Hematological Studies on Probiotic and Synbiotic in broilers

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Abstract:
Our study was carried out to evaluate the efficacy of probiotic (BIOPELLET – S ®) and synbiotic (MT – TRON ®) on some hematological parameters in broilers either in healthy state or experimentally challenged with *E. coli* (O78). 150 day old cobb chicks were divided into 6 equal treatments (A-F) 25 chicks per each and reared for 6 weeks. Group (A) control non-treated. Groups (B & E) were received probiotic (1 g/liter drinking water). Groups (C & F) were received synbiotic (0.5 ml/liter drinking water). Groups (D, E and F) were inoculated by intranasal route with 0.5ml of PBS (phosphate buffer saline) contains 4 X 10^6 CFU *E.coli O78* organism /ml at 3 weeks old. Hematological results revealed normocytic normochromic anemia in group challenged with *E. coli*. While, groups treated with probiotic and synbiotic showed no changes in hemogram. A significant leukocytosis, lymphocytosis and heteropenia were recorded in probiotic and synbiotic treated groups compared with group challenged with *E. coli* and that not infected.
Keywords: Probiotic–synbiotic–*E.coli* O78–Chicks–Hematological studies.

Introduction:

A great interest for poultry production as a source of economy in many countries. Bad environmental conditions and diseases causing severe economic loses **(Nava *et al.*, 2005)**. Poultry are vulnerable to potentially pathogenic microorganisms in its small intestine, which competes the host for nutrients **(Engberg *et al.*, 2000)**. Also, decrease the growth performance and increase disease incidence. Antimicrobial compounds have been used to improve health of bird by reducing population of the bacteria present in the gastrointestinal tract **(Fairchild *et al.*, 2001).** Growth stimulating antibiotics induce bacterial resistance that threat human health **(Turnidge, 2004).** Therefore, using of antibiotics as growth promotors restricted at many countries and many researches were done to found an alternatives food supplement such as probiotics, prebiotics, synbiotics, organic acids and phytobiotics available to poultry **(Giedrius *et al.*, 2008).**

Probiotics are live microorganisms affect beneficially on the host by improving the microbioal balance, produce lactic acid and decrease intestinal PH, produce antibiotic like substances and compete harmful microorganisms to adhere to intestine **(Mahdi *et al.*, 2015)**

The combination of a prebiotic and probiotic as a single administration is called synbiotic, which is characterized by antimicrobial, anticarcinogenic, antiallergic and immune stimulating actions. It also improves minerals absorption, prevent diarrhea and improve digestion ***(Gruzauskas et al., 2004)***.

E.coli infection caused as adverse response against ammonia moisture dust or secondary to a serious infection and cause septicemia, enteritis, perihepatitis, pericarditis and air sacculitis ***(Leitner and Heller, 1992)***.

This study conducted to evaluate the effect of probiotic (BIOPELLET-S ®) and synbiotic (MT-TRON®) on growth performance, some hematological, serum biochemical parameters and histopathological examination of broilers either in healthy state or experimentally challenged with *E. coli* (O78).

Material and methods:

Experimental chicks:

One hundred and fifty, one day old Cobb chicks weighting 45-50 g were obtained from Ismailia- Egypt Company for poultry. Chicks were classified into 6 groups 25 birds for each and reared for 42 days. Chicks housed on floor pens. Feed and water provided adlibitum. The diet formulated to achieve the nutrient needs that suggested by ***NRC (1994)***. Birds were given programmed vaccination.

Experimental design:

Table (1):

Groups	Treatment		
	Probiotic along the experiment	Synbiotic along the experiment	*E. coli* O78 at 3 weeks old
control (A)	-	-	-
Probiotic (B) *	+	-	-
Synbiotic (C) **	-	+	-
Control infected with *E. coli* (D)***	-	-	+
Probiotic + infected with *E. coli* (E)	+	-	+
Synbiotic + infected with *E. coli* (F)	-	+	+

*Probiotic: BIOPELLETS – S ®: 1 g/liter of drinking water containing *Bacillus Subtilis* (3.0 x 10^{10} CFU/g), *Enterococcus faecium* (3.0 x 10^{10} CFU/g) and dextrose (Up to 1 kg). (According to enclosed pamphlet).

** Synbiotic: MT – TRON®: 0.5 ml/liter of drinking water containing *Lactobacillus Acidophilus* (2 x 10^8 CFU/g (50 g)), *Lactobacillus planterum* (9.8 x 10^7 CFU/gm (25g)), *Lactobacillus reutrii* (2.1 x 10^8 CFU/gm (20g)), Yeast extracts (110 g), *Bacillus Subtilis* fermentation extracts (75g), *Aspergillus oryzae* fermentation extracts (75 g), M.O.S (50 g), betaine (30 g) and riboflavin (3250mg). (According to enclosed pamphlet).

*** *Escherichia coli* strain (O78) was kindly obtained from National lab. For quality control of poultry production – Dokki – Giza.

Experimental infection (Pathogenicity test):-

Colonies of *E.coli* strain were cultured on nutrient broth for 24 hours at 37 ^oC according to ***Macfaddin (1980).*** Chicken were inoculated 0.5 ml by intranasal route at 3 weeks old, according to ***Peighambari et al. (2000)***.

Sampling:

Blood samples:

Blood Samples were collected by bird heart puncture method from 6 birds of each group at 2nd, 4th and 6th week of age on dipotassium salt of EDTA ready for use and used for hematological studies.

Hematological studies:

A -The Erythrogram studies:

Blood examination done after 2 hours of blood collection. Total erythrocyte count (TEC) was determined by Neubauer hemocytometer by using Natt and Herrick's solution according to ***Natt and Herrick (1952)***.

Packed cell volume (PCV) measured by microhematocrit tube and high speed centrifuge according to ***Coles (1986).*** Hemoglobin estimation was performed using the sonnet-hemoglobin calorimetric method after centrifugation according to ***Zijlstra (1960)***. Red blood cells indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated.

B -The Leukogram studies:

Total leukocytic count (TLC) was performed using Neubauer hemocytometer and Natt and Herrick's solution as special diluent. The differential leukocytic count (DLC) detected by blood film examination that stained by giemsa stain and examined under oil immersion lens according to ***Feldman et al. (2000).***

Statistical analysis:

The obtained data from erythrogram and leukogram were statistically analyzed for mean and standard error (SE). Statistical comparisons between the mean of different weeks was made by (SPSS version 16) program ***(Coakes et al., 2009)***. Differences between means were carried out using one way ANOVA with Duncan multiple comparison tests according to ***Snedecor and Cochran (1989)***. Dissimilar superscript letters in the same column show a significance ($p \leq 0.05$).

Results and discussion:-

Probiotic and synbiotic used to maintain chicken health status and improve digestibility. Different studies reported that probiotic and synbiotic improve intestinal microbial balance and intestinal immunity. To evaluate the efficacy of tested probiotic and synbiotic, different hematological studies were done throughout this work.

Concerning the erythrogram picture, groups (B) and (C) didn't show any significant difference in Hb concentration, RBCs countand PCV along the experiment. Such result is similar to that obtained by ***La Ragione et al. (2001); Chen et al. (2005); Dimcho et al. (2005); Mátéová et al. (2008) and Doaa and Moshira (2015)***. Also, ***Alkhalf et al. (2010)*** explained that, administration of probiotic show non-significant changes in the Hb concentrations in broilers.

Group (D) exhibited normocytic normochromic anemia. This result agreed with ***Marcel (1994)*** and ***Shimaa et al. (2015)*** who reported that experimental infection of chickens with *E.coli* (O78) induced normocytic normochromic anemia. Moreover, breakdown of erythrocytes by the effect of hemolysis enzymes produced by *E.coli*, lead to decrease in the number of erythrocytes and consequently decrease the PCV % and hemoglobin concentration ***(Feldman et al., 2000 and Justice et al., 2006).***

Regarding leukogram, groups (B) and (C) showed a significant increase ($p \leq 0.05$) in total leukocytic count (Leukocytosis) and lymphocytes at 2, 4 and 6 weeks of age and a significant decrease ($p \leq 0.05$) in H/L ratio at 2 and 4 weeks of age. Leukocytosis is due to the stimulatory effect of probiotic to the bone marrow that producing leukocyte ***(Agnes, 2001; Kopp-Hoolihan, 2001*** *and* ***Gheith et al., 2011)***. Also, ***Shoeib et al. (1997)*** found an increase in the total leukocyte count on supplementation with a probiotic containing viable lactic acid bacteria. Leukocytosis observed in group (D) at 4^{th} week is due to heterophilia and monocytosis due to tissue distruction ***(Coles, 1986)***. Leukocytosis observed at 6^{th} week, was attriputed to an increase in the lymphocytes and monocytes. The lymphocytosis may be due to antigenic stimulation. Our results partially agree with ***Barry (1998)*** who reported that**,** leukocytosis with heterophilia is a response to *Escherichia coli* airsacculitis, acute staphylococcal infection and coccidiosis in chickens. Also the result goes in accordance with ***Manimaran et al. (2003)***; ***Hanan (2002)*** *and* ***Fatma (2005)***.

Table (2): *Effect of administration of probiotic and synbiotic on Erythrogram of broilers chickens at 2 weeks of age.*

Parameters / Groups	RBCs 10^6 / µl	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %
Group (A)	2.64 ± 0.20 [a]	8.95 ± 0.32 [a]	27.28 ± 1.40 [a]	103.33 ± 1.20 [a]	33.90 ± 2.40 [a]	32.89 ± 2.15 [a]
Group (B)	2.86 ± 0.13 [a]	8.77 ± 0.10 [a]	27.00 ± 1.20 [a]	94.29 ± 0.38 [b]	30.70 ± 2.26 [ab]	32.60 ± 2.31 [a]
Group (C)	2.60 ± 0.04 [a]	8.67 ± 0.50 [a]	27.39 ± 0.60 [a]	105.40 ± 0.40 [a]	33.47 ± 1.56 [a]	31.81 ± 2.76 [a]

Values are expressed as means ± standard error (SE); n=6. Means with the same letter in the same column are non-significant at ($p \leq 0.05$)

Table (3)*:* *Effect of administration of probiotic and synbiotic on Erythrogram of broilers chickens at 4 weeks of age.*

Parameters / Group	RBCs 10^6 / µl	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %
Group (A)	2.34 ± 0.14 [ab]	8.07 ± 0.38 [bc]	26.67 ± 1.86 [ab]	114.97 ± 2.51 [bc]	34.70 ± 1.76 [cd]	30.46 ± 1.54 [b]
Group (B)	2.56 ± 0.03 [a]	8.25 ± 0.47 [ab]	27.11 ± 1.76 [ab]	105.78 ± 2.10 [c]	32.19 ± 1.92 [d]	30.46 ± 0.23 [b]
Group (C)	2.44 ± 0.13 [a]	8.84 ± 0.67 [a]	27.50 ± 1.73 [a]	112.73 ± 2.49 [bc]	36.50 ± 2.21 [cd]	32.33 ± 1.90 [ab]
Group (D)	2.00 ± 0.09 [c]	7.10 ± 0.36 [d]	22.44 ± 1.33 [c]	112.20 ± 2.20 [bc]	35.50 ± 0.56 [d]	31.70 ± 0.69 [b]
Group (E)	2.10 ± 0.27 [bc]	8.00 ± 0.32 [bc]	23.50 ± 1.58 [bc]	112.14 ± 2.85 [bc]	38.50 ± 2.20 [bc]	34.00 ± 1.94 [a]
Group (F)	2.00 ± 0.17 [c]	8.30 ± 0.23 [ab]	25.00 ± 1.53 [abc]	125.45 ± 2.14 [ab]	41.50 ± 2.88 [a]	33.50 ± 1.46 [ab]

Values are expressed as means ± standard error (SE); n=6. Means with the same letter in the same column are non-significant at ($p \leq 0.05$)

Table (4): *Effect of administration of probiotic and synbiotic on Erythrogram of broilers chickens at 6 weeks of age.*

Parameters / Group	RBCs 10^6 / µl	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %
Group (A)	2.38 ± 0.09 [a]	8.76 ± 0.41 [ab]	24.33 ± 1.16 [a]	102.30 ± 1.18 [bc]	36.89 ± 1.63 [b]	36.06 ± 1.53 [a]
Group (B)	2.36 ± 0.14 [a]	8.82 ± 0.29 [ab]	24.39 ± 1.76 [a]	103.35 ± 2.31 [b]	37.30 ± 3.13 [b]	36.37 ± 2.51 [a]
Group (C)	2.33 ± 0.05 [ab]	8.95 ± 0.38 [a]	24.50 ± 1.53 [a]	105.04 ± 2.45 [ab]	38.42 ± 2.66 [ab]	36.67 ± 0.57 [a]
Group (D)	2.22 ± 0.25 [ab]	8.52 ± 0.30 [b]	23.39 ± 2.23 [a]	105.65 ± 2.80 [ab]	38.79 ± 3.54 [ab]	36.65 ± 2.60 [a]
Group (E)	2.36 ± 0.29 [a]	8.70 ± 0.37 [ab]	23.78 ± 2.79 [a]	100.76 ± 2.60 [bc]	37.30 ± 0.61 [b]	36.58 ± 0.91 [a]

Group (F)	2.35 ± 0.09 ^{ab}	8.63 ± 0.38 ^{ab}	24.67 ± 1.45 ^{a}	104.97 ± 2.29 ^{ab}	37.00 ± 2.04 ^{b}	34.98 ±1.49 ^{ab}

Values are expressed as means ± standard error (SE); n=6. Means with the same letter in the same column are non-significant at ($p \leq 0.05$)

Table (5): *Effect of administration of probiotic and synbiotic on Leukogram of broilers chickens at 2 weeks of age.*

Parameters / Group	TLC X 10^3 / µl	Heterophils X 10^3 / µl	Lymphocytes X 10^3 / µl	H/L ratio	Monocytes X 10^3 / µl	Eosinophils X 10^3 / µl	Basophils X 10^3 / µl
Group (A)	29.99 ± 2.96 ^{b}	12.43 ± 0.40 ^{a}	14.40 ± 2.00 ^{b}	0.86 ± 0.17 ^{a}	2.53 ± 0.28 ^{a}	0.50 ± 0.04 ^{a}	0.13 ± 0.03 ^{a}
Group (B)	33.67 ± 1.67 ^{a}	12.70 ± 1.80 ^{a}	18.33 ± 1.81 ^{a}	0.69 ± 0.21 ^{b}	2.23 ± 0.27 ^{a}	0.40 ± 0.11 ^{a}	0.00 ± 0.00 ^{a}
Group (C)	35.27 ± 0.33 ^{a}	12.70 ± 0.80 ^{a}	19.7 ± 0.95 ^{a}	0.65 ± 0.10 ^{b}	2.46 ± 0.22 ^{a}	0.38 ± 0.09 ^{a}	0.00 ± 0.00 ^{a}

Values are expressed as means ± standard error (SE); n=6. Means with the same letter in the same column are non-significant at ($p \leq 0.05$)

Table (6): *Effect of administration of probiotic and synbiotic on Leukogram of broilers chickens at 4 weeks of age.*

Parameters / Group	TLC X 10^3 / µl	Heterophils X 10^3 / µl	Lymphocytes X 10^3 / µl	H/L ratio	Monocytes X 10^3 / µl	Eosinophils X 10^3 / µl	Basophils X 10^3 / µl
Group (A)	40.51 ± 2.36 ^{b}	18.80 ± 2.10 ^{b}	19.46 ± 2.83 ^{d}	0.97 ± 0.05 ^{b}	1.70 ± 0.32 ^{c}	0.55 ± 0.18 ^{bc}	0.00 ± 0.00 ^{a}
Group (B)	44.50 ± 2.03 ^{a}	18.41 ± 1.05 ^{b}	23.64 ± 0.64 ^{bc}	0.78 ± 0.09 ^{c}	1.86 ± 0.34 ^{bc}	0.59 ± 0.13 ^{bc}	0.00 ± 0.00 ^{a}
Group (C)	43.65 ± 2.65 ^{a}	18.28 ± 1.46 ^{b}	22.40 ± 0.96 ^{c}	0.82 ± 0.08 ^{c}	1.71 ± 0.36 ^{c}	1.04 ± 0.18 ^{a}	0.23 ± 0.03 ^{a}
Group (D)	46.78 ± 2.84 ^{a}	24.03 ± 2.66 ^{a}	19.29 ± 1.78 ^{d}	1.25 ± 0.13 ^{a}	2.90 ± 0.27 ^{a}	0.56 ± 0.14 ^{bc}	0.00 ± 0.00 ^{a}
Group (E)	46.12 ± 2.40 ^{a}	18.19 ± 0.91 ^{b}	24.56 ± 2.23 ^{ab}	0.74 ± 0.07 ^{c}	2.55 ± 0.31 ^{a}	0.72 ± 0.10 ^{b}	0.09 ± 0.01 ^{a}
Group (F)	44.25 ± 2.18 ^{a}	15.59 ± 1.13 ^{c}	25.90 ± 1.57 ^{a}	0.6 ± 0.03 ^{d}	2.32 ± 0.36 ^{ab}	0.44 ± 0.07 ^{c}	0.00 ± 0.00 ^{a}

Values are expressed as means ± standard error (SE); n=6. Means with the same letter in the same column are non-significant at ($p \leq 0.05$)

Table (7): *Effect of administration of probiotic and synbiotic on Leukogram of broilers chickens at 6 weeks of age.*

Parameters / Group	TLC X 10^3 / µl	Heterophils X 10^3 / µl	Lymphocytes X 10^3 / µl	H/L ratio	Monocytes X 10^3 / µl	Eosinophils X 10^3 / µl	Basophils X 10^3 / µl
Group (A)	31.92 ± 2.70 ^{c}	12.02 ± 2.30 ^{a}	17.47 ± 1.48 ^{c}	0.69 ± 0.06 ^{a}	1.60 ± 0.02 ^{b}	0.78 ± 0.04 ^{ab}	0.05 ± 0.01 ^{ab}
Group (B)	36.52 ± 2.08 ^{b}	12.88 ± 2.40 ^{a}	20.98 ± 1.27 ^{b}	0.62 ± 0.10 ^{ab}	1.83 ± 0.13 ^{b}	0.83 ± 0.12 ^{ab}	0.00 ± 0.00 ^{b}
Group (C)	36.79 ± 2.40 ^{b}	12.77 ± 1.33 ^{a}	21.10 ± 1.67 ^{b}	0.61 ± 0.08 ^{ab}	1.73 ± 0.29 ^{b}	1.03 ± 0.14 ^{a}	0.15 ± 0.03 ^{ab}
Group (D)	40.11 ± 1.71 ^{a}	12.22 ± 1.96 ^{a}	24.04 ± 1.02 ^{a}	0.51 ± 0.06 ^{b}	2.62 ± 0.16 ^{a}	1.09 ± 0.21 ^{a}	0.14 ± 0.02 ^{ab}

Group (E)	36.48 ± 2.40 [b]	12.60 ± 1.00 [a]	22.90 ± 2.26 [ab]	0.57 ± 0.10 [ab]	1.36 ± 0.20 [b]	0.33 ± 0.08 [c]	0.18 ± 0.01[a]
Group (F)	38.73 ± 1.91 [b]	12.54 ± 1.23 [a]	23.10 ± 1.10 [ab]	0.59 ± 0.04 [ab]	1.69 ± 0.21 [b]	0.62 ± 0.16 [bc]	0.19 ± 0.02[a]

Values are expressed as means ± standard error (SE); n=6. Means with the same letter in the same column are non-significant at ($p \leq 0.05$)

Conclusion:

Probiotics and synbiotics have immunomodulatory effect and subsequently they increase the healthy status and growth performance. It is better to adding probiotic and synbiotic to chicken feeding program to decrease the severity of intestinal microbial infection of pathogenic bacteria such as *E.coli.* Addition of probiotic and synbiotic have no adverse effect on blood constituents. Probiotic seemed to be superior to synbiotic. So it is recommended to use of probiotics and /or synbiotics as growth promotors and antibacterial.

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دراسات هيماتولوجية على البروبيوتك و السينبيوتك فى الدواجن

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[1] المعمل القومي للرقابة البيطرية على الانتاج الداجني – الاسماعيلية – معهد بحوث صحة الحيوان.

هدفت الدراسة الحالية التعرف على أثر استخدام الخمائر (البروبيوتيك) والخليط التازرى (السينبيوتيك) على مكونات الدم وذلك في الدجاج السليم ظاهريا والمصاب تجريبيا بميكروب الايشريشيا القولوني . اجريت هذه الرسالة على عدد مائة وخمسون كتكوت تسمين (كب) عمر يوم وتم تقسيمهم عشوائيا الى 6 مجموعات كل مجموعة تضم 25 كتكوت لمدة 6 اسابيع. المجموعة (أ) : المجموعة الضابطة السالبة بدون اى اضافات. المجموعة (ب): اضيفت الخمائر (البروبيوتيك) طوال فترة التجربة. المجموعة (ج) : اضيف الخليط التأزري (السينبيوتيك) طوال فترة التجربة. المجموعة (د) : المجموعة الضابطة الموجبة بدون اى اضافات وتم اجراء العدوى بالميكروب القولوني عند الاسبوع الثالث. المجموعة (هـ): اضيفت الخمائر (البروبيوتيك) طوال فترة التجربة وتم اجراء العدوى بالميكروب القولوني عند الاسبوع الثالث. المجموعة (و) : اضيف الخليط التأزري (السينبيوتيك) طوال فترة التجربة وتم اجراء العدوى بالميكروب القولوني عند الاسبوع الثالث. و قد تم اخذ عينات دم كامل لعمل تحاليل قياس مكون الدم . اوضحت النتائج ان الطيور المصابة تجريبيا بالميكروب القولوني تعانى من أنيميا من النوع التى تتميزبحجم خلية وكمية هيموجلوبين طبيعتين. عدم وجود تغيرات معنوية فى عدد كرات الدم الحمراء و تركيز الهيموجلوبين وحجم الخلايا المضغوطة وذلك طوال فترة التجربة فى المجموعات المعالجة بالخمائر (البروبيوتيك) والخليط التازرى (السينبيوتيك). وجود زيادة معنوية فى العدد الكلى لخلايا الدم البيضاء والخلايا الليمفاوية وانخفاض نسبة خلايا الهتيروفيل الى الخلايا الليمفاوية بينما اظهرت المجموعة المصابة بميكروب الايشريشيا القولوني والمعالجة بالخمائر (البروبيوتيك) والخليط التازرى (السينبيوتيك) زيادة معنوية فى الخلايا الليمفاوية مع انخفاض معنوى فى نسبة خلايا الهتيروفيل الى الخلايا الليمفاوية مقارنة بالمجموعة المصابة بالميكروب القولونى وبدون علاج.

المفاتيح الكلامية: الخمائر – الخليط التأزري – الميكروب القولوني – كتكوت تسمين – محفزات النمو – اضافات الاعلاف