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Effect of Probiotics and Chelated Zinc on *E. coli* Infected Broilers

Laila Abd-El Rhman Shawkat*, Khaled Mohammed Fararh and Ayman Samir Farid****

***Clinical pathology Department, Faculty of Veterinary Medicine, Benha University, Egypt.*

ABSTRACT

This study was carried out to illustrate the effects of *Bacillus subtilis* probiotic (Baymix®) and chelated zinc (Availa zinc®) in prevention of *E. coli* in poultry farms and also to examine their effects on some blood biochemical, immunological parameters and antioxidant status in broiler chickens. One hundred and fifty one day old cobb chicks were used in this study. The chicks were divided into six groups, each group contain 25 chick, the 1st group was (control) non infected and fed balanced diet without additives, 2nd group was non-infected treated with probiotic (NPRO), 3rd group was non-infected treated with chelated zinc (NZn), 4th group was infected non treated (INT), 5th group was infected treated with probiotic (IPRO) and 6th group was infected treated with chelated zinc (IZn). The infection by *E.coli* (078) occurred at 21st day of age. Blood samples collected for biochemical parameters, phagocytic activity detection. Liver tissues collected for some antioxidant parameters (MDA and GPx), some immunological parameters interleukin-1*β* (IL-1*β*), interferon gamma (IFNγ) and interleukin-10 (IL-10), The results showed that were no significant changes in serum uric acid, creatinine, ALT and AST and significant decrease in malonaldehyde (MDA) and increases in glutathione peroxidase (GPx), phagocytic activity and immunological parameters (IL-1β, IFNγ, IL-10) in NPRO and NZn groups compared to control group and significant decreases in serum uric acid ,creatinine, ALT and AST and significant decrease in MDA and significant increase in glutathione peroxidase (GPx), phagocytic activity and immunological parameters IPRO and NZn groups compared to INT group.

From this study we could conclude that the use of *B. subtilis* probiotic and chelated zinc improve kidney and liver functions, antioxidant status, phagocytic activity and immune response in broilers chickens infected with *E. coli* (078).

Key words: probiotic, *Bacillus subtilis*, chelated zinc, *E. coli*, *broilers.*

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1. INTRODUCTION

Avian colibacillosis caused by enterotoxigenic *Escherichia coli* is a serious infectious disease occurring in different types of chickens and cause economic losses on poultry production worldwide *(He et al., 2014 and Otaki et al., 1995)*. Preventing or controlling of colibacillosis has been accompanied by the use of antibiotics, such as

colistin sulfate and enrofloxacin *(Asai et al., 2011).* However, the addition of antibiotics to the feeds, as animal growth promoters or as prophylactic measure, is a common practice in modern poultry industry and its random use can increase the resistance of many bacterial strains responsible for human diseases to antibiotic therapies *(Sarica et al., 2005).* So searching for safe alternative was necessary. This alternative approach is the use of probiotics which are products made from living microorganisms *(Patterson and Burkholder 2003). Teo and Tan (2006)* reported that *B. subtilis PB6* not only help in the maintenance of beneficial bacteria but also act as an alternative to antimicrobial growth promoters in broilers infected or uninfected with pathogenic strain of *E. coli*.Also probiotics found to be suitable for chickens to prevent oxidative stress *(Sohail et al., 2011).* The secretions of probiotics improve healthy intestinal linings and enable its interaction with pathogens as well as play a critical role in the development of immunity *(Rajput and Li 2012).*

On the other hand trace elements added in small amounts in animal feed, it supplemented as sulfates, oxides and carbonates to provide levels of minerals that prevent clinical deficiencies and allow the birds to reach their genetic growth potential *(Bao et al., 2007).* Zinc is an important trace mineral in poultry nutrition for growth, bone development, feathering, enzymes structure and function *(Batal et al., 2001).*The most important functions of zinc is related to its antioxidant role and its participation in the antioxidant defense system *(Powell 2000).* As Zinc is a cofactor of cytosolic and extracellular Zn/Cu super oxide dismutase (SOD) enzyme, which acts as ROS scavenger by catalyzing the dismutation of O_2 radical into the less harmful O_2 and H_2O_2 *(Mariani et al. 2008).*

Therefore, the aim of this study was to examine the effect of probiotic and chelted zinc on antioxidant parameters, phagocytic activity and immune responses in healthy and *E. coli* infected broilers.

2. MATERIALS AND METHODS

2.1. Birds

A total of 150, one day, old, chicks (Cobb breed) were used. They were obtained from (El-Dakhlea Poultry Company). The chicks were randomly divided into six groups (25 chicks / group). The chicks were housed in clean well-ventilated previously fumigated room. The room floor was bedded by fresh clean chopped wheat straw forming a deep litter of 3.5 cm depth, which was turned over weekly and changed every two weeks. Each group of bird was provided by suitable feeder and water. The broiler chicks were fed on well-balanced diet prepared from a corn-soybean meal based diet. Starter diet was given till the 20th day of age after that chicks were fed on grower and finisher diet which was given from the 21st day till the end of the experiment. The chicks were vaccinated against most common viral diseases, which infect the broiler chicks.

2.2. Drugs

2.2.1. *Probiotic*

Baymix® was applied in a dose of 100gm /1000 kg of diet to (NPRO) group and (IPRO) from 1st day of age till the end of experimental period. Baymix® contains *B. subtilis* (QST713)1x10^{10} CFU/g.

2.2.2. Chelated zinc

Availa Zn® 120 was added to diet in a dose 333 grams per metric ton of complete diet.

2.3. Bacterial agent

Escherichia coli (078) was kindly obtained as a gift from Centeral laboratory, Faculty of Veterinary Medicine, Benha University, Egypt.

Colonies of *E. coli* strain were grown in nutrient broth for 24 hours at 37 °C and viable number adjusted to $4x10^6$ colony-forming units CFU viable organism/ml by phosphate buffered saline (PBS) according to *Macfaddin(1980).* Chicks were inoculated with 0.5 ml by intranasal route at 21st day of age according to method described by *Peighambari et al. (2000).*

2.4. Sampling

2.3.1 Blood samples

Two blood samples were collected at1st, 2nd and 3rd week post-infection with *E. coli*. One of them on plain tubes for separation of serum to be used in biochemical tests and the other one on heparinized tube for phagocytic activity assay.

2.3.2. Tissue specimen

Specimens from liver were collected from all groups at 1st, 2nd and 3rd week post-infection with *E. coli* after sacrificing and preserved in clean dry labeled Eppendorf tubes and kept in deep freeze(-86° C) for antioxidants and interleukins assays.

2.5. Clinicopathological assays

2.5.1. Biochemical assays:

Commercial diagnostic kits used for estimation of concentration of different biochemical parameters. The diagnostic kit of creatinine was supplied by Stan Bio-Laboratory, USA. The diagnostic kits of serum uric acid was supplied by Centronic GmbH, Wartenberg, Germany. The diagnostic kits of alanine aminotransferase(ALT) and aspartate aminotransferase (AST) were supplied by Qumica Clinica Aplicada (QCA) (Spain).

2.5.2. Antioxidant parameters assay:

Commercial diagnostic kits used for estimation of concentration of hepatic lipid peroxidation and antioxidant enzymes activity. The diagnostic kits of malonaldehyde (MDA) and glutathione peroxidase (GPx) were obtained from Bio-diagnostic Company (Cairo, Egypt).

2.5.3. Phagocytic activity assay:

The phagocytic activity was detected according to *Goddeeris et al. (1986).*

2.5.4. Interleukines gene expressions:

RNA was extracted from chick liver by using RNeasy Mini Kit — for purification of total RNA from animal cells, animal tissues, and yeast, and for cleanup of RNA from crude RNA preps and enzymatic reactions (e.g., DNase digestion, proteinase digestion, RNA ligation, and labeling reaction). For PCR examination as follow, IFN-γ *(Suzuki et al., 2009),* IL-1*β* and IL10 *(Samy et al., 2015*). Primer sequences are shown in Table 1. At the end of each run, melting curve profiles were recorded.

2.6. Statistical analysis:

Statistical analysis was performed using the Statistical package for social science (SPSS) for windows (version 18.0; SPSS Inc., Chicago,III.). One way ANOVA test was used to determine significant differences among experimental groups with Duncan as a post hoc. Results are expressed as the mean ± standard error of mean (SEM). A P-value of less than 0.05 was considered significant *(Kinnear and Gray, 2006)*

3. RESULTS

Data demonstrating the effect of *B. subtilis* probiotic and chelated zinc on serum uric acid, creatinine, ALT and AST at 1st, 2nd and 3rdweek post infection are summarized in table (2, 3 and4). Chicks of NPRO and NZn groups showed no significant changes in serum creatinine, uric acid, ALT and AST compared to those of control group at 1st, 2nd and 3rd week post infection with *E. coli*. While INT group showed a significant increase in serum creatinine, uric acid, ALT and AST compared to control group at 1st, 2nd and 3rd week post infection with *E. coli.* On the other

hand IPRO group showed a significant decrease in serum creatinine and uric acid, as well as non significant changes in serum ALT and AST when compared with INT group. Also IZn group showed non significant changes in serum uric acid, ALT and AST, but showed a significant decrease in serum creatinine compared to INT group at 1st week post infection. But there were significant decrease serum creatinine, uric acid, ALT and AST in IPRO and IZn groups compared to INT group at 2nd and 3rd week post infection with *E. coli.*

Regarding to antioxidant parameters NPRO and NZn groups showed a significant decrease in MDA concentration significant increase in GPx activity compared to control group. On the other hand, there was a significant decrease in GPx activity and increase in MDA concentration in INT group when compared with control group. While IPRO and IZn groups showed a significant decrease in MDA concentration and increase in GPx activity compared to INT group at 2nd, 3rd week post infection as shown in table (5, 6).

Concerning to phagocytic activity, Phagocytic % and phagocytic index was significantly increased in NPRO and NZn groups compared to control group. While there was a significant decrease in them in INT group compared to control group.

IPRO and IZn groups revealed a significant increase phagocytic % and phagocytic index when compared with INT group as shown in table (7).

Regarding to interleukins results, IL-1β, IL-10 and IFNγ were significantly increased in NPRO and NZn groups when compared with control group. Also there was a significant increase in them in INT group when compared with control group.

While IL-1β was significantly increased by 260%, IFNγ increased by 300% and IL-10 increased by 265% in IPRO group when compared with INT group. IZn group also showed a significant increase in IL-1β by 130%, IFNγ by 240% and IL-10 by 180% when compared with INT group as shown in table (8).

Table (1): Primer sequence

Gene	Primer sequence (5'-3')	Reference
28SrRNA	GGCGAAGCCAGAGGAAACT	Suzuki *et al*., 2009
	GACGACCGATTTGCACGTC	
	(FAM) AGGACCGCTACGGACCTCCACCA (TAMRA)	
IL1B	GCTCTACATGTCGTGTGTGATGAG	Samy *et al*., 2015
	TGTCGATGTCCCGCATGA	
	(FAM)CCACACTGCAGCTGGAGGAAGCC-(TAMRA)	
IFN-ɣ	GTGAAGAAGGTGAAAGATATCATGGA	Suzuki *et al*., 2009
	GCTTTGCGCTGGATTCTCA	
	(FAM) GGCCAAGCTCCCGATGAACGA (TAMRA)	
IL10	CATGCTGCTGGGCCTGAA	Samy *et al*., 2015.
	CGTCTCCTTGATCTGCTTGATG	
	(FAM) CGACGATGCGGCGCTGTCA (TAMRA)	

Table (2): Effect of dietary supplementation of *B. subtilis* probiotic and chelated zinc on biochemical parameters of normal and *E. coli* experimentally infected broiler chickens at first week post- infection.

Groups	creatinine (mg/dl)	Uric acid (mg/dl)	ALT (U/L)	AST (U/L)
Control	0.23 ± 0.01^{c}	3.64 ± 0.27^{c}	22.50 ± 1.04^{c}	185.25 ± 3.64^{b}
NPRO	0.22 ± 0.01^{c}	3.44 ± 0.08^{c}	$23.75 \pm 0.63^{b,c}$	184.75 ± 3.54^{b}
NZn	0.22 ± 0.01^{c}	3.72 ± 0.26^{c}	21.25 ± 0.48^{c}	188.50 ± 3.50^{b}
INT	0.31 ± 0.01^{a}	6.30 ± 0.33^{a}	27.00 ± 0.41^{a}	226.50 ± 3.84^{a}
IPRO	0.28 ± 0.00^{b}	$5.25 \pm .016^{b}$	$25.50 \pm 0.87^{a,b}$	214.75 ± 6.65^{a}
IZn	0.29 ± 0.01^{b}	$5.74 \pm 0.20^{a,b}$	$26.50 \pm 1.44^{a,b}$	217.00 ± 5.70^{a}

Results are expressed as mean ±S.E.M. Different superscripts (a, b and c) within the same column indicate significant differences at $p < 0.05$. NPRO: Non infected treated with probiotic. NZn: Non infected treated with chelated zinc. INT: Infected with *E. coli* non treated group. IPRO: Infected with *E. coli* and treated with probiotic. IZn: Infected with *E. coli* and treated with chelated zinc.

Table (3): Effect of dietary supplementation of *B. subtilis* probiotic and chelated zinc on biochemical parameters of normal and *E. coli* experimentally infected broiler chickens at second week post- infection.

Groups	creatinine (mg/dl)	Uric acid (mg/dl)	ALT (U/L)	AST (U/L)
Control	0.22 ± 0.01^{c}	3.35 ± 0.19^{d}	$23.00 \pm 0.58^{b,c}$	182.75 ± 1.11^{c}
NPRO	0.22 ± 0.01^{c}	3.47 ± 0.13^{cd}	22.25 ± 0.85^{b}	183.25 ± 4.03^{c}
NZn	0.22 ± 0.01^{c}	4.18 ± 0.39^{c}	22.00 ± 1.08^{b}	182.25 ± 2.29^{c}
INT	0.32 ± 0.01^{a}	7.03 ± 0.30^{a}	29.50 ± 0.65^{a}	222.50 ± 2.90^{a}
IPRO	0.28 ± 0.01^{b}	5.15 ± 0.17^{b}	25.25 ± 1.03^{b}	210.50 ± 0.65^{b}
IZn	0.28 ± 0.01^{b}	5.02 ± 0.24^{b}	25.75 ± 1.11^{b}	202.75 ± 4.61^{b}

Results are expressed as mean ±S.E.M. Different superscripts (a, b and c) within the same column indicate significant differences at $p < 0.05$. NPRO: Non infected treated with probiotic. NZn: Non infected treated with chleated zinc. INT: Infected with *E. coli* non treated group . IPRO: Infected with *E.coli* and treated with probiotic. IZn: Infected with *E. coli* and treated with chelated zinc.

Table (4): Effect of dietary supplementation of *B. subtilis* probiotic and chelated zinc on biochemical parameters of normal and *E. coli* experimentally infected broiler chickens at third week post-infection.

Group	creatinine (mg/dl)	Uric acid (mg/dl)	ALT (U/L)	AST (U/L)
Control	0.21 ± 0.01^{c}	3.47 ± 0.18^{c}	22.00 ± 0.71^{c}	181.40 ± 1.33^{c}
NPRO	0.22 ± 0.01^{c}	3.28 ± 0.13^{c}	21.80 ± 0.66^{c}	185.75 ± 1.99^{c}
NZn	0.22 ± 0.00^{c}	3.34 ± 0.13^{c}	21.40 ± 0.51^{c}	181.80 ± 2.89^{c}
INT	0.28 ± 0.00^{a}	6.18 ± 0.48^{a}	29.80 ± 0.58^{a}	211.40 ± 4.05^{a}
IPRO	0.26 ± 0.01^{b}	5.13 ± 0.25^{b}	25.60 ± 0.40^{b}	200.40 ± 2.60^{b}
IZn	0.26 ± 0.01^{b}	4.95 ± 0.18^{b}	26.20 ± 1.1^{b}	200.00 ± 1.55^{b}

Results are expressed as mean ±S.E.M. Different superscripts (a, b and c) within the same column indicate significant differences at $p < 0.05$. NPRO: Non infected treated with probiotic. NZn: Non infected treated with chelated zinc. INT: Infected with *E. coli* non treated group. IPRO: Infected with *E. coli* and treated with probiotic. IZn: Infected with *E. coli* and treated with chelated zinc.

Table (5): Effect of dietary supplementation of *B. subtilis* probiotic and chelated zinc on antioxidant parameters of normal and *E. coli* experimentally infected broiler chickens at second week post- infection.

Groups	MDA	GPX
Control	114.90 ± 2.65^{b}	0.107 ± 0.00^{b}
NPRO	88.30 ± 1.12^{c}	0.124 ± 0.00^{a}
NZn	72.37 ± 4.52^{d}	0.127 ± 0.00^{a}
INT	134.47 ± 2.12^{a}	0.082 ± 0.00^{c}
IPRO	91.30 ± 1.94^{c}	0.103 ± 0.00^{b}
IZn	93.57 ± 1.94^{c}	0.101 ± 0.00^{c}

Results are expressed as mean ±S.E.M.

Different superscripts (a, b, c, and d) within the same column indicate significant differences at $p < 0.05$.NPRO: Non infected treated with probiotic. NZn: Non infected treated with chelated zinc. INT: Infected with *E. coli* non treated group. IPRO: infected with *E. coli* and treated with probiotic. IZn : Infected with *E. coli* and treated chelated zinc.

Table (6): Effect of dietary supplementation of *B. subtilis* probiotic and chelated zinc on antioxidant parameters of normal and *E. coli* experimentally infected broiler chickens at third week post- infection.

Groups	MDA	GPX
Control	130.36 ± 1.10^{b}	0.11 ± 0.00^{d}
NPRO	84.39 ± 5.43^{d}	0.12 ± 0.00^{c}
NZn	86.01 ± 6.21^{d}	0.20 ± 0.00^{a}
INT	165.78 ± 4.48^{a}	0.10 ± 0.00^{e}
IPRO	112.75 ± 8.47^{c}	0.12 ± 0.00^{d}
IZn	95.58 ± 1.15^{d}	0.13 ± 0.00^{b}

Results are expressed as mean ±S.E.M.

Different superscripts (a, b, c, d and e) within the same column indicate significant differences at $p < 0.05$. NPRO: Non infected treated with probiotic. NZn: Non infected treated with chelated zinc. NT: Infected with *E. coli* non treated group. IPRO: infected with *E. coli* and treated with probiotic. IZn: infected with *E. coli* and treated chelated zinc.

Table (7) :Effect of dietary supplementation of *B. subtilis* probiotic and chelated zinc on phagocytic activity of normal and *E. coli* experimentally infected broiler chickens at third week post- infection.

Groups	Phagocytic (%)	Phagocytic index
Control	62.75 ± 1.11^{c}	6.05 ± 0.10^{c}
NPRO	75.75 ± 0.85^{a}	7.45 ± 0.06^{a}
NZn	68.75 ± 1.11^{b}	6.73 ± 0.14^{b}
INT	39.25 ± 1.11^{f}	3.83 ± 0.14^{f}
IPRO	57.75 ± 0.85^{d}	5.60 ± 0.11^{d}
IZn	53.50 ± 0.65^{e}	5.15 ± 0.06^{e}

Results are expressed as mean ±S.E.M

Different superscripts (a, b, c, d, e and f) within the same column indicate significant differences at $p < 0.05$. NPRO: Non infected treated with probiotic. NZn: Non infected treated with chelated zinc. INT: Infected with *E. coli* non treated group. IPRO: infected with *E. coli* and treated with probiotic. IZn: Infected with *E. coli* and treated with chelated zinc.

Table (8): Effect of dietary supplementation of *B. subtilis* probiotic and chelated zinc on relative cytokines expression (IL-1*β,* IFNγ and IL-10) of normal and *E. coli* experimentally infected broiler chickens at third week post- infection.

Groups	IL-1*β*	IFNγ	IL-10
Control	1.00 ± 0.00^{e}	1.00 ± 0.00^{d}	1.00 ± 0.00^{d}
NPRO	3.12 ± 0.13^{c}	5.80 ± 0.45^{c}	3.99 ± 0.49^{c}
NZn	2.20 ± 0.25^{d}	4.13 ± 0.46^{c}	3.76 ± 0.52^{c}
INT	3.60 ± 0.29^{c}	4.70 ± 0.3^{c}	4.30 ± 0.15^{c}
IPRO	9.40 ± 0.33^{a}	14.90 ± 1.21^{a}	11.40 ± 0.4^{a}
IZn	4.80 ± 0.26^{b}	11.30 ± 0.77^{b}	8.09 ± 0.66^{b}

Results are expressed as mean ±S.E.M.

Different superscripts (a, b, c, d and e) within the same column indicate significant differences at $p \leq 0.05$. NPRO: Non infected treated with probiotic. NZn: Non infected treated with chelated zinc. INT: Infected with *E. coli* non treated group . IPRO: infected with *E. coli* and treated with probiotic. IZn: infected with *E. coli* and treated chelated zinc.

4. DISCUSSION

In last decades, antibiotic growth promoters have been widely used in poultry feed to improve growth performance and animal health status *(Baurhoo et al., 2009).* In European Union (EU) the use of antibiotics as growth promoters was banned in 2006 to promote the careful use of antibiotics *(Anadon et al.,* 2006).The ban of antibiotic growth promotors usage lead to increase interest to find alternatives that have the ability to improve disease resistance and enhance animal performance. These alternatives were probiotics *(Ganguly, 2013).*

In the present study, there were significant increase in serum creatinine and uric acid in INT groups compared to control. Similar findings obtained by *Petrov et al., (2011*). This occur due to kidney damage by toxic substances secreted by Entero-Pathogenic *E, coli* (EPEC) which cause damaging the endothelium of nephrons *(Okerman 1989).*

While our results showed non significant changes in serum creatinine and uric acid in NPRO groups compared to control. These results come in agreement with *Shareef and Al-Dabbagh., (2009)* and *Tang et al., (2017)* who found that probiotic supplementation had no effect on serum uric acid compared with control, and in consistent with *Hatab et al., (2016*) who found that there were non significant changes in serum creatinine of layer chickens treated with *B. subtilis and E. fascium* probiotics. While IPRO group showed improvement in kidney functions (significant decrease in serum creatinine and uric acid) compared to INT group. This may be attributed to the antioxidant effects of probiotic *(Wang et al., 2009).*

Concerning to antioxidant parameters our results showed an increase in

malonaldehyde (MDA) concentration and decrease in glutathione peroxidase (GPx) activity in INT group compared to control group. Similar findings reported by *Zheng et al., (2016)* who found there were increase in serum MDA and decrease in GPx activity in liver of broiler chickens experimentally infected by lipopolysaccharide (LPS) of *E. coli O55: B5.* These findings because LPS generates elevated levels of ROS which destroy the balance between pro-oxidant and antioxidant system and contribute to cell and tissue oxidative injuries.

On the other hand, present results showed significant decreases in MDA concentration and increases GPx Activity in NPRO group compared with control group. Also IPRO group showed improvement in them compared with INT. These results come in harmony with those obtained by *Wang et al. (2009)* and *Aluwong et al. (2013)* who found that there were increases in GPx activity in serum of pigs and chicks fed on probiotic supplemented diets. Also, agree with Rajput et al., (2013) who found that there was a decrease in serum MDA in broilers fed on diet supplemented with *Bacillus Subtilis* and *Saccharomyces boulardii* probiotics, As these micro-organisms could help in oxidation resistance by scavenging hydroxyl radicals and increasing antioxidant capacity *(Wen et al., 2011).*

Regarding to NZn group also showed a significant increase in GPx activity and decrease in MDA concentration compared with control group and same results reported in IZn group when compared with INT group. These results are in accordance with *Bun et al.,(2011)* who found significant increases in plasma GPx activity in male broiler fed on diet supplemented with organic zinc, and in accordance with *Ahmadi et al., (2014) and Sahin et al., (2005)* reported decreases in MDA concentration in serum of broilers and Japanese quails fed on zinc supplemented diets. These findings due to antioxidant action of zinc which act by competing with iron and copper to bind to the cell membrane and decrease the production of free radicals *(Tate et al., 1999).* Also zinc induces synthesis of metallothieonin (cystiene rich protein) which acts as free radicals scavenger *(Bales et al., 1994).*

Regarding to phagocytic activity, Our results of INT group showed that a significant decrease in phagocytic activity when compared with control group, similar findings reported by *Emery et al., (1991)* who found that turkeys experimentally with *E. coli* LPS showed significant decrease in phagocytic activity of systemic and pulmonary macrophages. And partially agree with *Higgins et al., (2007)* reported enhanced phagocytic activity in *salmonella*-infected broiler chicks fed on lactobacillus based DFMs diets. This is as bacterial endotoxin as LPS cause pathophysiological effect and induce suppression of antibacterial defense mechanism *(Baltzer et al., 1991).*

The *E. coli* infected and *L. rhamnosus* HN001-fed mice were significantly higher compared with the *E. coli* infected control mice. Improvement in phagocytic activity indicated that *Bacillus subtilis* based direct fed microbials (DFMs) promote maturation and activation of macrophages *(Lee et al., 2011).*

Concerning to NZn group showed a significant increase in phagocytic activity compared with control group. Also IZn group showed an improvement when compared with INT group. These findings come in harmony with *Ferket and Qureshi (1992) and Kidd et al., (1994)* who found that dietary zinc methionine supplementation in the turkey diet increase activity of macrophages phagocytosis in young turkeys.

Concerning to interleukins expression Concerning to interleukins expression,

Cytokines are immune regulatory peptides with relatively small molecular weights that participate in innate and adaptive host immune responses *Lee et al., (2015).*

The present study revealed also a significant increase in IL-1*β,* IFN*γ and* IL-10 in liver of INT group compared with control group. These results come in agreement with *Sadeyen et al. (2014)* who reported that there was up-regulation in IL-1*β,* IFN*γ* and IL-10in bird challenged with *E. coli*-O78-H9 strain compared to control birds. This may be due to *E. coli* infection stimulates the phagocytic cells of the innate immune system resulting in release of IL-10.

On the other hand, the present study showed an up-regulation in IL-1*β,* IFN*γ and* IL-10 in NPRO compared to control group. Also IPRO group showed a significant increase in IL-1*β,* IFN*γ*andIL-10compared to INT group. These results are in agreement with *Rodèguez-Lecompte et al., (2012)* reported a slight greater expression of IL-10 and IFNγ in cecal tonsils of chicks fed on diet contain probiotic mixture. *Rajput et al., (2013)* have reported that *Bacillus*-based DFM significantly induced inflammatory and anti - inflammatory cytokines in jejunum and ileum of broiler chickens. Also the *Guo et al., (2016)* who found that up regulation of IL-1*β,* IFN*γ and* IL-10in the thymus of duck fed *bacillus subtlis* supplemented diets. these findings may be attributed to that the innate immunity system is highly conservative, stimulated by the activation of PRRs (Pattern Recognition Receptors) as MDA5 and TLR3, The activation of PRRs induces the expression of cytokines and antiviral protein which play an essential role in defending against pathogenic microorganisms *Barbalat et al.,(2011).*

Also the present study results showed up-regulation of IL-1*β,* IFN*γ and* IL-10in group NZn group compared to control group. and IZn showed significant increase in them when compared with these results are in accordance with *Sundaresan et al., (2008),*who found up-regulation in pro-inflammatory cytokines as IL-1*β,* IL-6, TNF-α and IFN-γ in ovary and oviduct of chicken fed diets supplemented with zinc.

Previous studies of chicken cytokines suggest that they have similar properties to their mammalian counterparts *Cheeseman et al., (2007).* In the chicken, inflammation is controlled by IL-1*β (Weining et al., 1998)* and IL-6 *(Schneider et al., 2001).* The Th1 cytokines include IFN-γ *(Digby and Lowenthal 1995)* and IL-2 *(Sundick and GillDixon, 1997).*

In mammals zinc is found to induce IL-1*β,* IL-6 and TNF-α by direct interaction with monocytes (Well-Inghausen et al., 1996), the stimulation of of T-lymphocytes by zinc appears to occur via monocyte-released IL-1*β* and cell contact *(Boscolo et al., 2005).* Previous study revealed that zinc activates several signaling pathways that interacts with Signal transduction of toll-like receptors in monocytes and thereby induces the secretion of pro-inflammatory cytokines *(Haase and Rink 2007).*

5. CONCLUSION

From the present study, we can conclude that probiotics and zinc dietary supplementation used as growth promotors, improve antioxidant activity and act as immune stimulatory substances by enhancing cellular immunity and anti-inflammatory responses so they have a role in controlling *E. coli* infection in broilers.

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