

## Immune Response with Some Blood Parameters of Broiler Chicks Fed on Rations contained Different Levels of Zinc and Folic Acid.

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### Abstract:

The research was conducted to investigate the effect of Zinc and Folic Acid on Immune status as well as some blood parameters of broiler chickens. A total number of 400, one day old broiler chicks ( Ross 308 ) were distributed on 8 treatments, and fed on rations contained different levels of Zinc and Folic acid with their combination. Chicks were raised in to a semi closed house in the poultry farm which belongs to animal production Techniques in the Technical Colloge Al-Mussayab during the period which extended from the 2<sup>nd</sup> of march till the 12<sup>th</sup> of april 2018. Results revealed a highly significant surpass ( $p < 0.01$ ) of antibody titer against Newcastle disease, Infectious Bursal Disease and Infections Bronchitis in broilers supplemented with Zinc or combination of Zinc with folic acid. Moreover there was a significant amelioration ( $p < 0.05$ ) of Paced Cell volume with highly significant ( $p < 0.01$ ) amelioration of Heterophil / lymphocyte index as well as a highly significant elevation ( $p < 0.01$ ) of total plasma protein with highly significant ( $p < 0.01$ ) reduction of glucose, cholesterol and Triglycerides in the plasma.

### الاستجابة المناعية وبعض معايير الدم لفروج اللحم المغذى على علائق احتوت على مستويات مختلفة من الخارصين وحامض الفوليك

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### المستخلص:

أجري البحث لغرض دراسة تأثير الخارصين وحامض الفوليك في الحالة المناعية وبعض الصفات الدمية لفروج اللحم، استعمل بالبحث 400 فرخاً من أفراخ اللحم من سلالة روز (Roos) 308 بعمر يوم واحد وزعت على ثمانية معاملات وغذيت على علائق احتوت على مستويات مختلفة من الخارصين وحامض الفوليك وخليطهما. ربيت الأفراخ في قاعة شبة مغلقة تابعة الى قسم تقنيات الانتاج الحيواني في الكلية التقنية المسيب خلال المدة من 2 من آذار ولغاية 12 من نيسان 2018. أوضحت نتائج البحث الى تفوق عالي المعنوية ( $p < 0.01$ ) في مستويات المعيار الحجمي للأضداد الموجه ضد مرض النيوكاسل ومرض التهاب غدة فابريشيا المعدي ومرض التهاب الشعب الهوائية المعدي في معاملات إضافة الخارصين أو خليط الخارصين وحامض الفوليك فضلاً عن التحسن المعنوي ( $p < 0.05$ ) في قيم النسب المئوية لحجم خلايا الدم المرصوصة وتحسن عالي المعنوية ( $p < 0.01$ ) لدليل النسبة بين الخلايا المتغيرة / الخلايا اللمفية، وأرتفاع عالي المعنوية ( $p < 0.01$ ) في مستوى البروتين الكلي البلازما وانخفاض عالي المعنوية ( $p < 0.01$ ) في مستوى الكلوكوز والكوليستيرول والكليسيريدات الثلاثية في بلازما الدم.

### Introduction:

Researchers had been started to look for alternative antibiotic growth promoters for poultry, for this reasons vitamins and minerals are used to promote growth of birds [29]. One of the most important vitamins is vitamin B with all it's forms. Folic acid is termed Vitamin B<sub>9</sub>, this vitamin is important for poultry since it enhances embryogenesis and it improves growth of chicks as a coenzyme involved in biosynthesis of amino acids and deoxy nucleotides needed for DNA replication

and repair [14]. Scott [27] reported that folic acid is involved in participation of haemopoiesis, as well as it plays an important role in brain feeding with enhancement of regulation sophistication of nerve cells in chickens[30]. Zinc is a trace element that is necessary for normal growth and maintenance of birds. Moreover Zinc participates in constructing of more than 200 metallo enzymes, thus it plays an important role in bone formation, feathering, appetite regulation and homeostasis in all avian species [26].

Hudson [15] reported that Zinc is involved in biosynthesis of nucleic acids ( RNA and DNA) as well as gene expression. Zinc is used as organic additives in poultry rations such as Zinc oxide and Zinc sulfate but Zinc oxide is more accustomed to be used as feed additive for poultry [4&15].

The current study is aimed to use Zinc and folic acid with their combination at different levels in the rations in order to highlight on their effect on the immune status and some hematological traits of broilers.

### Materials and methods:

A total number of 400 one day old chicks (Ross 308) were used in the research, chicks were randomly allotted on eight equal treatments, each treatment included 50 chicks, each treatment contained two equal replicates. Chicks were raised in to a semi closed house in the poultry farm which belongs to animal production Techniques in the Technical College Al-Mussayab during the period which extended from the 2<sup>nd</sup> of March till the 12<sup>th</sup> of April 2018. The house was disinfected, fumigated and supplied with all requirements need for rearing before housing of chickens. All chick treatments fed on the starter ration which contained 22% crude protein and 3078 Kilo calorie/kg feed until 21 day of age, then the finisher ration was used, it contained 20% crude protein and 3125 Kilo calorie/kg. Starter and finisher rations were formulated according to [21]. Feed supplements were blended with the starter and finisher rations at different levels so that T1: with out any supplement (control), T2: 50 mg/kg folic acid, T3: 100 mg/kg folic acid, T4: 150 mg/kg folic acid, T5: 50 mg/kg Zinc, T6: 50mg/kg folic acid plus 50 mg/kg Zinc, T7: 100 mg/kg folic acid plus 100 mg/kg Zinc, T8: 150 mg/kg folic acid plus 50 mg/kg Zinc. Folic acid is a yellow powder( 996 mg/gm) was produced by DSM company, Switzerland. Mean while Zinc was a white powder( 99.5% Zinc oxide) produced by CHN Company, China. Folic acid and Zinc oxide were obtained from Al- Bayder Bureau, Baghdad.

Chicks in all treatment feed *adlib*. During the first week of age till the end the experiment at 42 day of age. Vaccination

program was applied to all chicks in treatments, chicks were vaccinated against Newcastle Disease(ND) at the 10<sup>th</sup> day and 20<sup>th</sup> day of age by drinking water and at the 12<sup>th</sup> day of age they were vaccinated against Infectious Bronchitis(IB). at 12 day of age. Vaccination against Infectious Burasli Disease (IBD) was applied at 15 day of age by drinking water.

Blood samples were collected from the brachial veins of chicks in all treatments at 35 day of age, Five blood samples were collected from each replicate by using test tubes with out anticoagulant, blood samples were put in the centrifuge (3000 RPM) for 15 minutes in order to get serum samples. Ebindroff tubes were used to keep serum samples frozen till application of the serological tests. Antibody titers against ND, IBD and IB were evaluated by Enzyme linked Immune Sorbent Assay (ELISA) according to [28].

Blood samples were also collected at 42 day of age by using test tubes containing EDTA to evaluate packed cell volume (PCV) by using heparinized capillary tubes and hematocrit [11]. Blood smears were stained by Wright Gimsa and Heterophil Lymphocyte ratio was calculated by a microscope X100 OI emersion [5].

Plasma samples were obtained to evaluate some biochemical tests. Total protein was evaluated by using Biolaba kit [31].

Glucose was evaluated [3]. Mean while cholesterol and Triglycerides were evaluated according[10]. Statistical analysis of results was applied according to the completely Randomized Design (CRD) by the Statistical program, SAS [24]. The significant differences between treatments were calculated according to Duncan [7].

### Results and Discussion:

Result of statistical analysis (Table 1) of immune response which are expressed as antibody titers of Newcastle disease were highly significant ( $p < 0.01$ ), this antibody titer was at lower level, this means it was 620 in the control treatment, but it was noticed an amelioration of this titer when folic acid is used. Further more this amelioration was the best by using Zinc, but amelioration of ND titer was more obvious in treatments of

folic acid and Zinc combination, it attained 1000, 1030 and 1080 in the 6<sup>th</sup>, 7<sup>th</sup>, 8<sup>th</sup> treatments respectively.

It was noticed that there was highly significant ( $p < 0.01$ ) differences between treatments regarding the antibody titer of IBD,

the lowest level of this titer was at the control treatment which was 410, but Zinc supplementation induced an amelioration of this titer but the combination of folic acid with Zinc

**Table 1: Antibody titers against ND, IBD and IB in broilers fed Zinc and folic acid supplements ( Mean± SE).**

Treatments	Antibody titers		
	ND	IBD	IB
T1:(control)	20.14±620 e	19.12±410 c	28.30±350 d
T2: (50 mg/kg folic acid)	9.12±630 e	14.20±440 c	20.10±430 c
T3: (100 mg/kg folic acid)	13.33±660 de	12.06±460 c	10.16±480 c
T4: (150 mg/kg folic acid)	12.14±690 d	10.20±480 c	20.11±480 c
T5: (50 mg/kg Zinc)	18.0±820 c	18.20±660 b	20.10±650 ab
T6: (50 mg/kg Zinc+50 mg/kg folic acid)	20.12±1000 b	60.10±930 a	10.20±680 ab
T7:( 50 mg/kg Zinc+100 mg/kg folic acid)	12.80±1030 ab	50.10±960 a	18.20±680 ab
T8: (50 mg/kg Zinc+150 mg/kg folic acid)	4505±1080 a	45.50±980 a	22.50±720 a
significance	**	**	**

-Means carrying different letters differ significantly in between.

\*\*( $p < 0.01$ )

caused highest levels which were 660, 930, 960, and 980 in the 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> treatment respectively. It was evidenced from table 1 that antibody titers of IB was 350 in the control treatment, then it was elevated to 430 in the second treatment and 480 in the third treatment and 490 in the fourth treatment, mean while it attained 650 in the fifth treatment which was supplemented with Zinc, this antibody titer attained 680 in the 6<sup>th</sup>, and 7<sup>th</sup> treatment. The highest antibody titer of IB was the highest level which was 720 in the 8<sup>th</sup> treatment, the differences of antibody titer of IB was highly significant ( $p < 0.01$ ) between treatments. The amelioration of the immune

response of bird which fed rations containing a combination of Zinc and folic acid may be attributed to the synergistic effect of both constituents, Since Zinc is an essential element which contributes in constructing of immunoglobulins specially IgG and it increases activity of macrophage phagocytosis in birds [6]. Moreover folic acid which plays an important role in improving the immunological response of birds since folic acid is required for methylation of homocysteine to form methionine as well as it participates in biosynthesis of immune proteins with a direct stimulation of the

immune system to produce antibody against ND, IBD and IB.

Additionally folic acid is involved directly in Interlukins formation which represent defensive lines against diseases [8].

Synergistic effect of Zinc with folic acid in the 6<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> treatments may be also attributed to the high levels of Zinc which stimulates activity of macrophages which represent a first defensive line against diseases, this was in consistent with [13]. The improvement of the immune response in the Zinc treatments may be referred to stimulation of T-helper cells and lymphocytes forming interferons which induce an immune response [9].

Results indicated also a significant effect ( $p < 0.01$ ) of the treatment on PCV trait (Table 2), so the highest value of PCV attained 33.60% in the 6<sup>th</sup> treatment, mean while the lowest value was in the first (control) treatment which was 27.50%.

The obvious amelioration of PCV in the 6<sup>th</sup> treatment may be attributed to Zinc with folic acid. Zinc plays an important role in formation of erythrocytes through increasing gene expression for biosynthesis of Erythropoietin which directly affects cellular division of erythroid and converting them to erythrocytes stroma resulting in increasing of PCV [28].

Moreover folic acid which is an essential element stimulating cellular division and methylation and biosynthesis of amino acid thus it increases the PCV [12 and 2].

It was indicated that Zinc and folic acid caused a highly significant effect ( $p < 0.01$ ) of H/L index, the highest index was 0.58 in the control treatment, then it was improved to become less than this value in other treatments specially the 6<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> treatments. Index of H/L is an important indication for evaluation of the health of birds since H/L index reveals the health of birds with the level of stress. Increased H/L indicates a stressful condition [1].

Increased H/L may be caused by increased secretion of Adrenocortical Hormone ACTH from the pituitary gland, this hormone causes reduction of lymphocytes with increased Heterophils [16].

The obvious improvement of H/L may be attributed to the effect of folic acid or to the Synergistic effect of Zinc with folic acid.

Folic acid stimulates the increased number of lymphocytes since it participated in constructing of lymphocytes with their division, so it increases lymphocyte numbers. Additionally, Zinc participate in constructing of Bursa gland in birds which plays an important role in amelioration of the immune response [12 and 19].

**Table 2: Effect of supplement Zinc with folic acid on PCV and H/L in broilers. ( Mean± SE).**

Treatments	PCV%	H/L
T1:(Control)	60±27.25 d	0.58±0.05 a
T2: (50 mg/kg folic acid)	31.00± 48 ab	0.33±0.02 b
T3: (100 mg/kg folic acid)	33.50±4.20 a	0.29±0.02 b
T4: (150 mg/kg folic acid)	33.50± 68 a	0.28±0.01 b
T5: (50 mg/kg Zinc)	33.60±3.10 ab	0.25±0.02 b
T6: (50 mg/kg Zinc+50 mg/kg folic acid)	33.60±2.80 a	0.23±0.01 b
T7:(50 mg/kg Zinc+100 mg/kg folic acid)	33.60±3.9 a	0.23±0.01 b
T8:(50 mg/kg Zinc+150 mg/kg folic acid)	33.80±3.90 a	0.25±0.01 b
significance	*	**

-Means carrying different letters differ significantly.

\*\*( $p < 0.01$ ) , \* ( $p < 0.05$ ).

Result of blood biochemical characteristics (Table 3) revealed highly significant differences ( $p < 0.01$ ) among treatments in relation to the total protein, the 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> treatments surpassed on the 1<sup>st</sup> and the 5<sup>th</sup> treatment, mean while there were no significant differences among the 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> treatments. The total protein is proportional to the antibodies levels as well as body weights of broilers, thus it may reflect

the metabolic activity specially the albumin which transmit carbohydrates, proteins and vitamins with some hormones such as thyroxin which plays an important role in metabolism [17], this may explain the increased level of total protein particularly in treatments of folic acid, this is in agreement with [18]. Folic acid is involved in many reactions in which single carbon units are incorporated in to large molecules [23].

**Table 3: Effect of folic acid with Zinc supplementation on blood biochemical characteristics. (Mean $\pm$  SE).**

Treatments	Total protein ( gm/ 100 ml)	Glucose ( gm/ 100 ml)	Cholesterol ( gm/100 ml)	Triglycerides ( gm/ 100 ml)
T1:(Control)	2.99 $\pm$ 0.03 b	188 $\pm$ 9.21 a	220 $\pm$ 8.77 a	135.10 $\pm$ 8.04 a
T2: (50 mg/kg folic acid)	4.69 $\pm$ .054 a	176 $\pm$ 2.19 ab	218 $\pm$ 7.76 ab	98.0 $\pm$ 5.12 b
T3: (100 mg/kg folic acid)	4.68 $\pm$ 0.12 a	174 $\pm$ 3.34 ab	201 $\pm$ 5.60 ab	95.51 $\pm$ 5.49 b
T4: (150 mg/kg folic acid)	4.70 $\pm$ 0.13 a	170 $\pm$ 3.90 b	191 $\pm$ 11.20 b	95.54 $\pm$ 3.25 b
T5: (50 mg/kg Zinc)	3.40 $\pm$ 0.20 b	176 $\pm$ 8.04 ab	154 $\pm$ 5.20 c	100.2 $\pm$ 2.89 b
T6: (50 mg/kg Zinc+50 mg/kg folic acid)	4.99 $\pm$ 0.16 a	161 $\pm$ 5.10 b	145 $\pm$ 5.73 c	103.5 $\pm$ 3.36 b
T7: (50 mg/kg Zinc+100 mg/kg folic acid)	4.97 $\pm$ 0.06 a	66 $\pm$ 149 c	139 $\pm$ 3.62 c	80.50 $\pm$ 3.95 c
T8: (50 mg/kg Zinc+150 mg/kg folic acid)	5.03 $\pm$ 0.26 a	5.40 $\pm$ 151 c	135 $\pm$ 3.61 c	80.20 $\pm$ 4.20 c
significance	**	**	**	**

-Means carrying different letters differ significantly.

\*\*( $p < 0.01$ ).

A highly significant reduction ( $p < 0.01$ ) was noticed in plasma glucose in the 7<sup>th</sup> and 8<sup>th</sup> treatments in which a combination of Zinc and folic acid were used, this may be attributed to the function of Zinc which plays an important role in constructing many enzymes particularly Thyroid stimulating Hormone (TSH) which stimulates Thyroxin hormone, this hormone stimulates glucose absorption by the tissues, subsequently, induced reduction of plasma protein [12]. The glucose reduction may be affected by folic acid which is required for methylation of homocysteine to from methionine, thus it ameliorates metabolism as well as it stimulate insulin activity which decreases glucose level [20].

Table 3 indicated a highly significant reduction of cholesterol and triglycerides,

this reduction may be related to effect of Zinc with folic acid or combination of them, It was noticed that Zinc increases adrenalin, testosterone and insulin functions, thus Zinc induce cholesterol reduction [17].

The increased reduction of triglycerides may be attributed to the effect of folic acid which increases homocysteine. Homocysteine levels have also been associated with increased oxidative stress [25]. Moreover Zinc participates in constructing of many enzymes, particularly those which increase triglycerides liberation and using it as energy alternatives instead of it's accumulation in the blood plasma [22].

It was concluded that folic acid with Zinc supplementation with their synergistic effect in broiler rations induce amelioration of the

immune response as well as other blood parameters.

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