with Kalandakanond-Thongsong et al. [13] who reported that the total white blood cell count was unaffected by treatments with probiotic (*Bacillus subtilis*). Concerning serum proteins, there was significant increase in Serum total protein and globulins and significant decrease in A/G ratio without change in albumin in NPRO group compared to control group, which may be due to stimulation of immunity. There was significant decrease in Serum total protein, albumin and A/G ratio and significant increase in globulins of INT group compared to control group, These results agree with Abd El-Baky [1] who reported hyperprotinemia due to hyperglobinemia as a result of using probiotic *(pediococcus acidilactici*). These results disagree with Al-Kassie et al. [3] who showed no significant differences in total protein, albumin and globulin between treatments with probiotics. From the results of gel electrophoresis it is clear that NPRO group characterized by high immunity through increasing gamma globulins due to effect of *bacillus subtilis* on immunity. Concerning to serum liver enzymes, our result revealed that there was

no change in the AST and ALT activities in group received probiotic. These results agree with Strompfova et al. [22] who reported that no effect on serum ALT and AST activities, after addition of probiotic (*Saccharomyces cerevisiae*) compared with control treatment. On the other hand our results disagree with Santoso et al. [19] who recorded that the probiotics lower levels of ALT and AST enzymes. Our result revealed a significant increase in AST and ALT enzymes as a result of challenge with *salmonella typhimurium* which act as hepatocellular damage indicator [12]. Significant reduction in glucose level in *bacillus subtilis* group compared with control one was observed. These results agree with Al-Kassie et al. [3] who recorded reduction in glucose in groups receiving probiotics compared with the control. On the other hand our results disagree with Abd El-Baky [1] and Gheith [10] who reported no change in glucose level in broiler treated with probiotic. Concerning serum lipids, there was a significant decrease in concentration of Serum triglycerides, total cholesterol, and low density lipoproteins cholesterol (LDL) and a significant increase in high density lipoprotein cholesterol (HDL) in *bacillus subtilis* group. Our results agree with the results of Shareef and Al-Dabbagh [20] who reported that supplementation of *B. Subtilis* in broiler diets decreased triglycerides in the serum. Salarmoini and Fooladi [18] explained that microorganisms such as *Bacillus 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. Our results disagree with Kawahara et al. [13] who did not find any lowering effect of probiotics on plasma cholesterol at the 4th or the 6th week. Concerning to kidney function, our results revealed that there was no significant change in uric acid and creatinine level in NPRO group thus indicates that *bacillus subtilis* doesn't have harmful effect on kidney. These results agree with Strompfova et al. [21] who reported that no effect on serum uric acid levels by the addition of probiotic (*Saccharomyces cerevisiae*) compared with control. On the other hand there was a significant increase of uric acid and creatinine after challenge with *salmonella typhimurium* as a result of renal damage. Our results disagree with Gevaert [9] who found no increase in plasma uric acid of pigeon infected with *Salmonella typhimurium* var. *Copenhagen*. Regarding to antibody titer against Newcastle ND, there was a significant increase in antibody titer against ND as a result of administration of *Bacillus subtilis*. These results agree with King and Seal [15] who reported that the antibody titers against ND in broilers fed with diets supplemented with probiotics containing *Bacillus subtilis* was significantly higher at 10 days post-immunization compared to the control birds. Our results disagree with Kalandakanond-Thongsong et al. [13] who found that there was no significant difference in the antibody titer responses to ND among groups. Concerning to phagocytic activity and phagocytic index, there was significant increase in phagocytic activity and phagocytic index in NPRO group. These results agree with Shareef and Al-Dabbagh [20] who recorded a significant increase in the phagocytic activity of leukocytes and the phagocytic index in experimental birds after the application of *Lactobacillus* probiotic. On the other hand, there was a significant decrease in phagocytic activity and phagocytic index in infected non-treated group. These results were improved by using probiotic. From these results we can conclude that probiotic did not induce any harmful effect on liver or kidney and decreased serum lipid. Probiotic can be considered as an immune-potentiates due to stimulation of immune system and it has the ability to reduce the

adverse effect of *Salmonella typhimurium* infection in broiler chicks.

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BENHA UNIVERSITY
FACULTY OF VETERINARY MEDICINE
مجلة بنها للعلوم الطبية البيطرية

دراسة التأثيرات الباثولوجية الإكلينيكية للبروبيوتك في بداري التسمين

هادية أحمد سالم[1]، أسامة علي محمد عبد الله[2]، خالد محمد مصطفي فراره[1]

[1] قسم الباثولوجيا الاكلينيكية –كلية الطب البيطري–جامعة بنها،

[2] قسم الباثولوجيا الاكلينيكية – كلية– الطب البيطري –جامعة قناة السويس

الملخص العربى

أجريت الدراسة على بدارى التسمين "سلالة هوبارد" لتقييم تأثير البروبيوتيك على كريات الدم البيضاء، وبعض العوامل البيوكيميائية والاستجابة المناعية. تم تقسيم ثمانون طائر إلى أربع مجموعات: المجموعة الضابطة، مجموعة البروبيوتيك، المجموعة المصابة بالسالمونيلا التيفيميوريم وغير معالجة، والمجموعة المصابة بالسالمونيلا التيفيميوريم ومعالجة بالبربيوتك (باسيلس سبتيليز). كشفت النتائج عن زيادة في كريات الدم البيضاء . كما أظهرت الفحوصات الكيميائية عن وجود زيادة معنوية في مستوي كلا من بروتين الدم الكلي والجلوبولين، وانخفاض كبير في الدهون الثلاثية، الكولسترول، والجلوكوز. لم يوجد تغيير معنوي في انزيمات الكبد، حمض البوليك والكرياتينين. اظهرت اختبارات المناعه زيادة في عدد الاجسام المضاده للفيروس المسبب لمرض النيوكاسل مع وجود زياده معنويه في قدره الخلايا الاكوله (مونوسيت) علي الابتلاع. خلصت النتائج إلى أن هناك آثار مفيدة لمكمل الغذاء البروبيوتيك على الوضع الصحي لبدارى التسمين.

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