Benha Veterinary Medical Journal 37 (2019) 197-203

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Effect of lycopene and vitamin E on hematological parameters, performance, bacterial count and histopathological alterations in *E. coli* infected broilers

Khalid M. Fararh[1], Adel M. Abd El-Aziz[2], Nagwan A. Alhelbawy[2] and Shereen Basiouni[1]

[1] *Clinical Pathology Department, Faculty of Veterinary Medicine, Benha University, Egypt.*

[2] *Animal Health Research Institute, Tanta, Egypt.*

ARTICLE INFO

Keywords

Broilers
E. coli infection
Hematological
Lycopene
Performance

Received 20/08/2019
Accepted 08/10/2019
Available On-Line 12/05/2020

ABSTRACT

The present study was conducted to evaluate the effects of lycopene and vitamin E on hematological parameters, performance, bacterial count and histopathological changes in broiler chicks experimentally infected with *E. coli O78*. A total of 120 twenty-one-day old chicks were divided into 6 groups. Group (1): was fed on commercial basal diet without any additives. Group (2): was fed on commercial basal diet plus lycopene (200 mg/kg of diet). Group (3): was fed on commercial basal diet plus vitamin E (200 mg/kg of diet). Group (4): was fed the commercial basal diet and challenged with *E. coli* O78 at 3 weeks old. Group (5): was fed on commercial basal diet plus lycopene and challenged with *E.coli* O78 at 3 weeks. Group (6): was fed on commercial basal diet plus vitamin E and challenged with *E.coli* at 3 weeks. Blood samples were taken for assaying hematological changes. Performance parameters were calculated. Samples from lung and intestine were taken under aseptic condition for *E. coli* count and other parts of these organs were taken on 10% formalin for histopathological examination. Results revealed macrocytic hypochromic anemia, leukocytosis, heterophilia, lymphocytosis and monocytosis in infected non-treated birds. It had poor performance, high liver and intestinal *E. coli* count and sever histopathological changes in lung and intestine. Treatment with lycopene or vitamin E modulated all of the above-mentioned parameters with more improvement in case of lycopene treatment therefore, they partially protect against the destructive effect of *E. coli* infection.

1. INTRODUCTION

Avian colibacillosis is an infectious disease of birds caused by *E. coli*, which is considered as one of the major causes of morbidity, mortality and heavy economic losses in the poultry industry by its association with various disease condition, either as primary or as secondary pathogen. Although *E. coli* is a normal inhabitant in the intestinal tract of birds, it is capable of producing disease under effect of predisposing factors like overcrowding, inadequate ventilation, thirst and higher temperature (Kabir, 2010). The infection of chickens with a pathogenic strain of *E. coli* is associated with poor performance in terms of body weight (BWT), body weight gain (BWG) (El-Kilany et al., 2018) and feed conversion ratio (FCR) (Teo and Tan, 2006). Considering the fact that, *E. coli* infections rise the uninvited economic costs in poultry industry and due to the high resistance of bacteria to the antibiotics and other chemical agents, it is rationale to survey the alternative approaches including natural and safe materials to limit these adverse complications (Tabatabaei et al., 2015).

Lycopene (LYC) is a bright red carotenoid pigment present in red fruits and vegetables like tomato and watermelon. It has potent antioxidant, anti-inflammatory, immune-stimulant and anticancer properties (Bayramoglu et al., 2015). LYC exhibits higher singlet oxygen quenching ability compared to β-carotene or α-tocopherol and to act as a potent antioxidant, preventing the oxidative damage of critical biomolecules including lipids, proteins and DNA. This could be attributed to its high number of conjugated double bonds (Palozza et al., 2010).

Vitamin E (VE) is a fat-soluble vitamin with immune-stimulant effects as well as antioxidant properties (Khan et al., 2012). Studies have shown that broiler feed supplemented with VE can prevent losses due to infections by *E coli* (Konjufca et al., 2004). Consequently, the aim of the present work was to study the modulatory effects of LYC and VE on hematological parameters, performance, bacterial count and histopathological changes in *E. coli* experimentally infected broilers.

2. MATERIAL AND METHODS

2.1. Experimental chicks and treatment:

100 broiler chicks, aged 21 days old and weighted 40-45 gm were used in this study. Chicks were housed in disinfected rooms and were divided into six groups of 20 birds as the following:

Group (1) was fed on commercial basal diet without additives (control negative).

Group (2): was fed on commercial basal diet plus LYC (50 mg LYCOPENE tablets, Purclinica. England) from the first

* Corresponding author: **Nagwan Abd-Elmogeeb Alhelbawy**. Animal Health Research Institute, Tanta, Egypt.

day at a dose of 200 mg/kg diet (Sahin et al., 2008) till the end of experiment (35 days).
Group (3): was fed on commercial basal diet plus VE *(*400 mg synthetic VITAMIN E capsules, Pharco. Pharmaceuticals. Egypt) daily from the first day till the end of experiment at a dose rate of 200 mg/kg diet (Niu et al., 2009).
Group (4): was fed on commercial basal diet and intramuscularly challenged with 0.3 ml of broth culture containing $3.6x10^8$ CFU *E. coli* O78 for one time (obtained from Bacteriology Department, Animal Health Research Institute) at 3 weeks old (Madian et al ., 2008).
Group 5: was fed on commercial basal diet plus LYC and were challenged with 0.3 ml of broth culture for one time at 3 weeks old.
Group 6: was fed on commercial basal diet plus VE and were challenged with 0.3 ml of broth culture for one time at 3 weeks old.

2. Sampling:
Blood samples were collected from wing vein of 5 chicks in each group 4 days and 2 weeks post infection (PI) on EDTA tube for hematological investigations. 5 gm samples from liver and intestine were taken for *E. coli* count and samples from lung and intestine were taken on 10% formalin for histopathological examination.

3. *Hematological studies:*
RBCs count, Hb concentration, PCV, Blood indices (MCV, MCH and MCHC), total and differential leukocytic count were determined using Automatic Vet Hematology Analyzer (Sysmex XT 2000 IV Corporation, KOBE, Japan)

4. Estimation of performance parameters:
The live BWT was determined by weighting 5 chicks in each group. The BWG was obtained by subtracting the initial weight from final weight. The feed intake (FI) was record then FCR was calculated (FI/BWG).

5- *E. coli* count:
Viable cell count was done by plate count method described by Cruickshank et al. (1975).

6- Histopathological examination:
Samples from lung and intestine were immediately taken after scarification of birds (2 weeks PI) and were fixed in 10% neutral buffered formalin to be examined microscopically (Bancroft and Stevens, 1996).

7- Statistical analysis:
Statistical analyses were performed by SPSS 19.0. Chicago. USA. Differences among the control and exposed groups were tested by one-way analysis of variance (ANOVA) followed by Tukey Post-hoc test for multiple comparison. All the values were expressed as mean± S.E.

3. RESULTS

3.1. Hematological results:
3,1, Erythrogram:
As shown in table (1) infected non-treated group (group 4) showed a significant decrease in Hb concentration and RBCs count 4 days and 2 weeks PI beside significantly decreased PCV 4 days PI. MCV and MCH revealed significant increases while MCHC revealed a significant decrease 2 weeks PI comparing to control birds reflecting a picture of macrocytic hypochromic anemia. LYC treated *E.coli* infected group (group 5) showed non-significant changes in all erythrogram parameters except a significant decrease in MCV 4 days and 2 weeks PI when compared to infected non-treated group. All values also not statistically differ from control values. Comparing VE treated *E.coli* infected group (group 6) to infected non-treated group (group 4) there were non-significant changes in erythrogram except MCV was significantly decreased and MCHC was significantly increased 2 weeks PI (All values not significantly differ from control values except PCV was significantly decreased).

3.1.2. Leukogram
As shown in table (2) LYC treated group (2) displayed a significant increase in leukocytic and lymphocytic count 4 days PI while VE treated group (3) revealed non-significant changes in leukogram comparing to control negative group. On the other hand, *E. coli* infected non-treated group (group 4) comparing to control group showed a significant increase in TLC and heterophils count 4 days PI while at 2 weeks PI there were a significant monocytosis. LYC treated *E.coli* infected group (group 5) when compared to infected non-treated group showed a significant decrease in heterophils count 4 days PI but at 2 weeks PI revealed a significant increase in lymphocytic count and a significant decrease in monocytic count. Comparing VE treated *E.coli* infected group to infected non-treated one there were a significant decrease in heterophils count 4 days PI and a significant decrease in monocytic count 2 weeks PI (all leukogram parameters not significantly differ from control).

2. Performance parameters:
As shown in table (3) LYC treated group revealed a significant increase in BWT, BWG and FCR while VE treated group had non-significant changes in these parameters except slight decrease in FCR comparing to control group. On the other hand, infected non-treated group (group 4) comparing to negative control group had significantly decreased BWT and BWG and significantly increased FCR. LYC and VE treated *E coli* infected groups (group 5 and 6) showed significant increases in BWT and BWG as well as significant decreases in FCR when compared to infected non-treated group. LYC treated groups had more improvement in performance than VE treated ones.

3. E. coli count:
Table (4) demonstrated that, LYC treated *E. coli* infected group (group 5) had significantly decreased liver and intestinal *E. coli* count comparing with infected non-treated group (group 4) either at 4 days or 2 weeks PI. On the other hand, VE treated *E. coli* infected group (group 6) comparing with infected non-treated group showed a significant decrease in liver *E. coli* count 4 days and 2 weeks PI, beside a significant decrease in intestinal count only at 4 days PI. Higher improvement was noticed in LYC treated birds than VE treated ones.

Table 1 Erythrogram in all groups 4 days and 2 weeks PI

	Group	TLC ($\times10^3$/µl)	Heterophils ($\times10^3$/µl	Lymphocyte ($\times10^3$/µl)	Monocyte ($\times10^3$/µl)
4 days PI	Control	2.02± 0.14 ^{b}	0.26 ± 0.11 ^{b}	1.69± 0.21 ^{b}	0.027± 0.01 ^{a}
	Lyc treated	3.33± 0.26 ^{a}	0.21± 0.06 ^{b}	3.10 ± 0.30 ^{a}	0.020± 0.01 ^{a}
	VE treated	2.91 ± 0.35 ^{ab}	0.21± 0.01 ^{b}	2.65± 0.33 ^{ab}	0.04± 0.010 ^{a}
	Infected	3.56± 0.35 ^{a}	0.90± 0.21 ^{a}	2.63± 0.47 ^{ab}	0.037± 0.01 ^{a}
	Lyc + infec	3.36± 0.25 ^{a}	0.25± 0.08 ^{b}	3.07 ± 0.21 ^{ab}	0.040 ±0.01 ^{a}
	VE+ infec	3.38 ± 0.11 ^{a}	0.20± 0.03 ^{b}	3.10± 0.12 ^{a}	0.04± 0.02 ^{a}
2 weeks PI	Control	2.99 ± 0.19 ^{ab}	0.430 ± 0.11 ^{a}	2.54± 0.18 ^{ab}	0.029 ± 0.006 ^{b}
	Lyc treated	2.92 ± 0.14 ^{ab}	0.322± 0.08 ^{a}	2.55 ± 0.11 ^{ab}	0.040 ± 0.005 ^{b}
	VE treated	3.35 ± 0.41 ^{a}	0.512± 0.07 ^{a}	2.82± 0.35 ^{ab}	0.026± 0.005 ^{b}
	Infected	2.30 ± 0.31 ^{b}	0.264 ± 0.08 ^{a}	1.79 ± 0.33 ^{b}	0.206± 0.006 ^{a}
	Lyc + infec	3.49 ± 0.37 ^{a}	0.330 ± 0.12 ^{a}	3.09± 0.27 ^{a}	0.044 ±0.009 ^{b}
	VE+ infec	2.87 ± 0.26 ^{ab}	0.300 ± 0.12 ^{a}	2.54± 0.21 ^{ab}	0.028± 0.004 ^{b}

Means (S.E.) carrying different alphabetic superscripts in the same column are statistically at p≤ 0.05 level.

Table 2 Leukogram in all groups 4 days and 2 weeks PI (Mean ± SE):

	Group	Hb (g/dl)	RBCs ($\times10^6$ µl)	PCV %	MCV (FL)	MCH (pg)	MCHC (g/dl)
4 days PI	Control	10.43±0.28 ^{a}	2.72±0.14 ^{a}	30.87± 0.87 ^{a}	113.6±2.47 ^{abc}	38.4±1.02 ^{a}	33.80± 0.49 ^{a}
	Lyc treated	10.37±0.22 ^{a}	2.76±0.09 ^{a}	30.53±0.48 ^{ab}	110.47±1.35 ^{bc}	37.5±0.71 ^{a}	33.97±0.55 ^{a}
	VE treated	9.43± 0.3 ^{ab}	2.50 ± 0.14 ^{ab}	27.7±0.93 ^{abc}	110.97±1.29 ^{bc}	37.8±0.50 ^{a}	34.07±0.37 ^{a}
	Infected	8.17± 0.22 ^{b}	2.10 ± 0.09 ^{b}	25.33 ±0.55 ^{c}	120.87± 1.09 ^{a}	38.93±0.50 ^{a}	32.23±0.45 ^{a}
	Lyc + Infect.	9.40 ± 0.21 ^{ab}	2.56±0.08 ^{ab}	28.13±0.75 ^{abc}	109.83 ±1.77 ^{c}	36.7± 0.42 ^{a}	33.43±0.43 ^{a}
	VE+ Infect.	9.03± 0.19 ^{ab}	2.38±0.05 ^{ab}	27.33±0.59 ^{bc}	114.7±1.36 ^{abc}	37.9±0.32 ^{a}	33.07±0.23 ^{a}
2 weeks PI	Control	10.5 ± 0.25 ^{a}	2.95 ± 0.1 ^{a}	29.67± 0.48 ^{ab}	100.7± 0.58 ^{b}	35.63±0.58 ^{b}	35.4± 0.21 ^{ab}
	Lyc treated	10.83± 0.32 ^{a}	2.94± 0.13 ^{a}	30.4 ± 0.31 ^{a}	103.4± 0.72 ^{b}	36.87±0.26 ^{ab}	35.63±0.24 ^{a}
	VE treated	10.13±0.44 ^{ab}	2.87±0.16 ^{ab}	29.3± 1.08 ^{ab}	102.0± 0.97 ^{b}	35.27 ±0.37 ^{b}	34.6±0.21 ^{ab}
	Infected	8.97± 0.20 ^{b}	2.35± 0.09 ^{b}	26.9± 0.31 ^{b}	114.5± 0.57 ^{a}	38.13± 0.47 ^{a}	33.33±0.32 ^{b}
	Lyc + Infect.	10.2± 0.31 ^{ab}	2.82±0.06 ^{ab}	29.67± 0.64 ^{ab}	105.37±1.76 ^{b}	36.23±0.58 ^{ab}	34.33±0.47 ^{ab}
	VE+ Infect.	9.7± 0.26 ^{ab}	2.61±0.14 ^{ab}	27.33± 0.66 ^{b}	104.73± 0.99 ^{b}	37.17±0.22 ^{ab}	35.53±0.39 ^{a}

Means (S.E.) carrying different alphabetic superscripts in the same column are statistically at p≤ 0.05 level.

Table 3 Growth performance in all groups at the end of experiment (Mean ± SE):

	Group	TLC ($\times10^3$/µl)	Heterophils ($\times10^3$/µl	Lymphocyte ($\times10^3$/µl)	Monocyte ($\times10^3$/µl)
4 days PI	Control	2.02± 0.14 ^{b}	0.26 ± 0.11 ^{b}	1.69± 0.21 ^{b}	0.027± 0.01 ^{a}
	Lyc treated	3.33± 0.26 ^{a}	0.21± 0.06 ^{b}	3.1 ± 0.3 ^{a}	0.020± 0.01 ^{a}
	VE treated	2.91± 0.35 ^{ab}	0.21± 0.01 ^{b}	2.65± 0.33 ^{ab}	0.04± 0.010 ^{a}
	Infected	3.56± 0.35 ^{a}	0.9± 0.21 ^{a}	2.63± 0.47 ^{ab}	0.037 ± 0.01 ^{a}
	Lyc + Infect.	3.36± 0.25 ^{a}	0.25± 0.08 ^{b}	3.07 ± 0.21 ^{ab}	0.040 ±0.01 ^{a}
	VE+ Infect.	3.38 ± 0.11 ^{a}	0.20± 0.03 ^{b}	3.1± 0.12 ^{a}	0.04± 0.02 ^{a}
2 weeks PI	Control	2.99 ± 0.19 ^{ab}	0.430 ± 0.11 ^{a}	2.54± 0.18 ^{ab}	0.029 ± 0.006 ^{b}
	Lyc treated	2.92 ± 0.14 ^{ab}	0.322 ± 0.08 ^{a}	2.55 ± 0.11 ^{ab}	0.040 ± 0.005 ^{b}
	VE treated	3.35± 0.41 ^{a}	0.512 ± 0.07 ^{a}	2.82± 0.35 ^{ab}	0.026± 0.005 ^{b}
	Infected	2.30 ± 0.31 ^{b}	0.264 ± 0.08 ^{a}	1.79 ± 0.33 ^{b}	0.206 ± 0.006 ^{a}
	Lyc + Infect.	3.49 ± 0.37 ^{a}	0.330 ± 0.12 ^{a}	3.09± 0.27 ^{a}	0.044 ±0.009 ^{b}
	VE+ Infect.	2.87 ± 0.26 ^{ab}	0.300 ± 0.12 ^{a}	2.54± 0.21 ^{ab}	0.028± 0.004 ^{b}

Means (S.E.) carrying different alphabetic superscripts in the same column are statistically at p≤ 0.05 level.

Table 4 Liver and intestinal E. coli count in infected groups 4 days and 2 weeks PI (Mean ± SE):

	Group	Liver count	Intestinal count
4 days PI	Infected	1.00 E09 ±2.88E07 ^{a}	4.67 E09±3.28E08 ^{a}
	Lyc + Infect.	5.67E05± 6.66E04 ^{c}	6.93E 07 ± 1.86E06 ^{b}
	VE + Infect	2.37E08 ±1.15E07 ^{b}	3.25 E08± 3.85E07 ^{b}
2 weeks PI	Infected	2.4E06 ±1.09E05 ^{a}	3.55E07 ±1.04E06 ^{a}
	Lyc + Infect.	6.00E03 ±6.67E02 ^{c}	1.93 E06 ± 1.86E05 ^{b}
	VE + Infect	3.87E05 ±3.18E04 ^{b}	2.87E07 ± 3.28E06 ^{a}

Means (S.E.) carrying different alphabetic superscripts in the same column are statistically at p≤ 0.05 level. *E0 means the number multiplied by 10?

Table 5 Growth performance in all groups (Mean ± SE):

Parameter	Control	LYC treated	VE treated	Infected	L YC + Infect.	VE + Infect.
BWT (g)	2080.0 ± 93.01^{b}	2448.0±75.17 ^{a}	2200.00±85.15^{ab}	1600.0±70.7^{c}	2100.0±79.06^{b}	2040.0±40.0^{b}
BWG (g)	2038.6± 92.53^{b}	2408.6±46.49^{a}	2158.4±84.77^{ab}	1558.5±70.64^{c}	2058.6±78.49^{b}	1998.4±39.78^{b}
FI (g)	3430.0±153.37^{a}	3463.0±67.04 ^{a}	3465.0±134.12^{a}	3090.0±136.56^{a}	3110.0±117.08^{a}	3145.0±61.67^{a}
FCR (g/g)	1.68± 0.18^{b}	1.44±0.12^{e}	1.61±0.18^{c}	1.97±0.28^{a}	1.51±0.21^{d}	1.57±0.19^{c}

Means (S.E.) carrying different alphabetic superscripts in the same column are statistically at p≤ 0.05 level.

4. Histopathological results:

Control, LYC treated and VE treated groups revealed normal histological structure of lungs (Fig 1; A, B and C respectively). Group (4) showed pulmonary emphysema and cellular exudate composed of RBCs admixed with leukocytes inside the alveolar lumen as represented in Fig (1D). On the other hand, LYC treated *E. coli* infected group (group 5) had improved picture when compared to infected non-treated birds as they had nearly normal lung except few leukocytes appeared in the pleura as seen in Fig 1E. Meanwhile, VE treated *E. coli* infected group (group 6) showed pulmonary emphysema and few leukocytic cells were seen inside the alveolar lumen as seen in Fig 1F.

Control, LYC treated and VE treated groups revealed normal histological structure of intestines (elongation of intestinal villi in group 2) (Fig 2; A, B and C). In contrast intestine of infected non-treated birds revealed edema with mononuclear leukocytic cells infiltration in the lamina propria as represented in Fig 2D. Meanwhile intestine of lycopene treated infected birds revealed few leukocytic cells infiltration as represented in Fig 2E**.** Intestine of VE treated birds in group (6) showed edema and mononuclear leukocytic cells infiltration in the lamina propria as shown in Fig 2F.

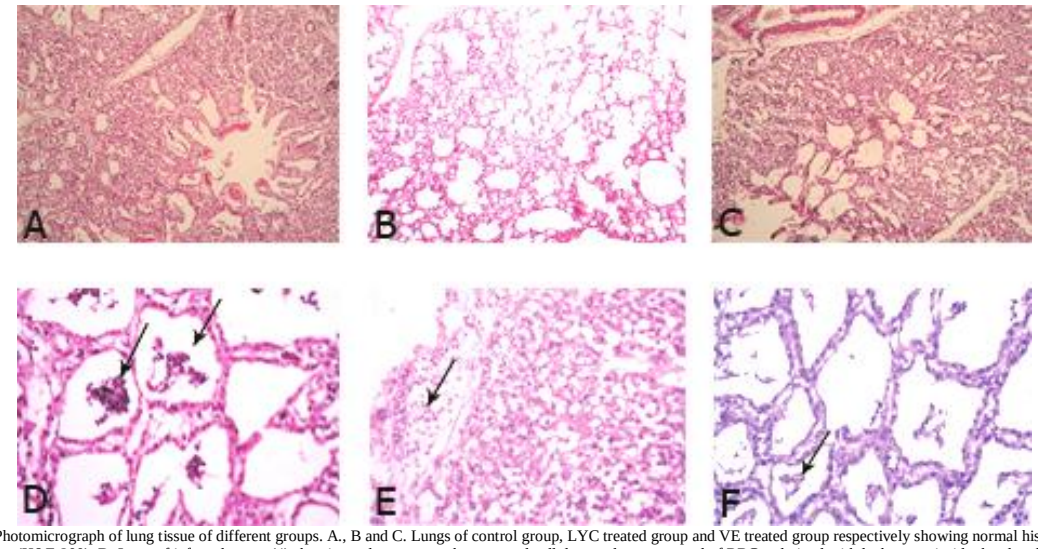

Fig 1 Photomicrograph of lung tissue of different groups. A., B and C. Lungs of control group, LYC treated group and VE treated group respectively showing normal histological structure (H&E 200). D. Lung of infected group (4) showing pulmonary emphysema and cellular exudate composed of RBCs admixed with leukocytes inside the alveolar lumen (H&E 400). E. Lung of LYC treated infected group (5) showing apparently normal structure except few leukocytes appeared in the pleura (H&E 200). F. Lung of VE treated infected group (6) showing pulmonary emphysema and few leukocytes in the alveolar lumen (H&E 400)

.

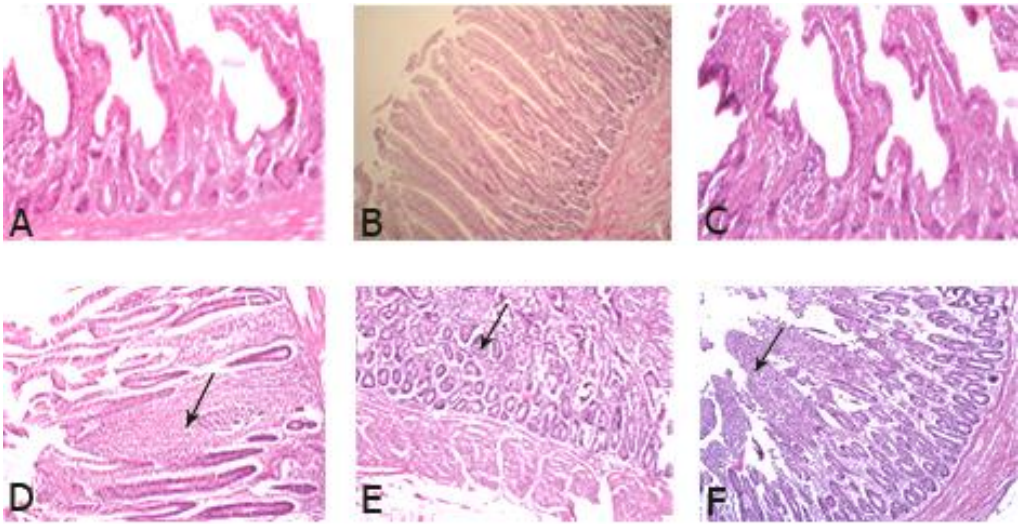

Fig 2 Photomicrograph of intestinal tissue of different groups. A, B and C. Intestines of control group (H&E 200), LYC treated group (H&E 100) and VE treated group (H&E 200) respectively showing normal histological structure with increased in the length of intestinal villi in lycopene treated group. D. Intestine of infected group (4) revealing edema with leukocytic cells infiltration (H&E 200). E. Intestine of LYC treated infected group (5) revealing few leukocytic cells infiltration in the lamina propria between intestinal glands (H&E 200). F. Intestine of VE treated infected group (6) revealing edema and mononuclear leukocytic cells infiltration in the lamina propria (H&E 200).

4. DISCUSSION

Regarding to hematological results *E. coli* infected non-treated group (group 4) showed a significant decrease in Hb concentration, RBCs count 4 days and 2 weeks PI beside significantly decreased PCV 4 days PI. MCV and MCH revealed significant increases 2 weeks PI comparing to control birds. Our results are in accordance with results obtained by Suvarna et al. (2017). On the other hand, Godbole et al. (2018) observed non-significant changes in HB, PCV in *E. coli* challenged broilers. This difference may be due to the infection dose or breed difference. These

changes occurred reflecting a picture of macrocytic hypochromic anemia. This Anemia may be due to break down of erythrocytes by hemolytic enzymes produced by *E. coli* (Justice et al., 2006). Macrocytosis may be due to increase in the reticulocytes count as a result of hemolysis (Nagao and Hirokawa, 2016). LYC treated *E. coli* infected group (5) revealed non-significant changes in erythrogram except a significant decrease in MCV when compared to infected non-treated group. Although changes didn't reach to statistical significance (except MCV), they brought the values toward normal control limits (normalization). Our results come in agreement with results of Yonar, (2017) who investigated that simultaneous treatment with LYC (10 mg/kg of fish weight for 28 days) alleviating the toxicity of cypermethrin on hematological parameters in carp. This normalization may be due to the antioxidant effect of LYC preventing lipid peroxidation in the cell membrane and maintaining cells integrity (Palozza et al., 2010). VE treated *E. coli* infected group (6) showed non-significant changes in erythrogram except a significant decrease in MCV and a significant increase in MCHC 2 weeks PI when compared to infected non-treated group. Although the changes were non-significant, it brought the values toward normal control values. These results agreed with Omonona and Jarikre, *(*2015*)* who observed that VE pretreatment (25 mg/kg BW) for 14 days in Carbendazim intoxicated African giant rats enhanced the blood parameters. This normalization may be due to the antioxidant effect of VE protecting cell membrane from oxidation and maintaining cells integrity (Kumari et al., 2013).

Regarding to Leukogram in our study LYC treated group (group 2) revealed significant leukocytosis and lymphocytosis 4 days PI. Similar results were obtained by Fachinello et al. (2018), who observed the immune-activating effect of LYC on finishing pigs. In contrast to our results Pozzo et al. (2013) found that male Hubbard broiler chicks received the basal diet supplemented with 500 mg LYC/kg for 35 days didn't showed any significant changes in leukogram. This difference may be due to dose difference. Infected non-treated group (group 4) revealed significant leukocytosis and heterophilia 4 days PI and monocytosis 2 weeks PI. Our results come in harmony with Gharieb and Youssef (2014). Indicated leukocytosis due to absolute heterophilia is mainly encountered in localized or generalized infections (Benjamin, 2013). Heterophils also contain a variety of granules that contribute to the first line host defense against bacteria, (Wakenell, 2010). Also, acute or chronic inflammatory disease is the predominant cause of monocytosis and heterophilia in pet birds because they play critical roles in defense and in maintaining homeostasis (Irizaary-Rovira, 2004). LYC treated *E. coli* infected group (group5) when compared to infected non-treated group showed a significant increase in leukocytic and lymphocytic count 2 weeks PI in addition to a significant decrease in heterophils count and monocytic count (restoration of their values toward control limits) 4 days and 2 weeks PI respectively. Our results come in agreement with Ibrahim and Banaee (2014), who observed that LYC treatment (10 mg/kg) for 28 days in diazinon exposed fish led to significant elevations in the diazinon induced decreases in WBCs and lymphocytes in addition, it decreased the elevated heterophils and monocytes. Increase TLC and lymphocytic count may be attributed to that LYC stimulates lymphocytes by increasing the production of interleukin-2 and interferon gamma, a potent activator of T lymphocytes (Yuksek et al., 2013). Also, LYC stimulate leukocytic proliferation and synthesis (Fachinello et al., 2018). VE treated *E. coli* infected group (group 6) when compared to infected non-treated group showed a significant decrease in heterophils and monocytic count 4 days and 2 weeks PI respectively (restoration of their values toward control limits). Our results come in harmony with Ibrahim and Banaee (2014), who documented that VE supplementation (50 mg/kg BW for 28 days) in the diazinon exposed Nile tilapia led to significant decreases in their monocytes and heterophils near to the control limits.

Regarding to performance parameters LYC supplemented group (group 2) had a significant increase in BWT and BWG beside decreased FCR comparing to control. Similarly, Mezbani et al. (2019) found that LYC supplementation in broiler diet (100 mg/kg diet) significantly improved BWT, BWG comparing with control. Dietary LYC could improve growth performance due to its positive effect on gut physiology (increase in villus height and villus height: crypt depth ratio) (Sun et al., 2015). Our histopathological results confirmed this theory. VE supplemented group showed only decreased FCR comparing with control group. Our results harmonized with Pompeu et al. (2018), who found non-significant differences in BWT and BWG by VE supplementation in broilers. On the other hand, Abou-Kassem et al. (2016) mentioned that dietary supplementation of VE (250 mg/kg diet) to growing Japanese quails improved live BWT and BWG at 6 weeks of age. Difference may be due to species. Infected non-treated birds (group 4) showed a significant decrease in BWT and BWG as well as significantly increased FCR comparing to control birds. LYC treated infected group (group 5) had significantly improved BWT, BWG and FCR comparing with infected non-supplemented group. Our results matched with Lee et al. (2016), who investigated that dietary LYC 10 and 20 mg/kg could mitigate the toxic effect of copper-mediated oxidation of low-density lipoprotein in broiler chicken and improve its growth performance. VE treated infected group (group 6) showed significant increases in BWT and BWG and significant decrease in FCR when compared to infected non-supplemented group. Our results come in agreement with Bou et al., (2004), who recorded that inclusion of VE at higher levels in broiler feed has resulted in positive effects on growth performance during heat stress. This effect may be due to that VE may reduce stress by suppressing the catabolic response of the body resulted in improvement of production effects (Rymer and Givens, 2005).

Regarding to results of bacterial cell count, LYC and VE supplemented infected group had significantly reduced *E. coli* count in liver and intestine comparing with infected non-supplemented group. Our results come in the same direction with Lee and Lee, (2014), who demonstrated the bactericidal effect of lycopene on *E. coli* in vitro. It was found that lycopene induced reactive oxygen species (ROS)-mediated DNA damage in *E. coli.* Also, Al-Salih et al., (2013) proved the antibacterial effect of VE (400IU) against *E. coli* in vitro. Decreased count may be due to the positive effect of VE in protecting chickens from lethal *E coli* infection by inhibiting the biosynthesis of prostaglandins, thereby activating humoral immunity and phagocytosis (Likoff et al., 1981).

Histopathological pictures of lung and intestine of birds in *E. coli* infected non-treated group came in same direction with those of El-Sheikh et al., (2007) and Tonu et al., (2011),

respectively. On the other hand, LYC treated *E. coli* infected groups had an improved histopathological picture comparing with infected non-treated group. This in agreement with Bas and Pandir, (2016), who demonstrated the protective effect of LYC against lung histopathological alterations caused by furan treatment in diabetic rats.

5. CONCULOSION

It could be concluded that the presence of Lycopene and vitamin E might be helpful in reducing the harmful effect of *E. coli* infection by maintaining optimum hematological values. LYC supplementation enhanced the performance, decreased liver and intestinal *E. coli* count and improved histopathological changes resulted from *E. coli* infection than VE supplementation. Consequently, we advise using lycopene as a supplement in chicken ration.

6. REFERENCES

1. Abou-Kassem, D.E., Mahrose, K.M. and Alagawany, M., 2016. The role of vitamin E or clay in growing Japanese quail fed diets polluted by cadmium at various levels. Animal Consortium, 10(3): 508–519.
2. Al-Salih, D.A., Bahir, A.F., Mshimesh, B.A. and Jehad, M.T., 2013. Antibacterial Effects of Vitamin E: in Vitro Study. Biotechnology Research, 7(2): 19-23.
3. Bancroft, G. and Stevens, A., 1996. Theory and Practice of Histopathology Technique. 4th Ed. Chuchill Living Stone. London, Melbourne and New York.
4. Bas, H. and Pandir, D., 2016. Protective effects of lycopene on furan-treated diabetic and non-diabetic rat lung. Biomedical and Environmental Science, 29(2): 143-147
5. Bayramoglu, G., Bayramoglu, A., Altuner, Y., Uyanoglu, M. and Colak, S., 2015. The effects of lycopene on hepatic ischemia/reperfusion injury in rats. Cytotechnology, 67(3): 487-91.
6. Benjamin, M. M., 2013. Outline of Veterinary Clinical Pathology. 3rd Ed. The Iowa State University Press Ames. Iowa, USA.
7. Bou, R., Guardiola, F., Tres, A., Barroeta, A.C. and Codony, R., 2004. Effect of dietary fish oil, α-tocopherol acetate and zinc supplementation on the composition and consumer acceptability of chicken meat. Poultry Science, 83(2): 282-292.
8. Cruickshank, R., Duguid, J.P., Marsion, B.P. and Swain, R.H., 1975. Medical Microbiology. 12th Ed. Churchill, Livingstone, Edinburgh, London and New York.
9. EL-Kilany, O., Youssef, F., Mabrouk, M. and Fares, I.M., 2018. Clinicopathological Studies on the Effect of Some Antibacterial Medicinal Plants in Broilers. Journal of Clinical Pathology Forecast. Science Forecast Publications, 1(1):1003
10. El-Sheikh, S.M., Abdel-Alim, A.F., Shabana, M.S. and El-Shazly, D.A., 2007. The effect of the concurrent use of marbofloxacin and jojoba oil in quails. Zagazig Veterinary Journal; 35(3): 27-39.
11. Fachinello, M.R., Fernandes, N.L.M., Souto, E.R., Santos, T.C., Costa, A.E.R. and Pozza, P.C., 2018. Lycopene affects the immune responses of finishing pigs. Italian Journal of Animal Science, 1-1 0.
12. Gharieb, M.M. and Youssef, F.M., 2014. Effect of *Echinacea purpurea* and garlic on growth performance, immune response, biochemical and hematological parameters in broiler chicks. Vetrinary. Medicine. Journal; 60(140): 218-228.
13. Godbole, P.V., Hajare, S.W., Poonam, B., Madhuri, H., Ingawale, M.V., Ingole, R.S., Prajakta, K. and Bhojane, N.M., 2018. Effect of curcumin on hemato-biochemical alterations after induced *E. coli* infection in broilers. Journal of Pharmacognosy and Phytochemistry, 7(1): 484-486.
14. Ibrahim, A.T. and Banaee, M., 2014. Ameliorative effect of lycopene and vitamin E on some hematological and biochemical parameters of *Oreochromis niloticus* against diazinon toxicity. Advanced Plants and Agriculture Research, 1(3): 00014.
15. Irizaary-Rovira, A.R., 2004. Avian and reptilian clinical pathology (Avian hematology & biochemical analysis). Section XI: 282–313. *In* R.L. Cowell, (ed.).
16. Justice, S., Hunstad, D., Seed, P. and Hultgren, S., 2006. Filamentation by *Escherichia coli* subverts innate defenses during urinary tract infection. Proceedings of the National Academy of Science. USA, 103(52): 19884-9
17. Kabir, S.M.L., 2010. Avian collibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health importance. International journal of Environmental Research and Public Health, 7: 89-114.
18. Khan, R., Rahman, Z., Nikousefat, Z., Javdani, M., Tufarelli, V., Dario, C., Selvaggi, M. and Laudadio, V., 2012. Immunomodulating effects of vitamin E in broilers. Worlds. Poult. Sci J, 68(01):31-40.
19. Konjufca, V.K., Bottje, W.G., Bersi, T.K. and Erf, G.F.,2004. Influence of dietary vitamin E on phagocytic functions of macrophages in broilers. Poult Sci, 83(9):1530-1534.
20. Kumari, R.R., Kumar, P. and Mondal, T.K., 2013. Effect of Vitamin E and Selenium on Hematological Parameters in Sub-acute Toxicity of Hexavalent Chromium in Broiler Chick. National Journal of Physiology, Pharmacy & Pharmacology, 3(2): 158 – 161.
21. Lee, K.W., Choo, W.D., Kang, C.W. and An, B.K., 2016. Effect of lycopene on the copper-induced oxidation of low-density lipoprotein in broiler chickens. Springerplus, 5: 389-396.
22. Lee, W. and Lee, D.G., 2014. Lycopene-Induced Hydroxyl Radical Causes Oxidative DNA Damage in *Escherichia coli*. Microbiology and Biotechnolology Journal, 24(9): 1232–1237.
23. Likoff, R.O., Guptill, D.R., Lawrence, L.M., Tengerdy, R.P., 1981. Vitamin E and aspirin depress prostaglandins in protection of chickens against *Escherichia coli* infection. American Journal of Clinical Nutrition, 34(2): 245-51.
24. Madian, K., Abd El-Ghany, W.A. and Kamel, G.M., 2008. Efficacy of pefloxacin for the treatment of broiler chickens experimentally infected with *Escherichia coli* O78: K80. 3rd Scientific Congress of the Egyptian Society for Animal Management. 28th – 29th, 94-105.
25. Mezbani, A., Kavan, B.P., Kiani, A. and Masouri, B., 2019. Effect of dietary lycopene supplementation on growth performance, blood parameters and antioxidant enzymes status in broiler chickens. Livestock Research for Rural Development, 31 (12): 3101-3112.
26. Nagao, T. and Hirokawa, M., 2016. Diagnosis and treatment of macrocytic anemias in adults. Journal of general and family medicine, 18(5): 200–204.
27. Niu, Z., Liu, F., Yan, Q. and Li, W., 2009. Effects of different levels of vitamin E on growth performance and immune responses of broilers underheat stress. Journal of Poultry Science, 8(10): 2101-2107.
28. Omonona, A.O. and Jarikre, T.A., 2015. Effect of Carbendazim Exposure and Vitamin E Supplementation in African Giant Rats. Journal of Agriculture and Ecology Research International, 4(1): 1-9.
29. Palozza, P., Simone, R., Catalano, A., Boninsegna, A., Böhm, V., Fröhlich, K., Mele, M.C., Monego, G. and Ranelletti, F.O., 2010. Lycopene prevents 7-ketocholesterol-induced oxidative stress, cell cycle arrest and apoptosis in human macrophages. J. Nutr. Biochem, 21:34–4.
30. Pompeu, M.A., Cavalcanti, L.F.L. and Toral, F.L.B., 2018. Effect of vitamin E supplementation on growth performance, meat quality, and immune response of male broiler chickens: A meta-analysis. Livestock Science, 208: 5-13.
31. Pozzo, L., Tarantola, M., Biasibetti, E., Capucchio, M.T., Pagella, M., Mellia, E., Bergagna, S., Gennero, M.S.,

Strazzullo, G. and Schiavone, A., 2013. Adverse effects in broiler chickens fed a high lycopene concentration supplemented diet. Canadian Journal of Animal Science, 93: 231-241.

32. Rymer, C. and Givens, D.I., 2005. N-3 fatty acid enrichment of edible tissue of poultry. Livestock Science. A review. Lipids, 40: 121-130.
33. Sahin, N., Orhan, C., Tuzcu, M., Sahin, K. and Kucuk, O., 2008. The effects of tomato powder supplementation on performance and lipid peroxidation in quail. Poultry Science, 87: 276-283.
34. Sun, B., Chen, C., Wang, W., Ma, J., Xie, Q., Gao, Y., Chen, F., Zhang, X. and Bi, Y., 2015. Effects of lycopene supplementation in both maternal and offspring diets on growth performance, antioxidant capacity and biochemical parameters in chicks. Journal of Animal Physiology and Animal Nutr, 99:42–49.
35. Suvarna, S., Ingole, R.S., Madhuri, H., Rathod, P.R., Hajare, S.W. and Ingawale, M.V., 2017. Ameliorative effect of *Andrographis paniculata* on hematobiochemical parameters in *Escherichia coli* induced broilers. Journal of Pharmacognosy and Phytochemistry, 6(6): 1284-1288.
36. Tabatabaei, S.M., Badalzadeh, R., Mohammadnezhad, G.R. and Balaei, R., 2015. Effects of Cinnamon extract on biochemical enzymes, TNF-α and NF-κB gene expression levels in liver of broiler chickens inoculated with *Escherichia coli*. Brazillian Journal of Vetrinary Research, 35(9):781-787.
37. Teo, A. and Tan, H.M., 2006. Effect of Bacillus subtilis PB6 (CloSTAT) on broilers infected with a pathogenic strain of *E. coli*. Journal of Applied Poultry Research, 15:229-235.
38. Tonu, N.S., Sufian, M.A., Sarker, S., Kamal, M.M., Rahman, M.H. and Hossain, M.M., 2011. Pathological study on colibacillosis in chickens and detection of *Escherichia coli* by PCR. Bangladesh Journal of Veterinary Medicine, 9(1): 17 – 25.
39. Wakenell, P.S., 2010. Hematology of chickens and turkeys. Chapter 122: 957–967. *In* D.J. Weiss and K.J. Wardrop, (eds.).Veterinary Hematology; 6th ed. John Wiley & Sons. Ames, Iowa, USA.
40. Yonar, M.E., 2017. Ameliorative Effect of Lycopene on Hematological Indices of Common Carp Cyprinus carpio, Linnaeus, 1758 Exposed to Cypermethrin. Turkish Journal of Agriculture Food Science and Technology, 5(10):1161-1164.
41. Yuksek, V., Dede, S. and Ceylan, E., 2013. The electrophoretic determination of serum protein fractions in lycopene treated experimental diabetic rats. Cell Biochemistry and Biophysics, 67:1283–12.