Assiut University web-site: <u>www.aun.edu.eg</u>

PHYSIOLOGICAL EFFECT OF MULTIVITAMINS SUPPLEMENTATION ON HEMATOLOGICAL PARAMETERS, LIPID PROFILE, HEPATO-RENAL FUNCTION OF ROSS 308 BROILERS

MARAH SALIM HAMEED¹; SHAYMAA JABBAR HASSON²; MOHAMMED ABED MAHMOOD³ AND ALI IBRAHIM ALI AL-EZZY³

¹Department of Physiology, College of Veterinary Medicine, University of Diyala, Iraq ORCID: 0000-0002-4317-6327

² Department of Microbiology, College of Veterinary Medicine, University of Diyala, Iraq ORCID: <u>0000-0002-</u> 0582-1006

³Department of Pathology, College of Veterinary Medicine, University of Diyala, Iraq ORCID: 0000-0002-

8910-3772

³ Department of Pathology, College of Veterinary Medicine, University of Diyala, Iraq ORCID: 0000-0003-4496-1949

Received: 22 July 2024; Accepted: 18 September 2024

ABSTRACT

Increasing poultry performance and productivity represent the cornerstone in the poultry industry. The study aimed to evaluate the effect of multivitamins (A, B1, B6, D3, E) supplementation on improving the health and immune status of broilers via hematological parameters, lipid profile, and hepato-renal functions of Ross 308 broilers. Forty Ross 308 broilers of both sexes were divided equally into two groups, control group given standard ration and water supply and the supplemented group given standard ration and water supply plus 10 ml of multivitamins per 1 liter of water (vitamin A 1 MUI; vitamin D 30.1 MUI; vitamin E 400 UI; vitamin B1250 mg; vitamin B6 250 mg) for 28 days. Blood was collected at the end of day 28 of the experiment. Complete blood count, liver enzymes, lipid profile and kidney functions were evaluated. Significant differences were reported between the control and multivitamins supplemented group regarding WBCs, RBCs, MCV and MCH, ALP, direct bilirubin. Significant decreases of cholesterol (P=0.007), triglyceride (P=0.011), HDL (P=0.000), LDL (P=0.004) and urea (P=0.000) were also reported between the control and multivitamins supplemented group. In conclusion, the use of multivitamins has a significant positive effect on hematological parameters, WBCs, RBCs, MCV and MCH, level of alkaline phosphatase, direct bilirubin, urea and LDL. The use of multivitamins has a significant effect on decreasing the levels of cholesterol, triglyceride, and HDL. Therefore, the study recommends supplementation of multivitamins to promote the growth and health of broilers.

Keywords: Multivitamins, Hematological Parameters, Lipid Profile, Hepato-Renal Function, Broilers

INTRODUCTION

Prevention has traditionally been favored above cure due to the high expenses of illness supplementation and their possible detrimental effects on health (Hameed. *et al.*, 2024; Shojadoost *et al.*, 2021). Producing hens with healthy immune systems is crucial for poultry production because of the immune system's critical role in preventing sickness and promoting optimal growth (Rheinberger *et al.*, 2016). As a result, there is a greater immune response to vaccinations and defense

Corresponding author: Ali Ibrahim Ali Al-Ezzy *E-mail address:* alizziibrahim@gmail.com *Present address:* Department of Pathology, College of Veterinary Medicine, University of Diyala, Iraq

against infections. The vital roles that vitamins play in the immune system's regular operation have been thoroughly researched and are widely known (Hameed & Al-Ezzy 2024). Deficits in certain vitamins can also cause immune system malfunction, which can raise the risk of infection or inflammation and eventually stunt growth (Hameed & Al-Ezzy 2024; Rheinberger *et al.*, 2016)

Chickens are vulnerable to vitamin deficiencies, due to their intense rearing techniques and the limited amount of vitamins that their gut flora can manufacture (Islam & Nishibori, 2017). Merely trace levels of vitamin A, riboflavin, and B12 are often found in poultry diets. In addition, farmers are haphazardly employing hormones, antibiotics, enzymes, growth boosters, and protein concentrates containing heavy metals to induce fast growth in chickens, despite the fact that these additives may be hazardous to both humans and poultry. multivitamins supplements are an essential part of designed diets because mixing feed ingredients cannot guarantee that all the important vitamins-like folic acid, pantothenic acid, pyridoxine, riboflavin, and so forth-are present in the right amounts for hens. Multivitamins supplementation in broiler diets lowers mortality, prevents deficiency illnesses, and boosts body weight and meat yield.

Through a number of processes, vitamins A, D, E, and C have been shown to have the largest effects on immune system function out of all the vitamins. Vitamin A is crucial for preserving the integrity of epithelial cells and supports a number of immune-related processes, including boosting mucosal immunity and lowering free radical levels in mice and chickens (Kam et al., 2012; Lucas et al., 2014). This vitamin also has the ability to induce opposite effects in a dose-dependent way; in mice and chickens, Vitamin A has immunostimulatory activity at lower doses and anti-inflammatory activity at higher doses (Yuan et al., 2014). Since vitamin D lowers the levels of proinflammatory cytokines like interleukins 1 (Morris et al., 2014); interleukin 6 (Zhang et al., 2012) and gamma Table 1: The used multivitamins in water

interferon (Boodhoo *et al.*, 2016), it is also widely recognized to have anti-inflammatory properties.

In poultry production, antioxidants are crucial for nutrition and performance. In poultry, an antioxidant deficiency leads to a number of illnesses, including impaired absorption of selenium from food, encephalomalacia, exudative diathesis for vitamin E, polyneuritis, curled toe paralysis, perosis, and slowed growth, weakness, ruffled feathers, blindness, xerophthalmia (Alagawany et al., 2021). Supplementing of poultry diets with vitamin E at levels beyond the (National Research Council, 1994) recommendations improved immunity (Lin et al., 2004) and overall health(Fu et al., 2022). Administration of higher doses of vitamin E (150 ppm) caused a significant effect on quail productivity, whereas lower doses of vitamin E (15 ppm) had no beneficial effects (Abou-Kassem et al., 2016)

The aim of the current study was to evaluate the following hypothesis that supplementation of broilers with adequate concentrations of multivitamins including, vitamin A, vitamin D3, vitamin E, vitamin B1 and vitamin B6 would improve the immune status of the broilers including the positive effects on hematological parameters, lipid profile, and hepato-renal functions of Ross 308 broilers which reflect the health safety of investigated broilers.

MATERIALS AND METHODS

Ethical Approval:

The current study was approved according to ethics license No. CVM 2023/PD110, by ethics committee at the department of pathology, college of veterinary medicine, University of Diyala, Iraq

Source of multivitamins:

Multivitamins (A,B1,B6,D3,E) combination were purchased from Goovet Group Joint Stock Company; Vietnam (GOOVET, 2024).The main component was illustrated in Table (1):

	U U	
Vitamin	Conc. per 100ml water	Conc. per 100ml water
Vit. A	1 MUI	0.0003mg
Vit. D3	0.1 MUI	0.00003 mg
Vit. E	400 UI	0.12 mg
Vit. B1	250 mg	250 mg
Vit. B6	250 mg	250 mg

Table 1: The used multivitamins list added to drinking water.

Study Design:

Forty Ross 308 broilers of both sexes of one day old and weighed 45 ± 5 gm on average were used. They were separated randomly into experimental and control groups equally. The experimental group was fed a conventional broiler diet supplemented with 10 milliliters of multivitamins per liter of water according to manufacturer instructions (GOOVET, 2024).

Chicks were raised in cages with an automated watering system, 10 chicks per square meter of space, and no feeding in front of the chicks. From days 1 through 3, the lighting regime lasted 24 hours. From that point on, it lasted 23 hours until the experiment's conclusion.

The beginning temperature was set to 33 ± 1 °C for the first three days and subsequently lowered by 3 °C per week until it reached 24 °C at the conclusion of the experiment (Aviagen, 2018). Approximately 60% humidity was sustained over the entire experiment.

Blood collection:

Five chicks were randomly chosen from experimental and control groups at the end of the 28-days study. Blood was collected from the wing vein and separated as follows: one part was collected into clean and dry 15 ml falcon tubes without the use of an anticoagulant, allowed to clot at room temperature, and then centrifuged for five minutes at 3000 rpm for serum separation and kept at -20 °C until biochemically analyzed lipid profiles, liver functions, and renal function profiles. Another part of blood was collected in sterile tubes with (EDTA) for hematological examination (Al-Ezzy *et al.*, 2020; Hassan *et al.*, 2018)

Hematological study:

Collected blood was used for evaluation of total white blood cells, total red blood cells, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width-standard deviation, hemoglobin and platelets (Al-Khalidi *et al.*, 2020; Hameed & Al-Ezzy 2024)

Liver Function tests:

Serum was used for evaluation of liver enzymes: Alkaline phosphatase (ALP),Alanine transaminase (ALT), Aspartate transaminase (AST) according to (Al-Khalidi *et al.*, 2020; Hameed *et al.*, 2020) by spectrophotometric measurement using Roche Cobas c501, series BX1432 (Roche Diagnostics, Mannheim, Germany). ALP (Roche Cat No. 03333752 190),ALT (Roche Cat No. 32233) and AST (Roche Cat No. 46985).

Renal function tests:

Serum was used for evaluation of renal function, which includes: urea and creatinine according to (Hameed & Al-Ezzy 2024; Hameed *et al.*, 2020; Hassan *et al.*, 2018) by spectrophotometric measurement using Roche Cobas Integra 400 Plus , Urea (Roche Cat No 04460715190) ; creatinine (Roche Cat No 0766127)

Lipid profile

Serum was used for evaluation of lipid profile including : total serum bilirubin, direct bilirubin, indirect bilirubin, cholesterol, triglyceride, high-density lipoprotein, and low density lipoprotein according to (Al-Ezzy *et al.*, 2016; Al-Khalidi *et al.*, 2020; Hameed *et al.*, 2020) by spectrophotometric measurement using Roche Cobas b101system (Roche Diagnostics, Mannheim, Germany).

Statistical Analysis:

Data were analyzed using the "Statistical Package for Social Sciences (SPSS version 18.0)(Hameed & Al-Ezzy, 2019; Hassan *et al.*, 2020)". T-test was used to determine the presence of a significant difference at p<0.05 (Al-Ezzy, 2017; Hameed *et al.*, 2020)

RESULTS

Evaluation of hematological parameters

Table (2) revealed that total WBC count was significantly increased in multivitamins supplemented group (214.50 \pm 19.44 x103/µl)) compared with control (169.46 \pm 6.73 x103/µl)), P (0.013). Total RBCs count significantly increased in multivitamins supplemented group $(2.91 \pm 0.38(x106/\mu l))$ compared with control $(2.35 \pm 0.107(x106/\mu l))$, P (0.046). HCT% increase in multivitamins supplemented group (35.62 ± 4.68) compared with the control group (30.76 ± 1.66). MCV was significantly decreased in multivitamins supplemented group (122.70 \pm 4.30) compared with control (130.50±4.00), P (0.029). MCH was significantly decreased in supplemented group (46.67 \pm 0.99) compared with the control group (50.83±0.76), P (0.000). MCHC was non-significantly decreased in multivitamins supplemented group (38.12 \pm 1.03) compared with control (39.03±0.89), P (0.139). RDW-SD was non-significantly decreased in multivitamins supplemented group (39.60 ± 5.24) compared with control (44.83±3.69), P (0.101). Hb was non-significantly increased in multivitamins supplemented group (13.57 ± 1.61) compared with control (12.00 \pm 0.60), P (0.131). Platelets were non-significantly increased in multivitamins supplemented group (7.50 ± 3.78) compared with control (4.33 ± 0.55), P (0.118).

Evaluation of liver enzymes

Table (3) revealed that ALP was significantly increased in multivitamins supplemented group ($3201.92 \pm 157.19U/L$) compared with control ($1104.25 \pm 124.96 U/L$), P (0.0000). ALT was non-significantly increased in multivitamins supplemented group (1.21 ± 1.11 U/L) compared with the control group ($1.2067 \pm 0.43 U/L$), P (0.3996). AST was non-significantly decreased in multivitamins supplemented group $(219.7160 \pm 46.71875 \text{ U/L})$ compared with control $(255.33 \pm 8.30 \text{ U/L})$, P (0.1432).

Evaluation of lipid profile

Table (4) revealed that total serum bilirubin was not significantly decreased in multivitamins supplemented group (0.0360 ± 0.019) mg/dL) compared with the control group $(0.05\pm0.025 \text{ mg/dL})$, (P= 0.281). Direct bilirubin was significantly increased in multivitamins supplemented group (0.0360 ± 0.019) mg/dL) compared with control (0.0125 ± 0.005 mg/dL), (P=0.026). Indirect bilirubin was non significantly increased in multivitamins supplemented group (0.0360±0.01 mg/dL) compared with the control group $(0.0350 \pm 0.026 \text{ mg/dL}), (P=0.474).$ Cholesterol was significantly decreased in multivitamins supplemented group (125.94 \pm 15.513 mg/dL) compared with control (157. \pm

12.98 mg/dL, (P= 0.007).

Triglyceride was significantly decreased in multivitamins supplemented group (42.48 \pm 7.78 mg/dL) compared with control (109.5 \pm 51.72 mg/dL), (P= 0.011).

HDL was significantly decreased in multivitamins supplemented group $(20.98\pm20.81 \text{ mg/dL})$ compared with control $(97.75\pm16.27 \text{ mg/dL})$, (P= 0.000). LDL was significantly increased in multivitamins supplemented group $(75.78\pm5.51 \text{ mg/dL})$ compared with control $(58.50\pm9.183 \text{ mg/dL})$, (P= 0.004).

Evaluation of Renal Function

Table (5) revealed a significant increase in urea among multivitamins supplemented group (5.01 \pm 0.36mg/dL) compared with control (2.18 \pm 0.86 mg/dL), P=0.000). Creatinine was stable in multivitamins supplemented group and control (0.36 \pm 0.00 mg/dL). **RBC** (x10⁶/µl)

НСТ%

MCV

MCH

MCHC

RDW-SD

HB (g/dl)

Platelet (x10³/µl)

0.046

0.114

0.029

0.000

0.139

0.101

0.131

0.118

 $2.91 \pm .384$

 35.62 ± 4.68

 122.70 ± 4.30

 46.67 ± 0.99

 38.12 ± 1.03

 39.60 ± 5.24

 13.57 ± 1.61

 7.50 ± 3.78

group in broilers.			1
Parameters	Control group	Multivitamins sup- plemented group	P value
WBC (x10 ³ /µl)	169.46 ± 6.739	214.50 ± 19.442	0.013

 2.35 ± 0.107

 30.76 ± 1.66

 130.50 ± 4.00

 50.83 ± 0.76

 39.03 ± 0.89

 44.83 ± 3.6936

 12 ± 0.60

 4.33 ± 0.55

Table 2	2:	Haematological	parameters	for	multivitamins	supplemented	group	versus	control
		group in broilers	5.						

Table 3: Liver enzymes pro	file of multivitamins supplemented g	group versus control group in
broilers.		

Parameters Control group		Multivitamins supple- mented group	P value
ALP (U/L)	1104.25 ± 124.96	3201.92 ± 157.19	0.0000
ALT (U/L)	1.2067 ± 0.43	1.2160 ± 1.119	0.3996
AST (U/L)	255.33 ± 8.30	219.7160 ± 46.718	0.1432

Table 4: lipid profile in broilers for control group versus multivitamins supplemented group.

ameters (mg/dL) Control group		P value
0.05 ± 0.025	$0.0360 \pm .019$	0.281
0.0125 ± 0.005	$0.0360 \pm .019$	0.026
$0.035{\pm}0.026$	$0.0360 \pm .0194$	0.474
157 ± 12.987	125.9440 ± 15.51	0.007
109.5 ± 51.72	42.4840 ± 7.789	0.011
97.75 ± 16.27	20.9820 ± 20.817	0.000
58.50 ±9.18	75.7840 ± 5.51	0.004
	Control group 0.05 ± 0.025 0.0125 ± 0.005 0.035 ± 0.026 157 ± 12.987 109.5 ± 51.72 97.75 ± 16.27 58.50 ± 9.18	$\begin{array}{r c c c c c c c c c c c c c c c c c c c$

Table 5:	The evaluation	of renal	function	profile	in	broilers	for	multivitamins	supplement	ted
	group versus co	ontrol gro	oup							

Parameters (mg/dL) Control group		Multi vitamins sup- plemented group	P value		
Urea	$2.18{\pm}0.86$	5.01 ±.36	0.000		
Creatinine	$0.36{\pm}0.00$	0.36±0.00	ND		

ND: not detected

DISCUSSION

The current study revealed that total WBCs count was significantly increased (P = 0.013) in multivitamins supplemented group $(214.50 \pm 19.44 \text{ x}10^3/\mu \text{l})$ compared with control (169.46 \pm 6.73 x10³/µl), which agreed with Khoso et al. (2018), who stated that total

WBCs count was significantly higher in multivitamins supplemented group compared with control. Similar result was reported by (Iftitah et al., 2022). The current study disagreed with Khudhair & Alwan (2019), who found no significant difference in WBCs count among broilers treated with AD3E supplements compared with control. The total WBCs count increased in AD3E,B1,B6 treated group as the synthesis in bone marrow requires nutrients, mainly vitamins and minerals, which enhance the biosynthesis of WBCs compared with the control group (Iftitah *et al.*, 2022).

In the current study, the total RBCs count was significantly increased in multivitamins supplemented group $(2.91 \pm .38 \times 106/\mu l)$) compared with control group (2.35 ± 0.107) $x106/\mu l$), which come in accordance with that reported by (Khoso et al., 2018), who stated that Ross 308 broilers supplemented for 6 weeks with multivitamins (vitamin A 12 MIU, vitamin D3 2 IU, vitamin. E 4 000 IU, vitamin. K3 4 000 mg, vitamin B2 3 000 mg, vitamin C 5 000 mg) causing a significant increase in RBCs count compared with control. The result of the current study comes in accordance with (Das et al., 2014), who stated that total RBCs count was significantly increased compared with control. Current study disagreed with (Khudhair & Alwan 2019; Kumari et al., 2013), who found no significant difference in RBCs count among broilers supplemented with AD3E compared with control. The differences in RBCs count are attributed to the fact that AD3EB1.B6 vitamins support the erythropoiesis beside amino acids , minerals , iron and hemopoietin hormone (Iftitah et al., 2022). Several factors including the dose of supplemented vitamins, the strain of birds, age, climate, immune status, general health status, environment of rearing, management system applied, and the duration of experiment (Khudhair & Alwan 2019)

In the current study, HCT% was non significantly increased in multivitamins supplemented group (35.62 ± 4.68) compared with control (30.7667 ± 1.66547) which comes in accordance with (Horhoruw & Kewilaa, 2024), who stated that the normal HCT% 22-35%, which was related to the total RBCs count and Hb (Enos & Moore, 2022). A positive correlation between the size of the erythrocytes and the hematocrit value was recorded by (Ulupi & Ihwantoro, 2014).

Nonetheless, there is a negative correlation between this hematocrit number and the chicken's body fluid content. Hematocrit levels will rise in chickens that are deficient in blood fluids. On the other hand, if the chicken has too much fluid in it, the hematocrit number may drop (Iftitah et al., 2022). This reduction suggests that heat stress is wearing down broiler birds. Increased ambient temperature-induced heat stress produces reactive oxygen compounds or free radicals, which in turn cause oxidative stress. The quality of red blood cells (erythrocytes) decreases due to the rise in free radical generation (Reactive Oxygen Species), which is increased with rising ambient temperature. Hematocrit readings might drop as a result of damaged erythrocyte circumstances(Iftitah et al., 2022).

In the current study, MCV was significantly decreased in multivitamins supplemented group (122.70 ± 4.30) compared with the control (130.50 ± 4.00) . The current results disagreed with that reported by Kumari *et al.* (2013), who stated no significant effects of 200mg of vitamin E on MCV level of broiler compared with the control. The difference might be due to the presence of damaged erythrocytes.

In the present study, MCH was significantly decreased in multivitamins supplemented group (46.67±0.99) compared with control (50.83±0.76). MCHC was non significantly decreased in multivitamins supplemented group (38.1250 ±1.03) compared with control (39.03 ±0.89). RDW-SD was non significantly decreased in multivitamins supplemented group (39.6±5.24) compared with control (44.83 ±3.69) which came in line with that reported by (Abdul-Majeed & Abdul-Rahman, 2022; Kumari *et al.*, 2013).

Hb was non significantly increased in multivitamins supplemented group (13.57 ± 1.61) compared with the control (12 ± 0.60) which come in contrary with that reported by (Abdul-Majeed & Abdul-Rahman, 2022), who stated that Hb was significantly increased in Ross 308 broilers after in ovo injection of vitamin E and oppose with (Khoso *et al.*, 2018; Tayeb & Qader, 2012)

who stated a significant increase in Hb after supplementation with multivitamins containing vitamin E at a concentration 4000IU. The current results come in accordance with Setivaningsih et al. (2023), who reported significant variations in hemoglobin levels acRoss supplementations group with 4500 IU of vitamin D3. The variation of the hemoglobin level ranged between 10.64 and 12.08 g/dl, which is acceptable. In the current study, Hb value was not sigificantly increased, compared to the control, which come in accorance with (Das et al., 2014) and (Douglas & Wardrop, 2010), who stated that the hemoglobin content in chickens (Gallus domesticus) varied between 7 and 13 g/dl.

The current Hb level is consistent with reports from (Horhoruw & Kewilaa, 2024) indicating that physiological variations, such as age and activity, environment, temperature and humidity, and feed composition or content, are the primary causes of variations in hemoglobin levels. Blood hemoglobin levels are influenced by bodily activity; the more active the body, the greater the hemoglobin level (Aprihatin & Imral, 2021). Non significant increase in Hb was explained by Nayaka et al. (2013), who stated a significant difference between vitamin E supplemented group with 0.02% and control group of broiler chicks which indicates an antoxidant activity for vitamin E which lead to the minimization of free radicals in the blood and increase Hb synthesis (Kandpal et al., 2019). The Hb value of current study was higher than that recorded by Roy & Mishra, (2011), who stated that the range of hemoglobin levels in the various broiler groups was 9.9 to 10.13 g/dl. The current study disagree with that reported by (Kumari et al., 2013), who stated that no significant effect of vitamin E and selenium on the hematological parameters of broilers in subacute toxicity of hexavalent chromium in broiler chick. Based on the results, it is evident that vitamin E has a considerable impact on Hb percent. This effect may have been caused by a dose and duration of exposure dependent antioxidant activity of vitamin E which reduces free radicals and leads to inhibits their peroxidative impact on the unsaturated lipid in the membrane, protecting the integrity of the WBCs and RBCs membranes. The tocopherol chromanol ring oxidizes to quinone form after donating its phenolic hydrogen to lower the free radical and finally good influence in improving hematological parameters, or by increasing PCV, which raises Hb count.

In the current study, platelets were non-significantly increased in multivitamins supplemented group (7.50 \pm 3.78) compared with control(4.33 \pm 0.55),which come in contrary with Khudhair & Alwan (2019), who stated a significant difference in platelet count and this difference attributed to the dose of AD3E supplemented to broilers and the addition of amino acids to the ration beside the duration of the experiment.

In the current study, no significant change reported between multivitamins was supplemented group compared with control regarding the levels of ALT and AST .On the other hand ALP was significantly increased in multivitamins supplemented group $(3201.92 \pm 157.19 \text{ U/L})$ compared with control group (1104.25 \pm 124.96 U/L). These results disagree with that reported by Das et al. (2014), who stated that AST, ALT were lower in vitamin tracted group compared with control. In the current study, increased level of ALP in AD3E-B1-B6 supplemented group compared with control which disagree with (Arslan et al., 2001; Paul et al., 2010), who stated that the ALP levels in broiler showed a gradual decrease as the age increased. which is contradicted by the findings of (Senanayake et al., 2015), who stated that ALP level increased with the age of broiler. This has also been confirmed by previous studies (ArRashid et al., 2015; Franchini et al., 1990). In a study by (Arslan et al., 2001; Guo et al., 2023), they stated that although the ALP levels of the experimental groups were higher than those of the control group, this difference was found not to be significant. The current results of ALP agreed with that recorded by Kalaba et al. (2024), who reported that the level of ALP was significantly increased in vitamin B1 treated broiler, compared to the control. It has been declared that the plasma ALP levels of hens increased as the level of vitamin E increases (Franchini et al., 1988; ArRashid et al., 2015; Franchini et al., 1990). The increased level of ALP in growing broilers is closely related to increase the activity of bone formation and growth and increase the metabolic activity as the broilers vitamin supplemented with are D (Senanayake et al., 2015). In the current study, ALT was non-significantly increased in multivitamins supplemented group which agrees with that reported by (Guo et al., 2023; Sahin et al., 2001).

The current results revealed no significant difference in the level of total bilirubin between multivitamins supplemented group and control which agreed with (Guo *et al.*, 2023), stated no significant difference in the level of total bilirubin between vitamin A supplemented group and control. Although the current study reported a significant increase of direct bilirubin in multivitamins supplemented group ($0.036 \pm 0.01 \text{ mg/dl}$), compared to the control ($0125 \pm 0.005 \text{ mg/dl}$).

In the current study, cholesterol was significantly low in AD3EB1B6 vitamins supplemented group $(125.94 \pm 15.51 \text{ mg/dl})$ versus control (157.0±12.98 mg/dl), (P=0.007) which disagreed with Guo et al. (2023) and Jebur et al. (2018), who stated no significant difference. Triglyceride was significantly low in multivitamins supplemented group (42.48 \pm 7.789 mg/dl) versus control (109.5 \pm 51.72mg/dl), (P=0.011), which disagreed with that reported by (Guo et al., 2023; Jebur et al., 2018), who stated no significant difference. HDL was low in multivitamins supplemented group $(20.98 \pm 20.81 \text{mg/dl})$ versus control (97.7 \pm 16.27 mg/dl) with a significant difference (P=0.000) which disagreed with (Guo et al., 2023; Jebur et al., 2018), who stated no significant difference. LDL was elevated in multivitamins supplemented group (75.78± 5.51mg/dl) versus control (58.5 ±9.18mg/dl) with a significant difference (P=0.004) which come in agreement with (Guo et al., 2023; Jebur et al., 2018). The proposed mechanism of low levels of lipid indices might be due to the effect of vitamins mainly E on reduction of lipid peroxidation

process and maintenance of cellular integrity.

In the current study , urea was significantly elevated in multivitamins supplemented group $(5.01\pm.36 \text{mg/dl})$ versus the control $(2.18\pm0.86 \text{mg/dl})$, (P=0.0001), which agreed with Khudhair & Alwan (2019; Naimi & Vakili, (2014), who attribute the differences to age, strain, climate and drugs that may be used accidentally at the time of production cycle.

CONCLUSIONS

The use of multivitamins has a significant positive effect on WBCs, RBCs, MCV and MCH, Alkaline phosphatase, direct bilirubin, urea and LDL. The use of multivitamins has a significant effect on decreasing the levels of cholesterol, triglyceride, and HDL. Therefore, the study recommends supplementation of multivitamins to promote the growth and health of broilers.

Conflict of interest: Authors declare no conflict of interest

Acknowledgement: authors appreciated for the support of Pathology Department, College of Veterinary Medicine, University of Diyala, Iraq

Author Contributions: Authors are equally contributing in all steps of manuscript construction.

Funding: Current study was funded by authors only.

REFERENCES

- Abdul-Majeed, A. and Abdul-Rahman, S. (2022): Impact of vitamin e and selenium treatment in-ovo and after hatching of broiler. *Iraqi Journal of Agricultural Sciences*, 53(4), 810-818.
- Abou-Kassem, D.E.; Mahrose, K. and Alagawany, M. (2016): The role of vitamin E or clay in growing Japanese quail fed diets polluted by cadmium at various levels. (Vol. 10). Animal
- Al-Ezzy, A.I.A. (2017): Immuno histopathological Role Of Bcl2 And P53 Gene Expression In Helicobacter

Pylori Cytotoxin-Associated Gene A Positive Versus Cytotoxin-Associated Gene A Negative Antral Predominant non-atrophic gastritis in Iraqi patients. *Asian J Pharm Clin Res*, 10(3), 142-148.

- Al-Ezzy, A.I.A.; Al-Khalidi, A.A.H. and Hameed, M.S. (2020): Evaluation of C-Reactive Protein in Iraqi Children Presented with Acute Enteropathogenic Escherichia Coli Associated Diarrhea with Special Emphasis to Age and Gender. Gazi Medical Journal, 31(2).
- Al-Ezzy, A.I.A.; Hameed, M.S., Jalil, W.I. and Mohamad, W.M. (2016): Pathophysiological effects of vitamin C and E-selenium combination on lipid profile and serum glucose of experimentally induced sodium nitrate intoxication in mice Research Journal of Pharmaceutical Biological and Chemical Sciences, 7(2), 958-964.
- Al-Khalidi, A.A.H.; Hameed, M.S.; Ali, A.-E. A.I. and Ibrahim, S.N. (2020): Effects of Saccharomyces cerevisiae as probiotic on blood indices, humoral immunity and performance of Isa brown laying hens in Diyala province, Iraq. Biochemical and Cellular Archives, 20(1).
- Alagawany, M.; Elnesr, S.S., Farag, M.R., Tiwari, R.; Yatoo, M.I.; Karthik, K. and Dhama, K. (2021): Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health–a comprehensive review. Veterinary Quarterly, 41(1), 1-29.
- Aprihatin, Y. and Imral, M. (2021): The Relationship of Body Mass Index to Hemoglobin Levels. 1st International Conference on Sport Sciences, Health and Tourism (ICSSHT 2019),
- ArRashid, M.H.; Ahmad, N.; Amin, M.R. and Mollah, M.L. (2015): Effects of selected vitamins and minerals on growth rate and hematological parameters in broilers. Asian Journal of Medical and Biological Research, 1(3), 487-494.
- Arslan, M.; Özcan, M.; Matur, E.; Çötelİoğlu, Ü. and Ergül, E. (2001): The effects of

vitamin E on some blood parameters in broilers. *Turkish Journal of Veterinary and Animal Sciences*, 25(5), 711-716.

- Aviagen, W.R. (2018): 308: Broiler's Management and Nutrition Specification. AOAC International Aviagen Inc.
- Boodhoo, N.; Sharif, S. and Behboudi, S. (2016): 1α, 25 (OH) 2 vitamin D3 modulates avian T lymphocyte functions without inducing CTL unresponsiveness. *PloS one*, 11(2), e0150134.
- Das, M.R.; Sarker, M.; Rashid, M. and Miah, M. (2014): Effect of enzyme, multivitamins and growth promoter on growth performance and hematobiochemical parameters in broiler chicken. International Journal of Natural Sciences, 4(1), 01-04.
- Douglas, J.W. and Wardrop, K.J. (2010): Schalm's veterinary hematology.
- Enos, K.E. and Moore, D.M. (2022): Hematology of laboratory animals. Schalm's Veterinary Hematology, 1058-1072.
- Franchini, A.; Giordani, G.; Meluzzi, A. and Manfreda, G. (1990): High doses of vitamin E in the turkey diet.
- Franchini, A.; Meluzzi, A.; Bertuzzi, S. and Giordani, G. (1988): High doses of vitamin E in the broilers diets. Archiv fuer Gefluegelkunde (Germany, FR), 52(1).
- Fu, Z.; Zhong, T.; Wan, X; Xu L, H; Y., Han H. and Z, W. (2022): Effects of dietary vitamin E supplementation on reproductive performance, egg characteristics, antioxidant capacity, and immune status in breeding geese during the late laying period. 11(10), 2070.
- GOOVET (2024): Oral solution -colivit. Retrieved 24 march from https://goovetvn.com/en/veterinarymedicine/oral-solution/colivit-en.html
- Guo, S.; He, L.; Zhang, Y.; Niu, J.; Li, C.; Zhang, Z. and Ding, B. (2023): Effects of Vitamin A on Immune Responses and Vitamin A Metabolism in Broiler Chickens Challenged with Necrotic Enteritis. Life, 13(5), 1122.

- Hameed, M. and Al-Ezzy, A. (2019): Evaluation of possible stress factors affecting physiological level of gamma interferon during first six months of life in healthy calves. Adv. Anim. Vet. Sci, 7(5), 370-377.
- Hameed, M.S.; Al-Ezzy, A.I.; Ali, Jalil; W. I. and Al Khalidi; A.A.H. (2020): Impact of Stress Factors on Physiological Level of Interleukin 10 in Healthy Calves in Diyala Province–Iraq. International Journal of Pharmaceutical Research, 12(2). https://doi.org/https://doi.org/10.31838 /ijpr/2020.SP2.362
- Hameed, M.S. and Al-Ezzy, A.I.A. (2024): Evaluation of antioxidant; nephroprotective and immunomodulatory activity of vitamins C and E sodium selenite in mice intoxicated with sodium nitrate. Adv. Anim. Vet. Sci, 12(6), 1018-1027.
- Hameed, M.S.; Al-Ezzy, A.I.A.; Jalil, W.I.; and Al-Khalidi, A.A.H. (2020): Physiological protective effects of ascorbic acid versus Dl-A-tocopheryl acetate-sodium selenite combination in mice under experimental sodium nitrate intoxication. *Biochem. Cell. Arch*, 20(1), 2593-2601.
- Hameed, M.S.; Raad Mahmood Hussein AlZubaidi and Ali Ibrahim Ali Al-Ezzy (2024): Effectiveness of Aqueous Versus Alcoholic Extracts of Melia azedarach in Amelioration of Lipid Profile, Liver Enzymes and Innate Inflammatory Indices for White New Zealand Rabbits. Advances in Animal and Veterinary Sciences, 12(7), 1256.
- Hassan, A.A.; Hameed, M.S. and Al-Ezzy, A.I.A. (2018): Correlation between aspergillosis and renal function profile analysis in broilers of Diyala province– Iraq.
- Hassan, A.A.; Hameed, M.S. and Al-Ezzy, A.I.A. (2020): Effect of drinking water quality on physiological blood parameters and performance of laying hens in diyala province, Iraq. Biochemical and cellular archives, 20(1), 2649-2654.

- Horhoruw, W. and Kewilaa, A.I. (2024): Hematological Profile in Free-Range Chickens at The Age Of 4, 8, 12 Weeks. *Advances In Social Humanities Research*, 2(5), 731-736.
- Iftitah, D.; Arisandi, B. and Widyani, R.R. (2022): Physiological Conditions of Broiler Chickens During Transportation with Vitamin Treatment and Distance Difference. Jurnal Ilmu-Ilmu Peternakan, 32(3), 313-327.
- Islam, M. and Nishibori, M. (2017): Use of multivitamins, acidifier and Azolla in the diet of broiler chickens. Asian-Australasian journal of animal sciences, 30(5), 683.
- Jebur, S.; Mohammed, T. and Al-khalanı, F. (2018): Effect of Vitamin E, C and Aspirin in the Performance, Lipid Peroxidation and Blood Biochemistry Traits of Broiler in Heat Stress. The Eurasia Proceedings of Science Technology Engineering and Mathematics(3), 145-151.
- Kalaba, Z.; El-Hadad, E.S.; Gomaa, M.H. and Farouk, Z.M. (2024): Influence of In-ovo Feeding Vitamins E, B1, and B2 to Broiler Embryos on Hatchability, Chick Quality, and Blood Biochemical Parameters of Hatched Chicks. Journal of Animal and Poultry Production, 15(3), 57-65.
- Kam, R.K.T.; Deng, Y.; Chen, Y. and Zhao, H. (2012): Retinoic acid synthesis and functions in early embryonic development. Cell and bioscience, 2, 1-14.
- Kandpal, V.; Kumar, D. and Bisht, R. (2019): Protective effect of vitamin E on haematological parameters in chronic toxicity of hexavalent chromium in laboratory chicks. Journal of Drug Delivery and Therapeutics, 9(3), 388-392.
- Khoso, P.A.; Memon, A.A.; Baloch, A.A. Mangi, A.R. and Khoso, Z.A. (2018): Effect of Mineral and Vitamin Supplementation on Performance and Haemotological Values in Broilers. Journal of Northeast Agricultural University (English Edition), 25(1), 33-39.

- Khudhair, N.A. and Alwan, M.T. (2019): Study Effect Of Vitamin Supplementation (Ad3e) On Physio-Biochemical Parameters In Broiler Chickens In Basrah Governorate. Basrah Journal of veterinary Research, 18(1), 410-418.
- Kumari, R.R.; Kumar, P. and Mondal, T.K. (2013): Effect of vitamin E and selenium on haematological parameters in sub-acute toxicity of hexavalent chromium in broiler chick. National Journal of Physiology, Pharmacy and Pharmacology, 3(2), 158-158.
- Lucas, A.; Morales, J. and Velando, A. (2014): Differential effects of specific carotenoids on oxidative damage and immune response of gull chicks. Journal of Experimental Biology, 217(8), 1253-1262.
- Morris, A.; Shanmugasundaram, R.; Lilburn, M.S. and Selvaraj, R.K. (2014): 25-Hydroxycholecalciferol supplementation improves growth performance and decreases

inflammation during an experimental lipopolysaccharide injection. *Poultry Science*, 93(8), 1951-1956.

- Naimi, D. and Vakili, R. (2014): The Study of the Effects of Different Levels of Vitamin Ad3e on Intestinal Adhesion, Growth Performance, Feed Intake and Feed Conversion Ratio in Broilers. Spectrum, 3(8).
- National Research Council (1994): Nutrient Requirements of Poultry: Ninth Revised Edition, 1994. The National Academies Press. https://doi.org/doi:10.17226 /2114
- Nayaka, H.S.; Umakantha, B.; Ruban, S.W. Murthy, H. and Narayanaswamy, H. (2013): Performance and hematological parameters of broilers fed neem, turmeric, vitamin e and their combinations. *Emirates Journal of Food and Agriculture*, 25(6), 483.
- Paul, R.; Ahmad, N.; Moinuddin, M. and Hasan, N. (2010): Effects of administration of multivitamins and enzymes for broilers either singly or in combination on body weight and haematobiochemical parameters.

Journal of the Bangladesh Agricultural University, 8(1).

- Rheinberger, C.M.; Herrera-Araujo, D. and Hammitt, J.K. (2016): The value of disease prevention vs treatment. Journal of health economics, 50, 247-255.
- Roy, S. and Mishra, S.C. (2011): Effect of Antistress agents on Haemato-Biochemical profiles of broiler and breeder hen druing summer. Veterinary World, 4(2), 60.
- Sahin, N.; Sahin, K. and Küçük, O. (2001): Effects of vitamin E and vitamin A supplementation on performance, thyroid status and serum concentrations of some metabolites and minerals in broilers reared under heat stress (32 degrees C). Veterinarni medicina, 46.
- Senanayake, S.; Ranasinghe, J.; Waduge, R.; Nizanantha, K. and Alexander, P. (2015): Changes in the serum enzyme levels and liver lesions of broiler birds reared under different management conditions. *Tropical Agricultural Research*, 26(4), 584 – 595.
- Setiyaningsih;, N.; Sumiati, Anuraga Jayanegara and Wira Wisnu Wardani (2023): Effects of a Vitamins D and C Supplement on Performance, Hatchability, and Blood Profiles of Broiler Breeders. Journal of World's Poultry Research, 13(1), 71-80.
- Shojadoost, B.; Yitbarek, A.; Alizadeh, M.; Kulkarni, R.R.; Astill, J.; Boodhoo, N. and Sharif, S. (2021): Centennial Review: Effects of vitamins A, D, E, and C on the chicken immune system. Poultry Science, 100(4), 100930.
- Tayeb, İ. and Qader, G. (2012): Effect of feed supplementation of selenium and vitamin E on production performance and some hematological parameters of broiler. *KSÜ Doğa Bilimleri Dergisi*, 15(3), 46-56.
- Ulupi, N. and Ihwantoro, T. (2014): Gambaran darah ayam kampung dan ayam petelur komersial pada kandang terbuka di daerah tropis. Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan, 2(1), 219-223.

Yuan, J.; Roshdy, A.R.; Guo, Y.; Wang, Y. and Guo, S. (2014): Effect of dietary vitamin A on reproductive performance and immune response of broiler breeders. *PloS one*, 9(8), e105677.

Zhang, Y.; Leung, D.Y.; Richers, B.N.; Liu, Y.; Remigio, L.K.; Riches, D.W. and Goleva, E. (2012): Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *The Journal of Immunology*, 188(5), 2127-2135.

التأثير الفسيولوجي لمكملات الفيتامينات المتعددة على مؤشرات الدم ومستوى الدهون والوظائف الكبدية الكلوية لفروج اللحم روس ٣٠٨

مرح سالم حميد '، شيماع جبار حسون' ، محمد عبد محمود "، علي ابراهيم علي العزي" أ قسم الفسيولوجيا، كلية الطب البيطري، جامعة ديالي، العراق قسم الأحياء الدقيقة، كلية الطب البيطري، جامعة ديالي، العراق قسم علم الأمراض، كلية الطب البيطري، جامعة ديالي، العراق

Email: alizziibrahim@gmail.com Assiut University web-site: www.aun.edu.eg

يمثل تحسين أداء الدواجن وإنتاجيتها حجر الزاوية في صناعة الدواجن. هدفت هذه الدراسة الى تقييم تأثير مكملات الفيتامينات المتعددة (أ، ب١، ب٦، ٣٦، هـ) على تحسين الحالة الصحية والمناعة للدجاج اللاحم من خُلُّل المعايير الدموية ومستويات الدهون ووظائف الكبد والكلي للدجاج اللاحم من سلالة روس ٣٠٨. تم تقسيم أربعين دجاجة لاحم من سلالة روس ٣٠٨ من كلا الجنسين بالتساوي إلى مجموعتين، المجموعة الضابطة أعطيت العليقة القياسية و المياة والمجموعة المعالجة أعطيت العليقة القياسية والمياة مضافا اليه ١٠ مل من الفيتامينات المتعددة لكل لتر من الماء (فيتامين أ ١ MUI؛ فيتامين د ٣٠,١ MUI؛ فيتامين هـ ٤٠٠ UI؛ فيتامين ب ١٢٥٠ مجم؛ فيتامين ب ٢٥٠٦ مجم) لمدة ٢٨ يومًا. تم جمع الدم في نهاية اليوم الثامن والعشرين من التجربة. تم تقييم تعداد الدم الكامل، إنزيمات الكبد، الدهون ووظائف الكلي. أفادت النتائج وجود زيادات معنوية بين المجموعة الضابطة والمجموعة المعالجة بالفيتامينات المتعددة فيما يتعلق بخلايا الدم البيضاء، خلايا الدم الحمراء، حجم الدم الوسطى وتكوين الدم الوسطى، الفوسفاتيز القلوي، البيليروبين المباشر. كما أفادت التقارير عن وجود انخفاضات معنوية بين المجموعة الضابطة والمجموعة المعالجة بالفيتامينات المتعددة فيما يتعلق بالكوليسترول (القيمة الاحتمالية = ٠,٠٠٧)، الدهون الثلاثية (القيمة الاحتمالية = ٠,٠١١)، البروتين الدهني مرتفع الكثافة (القيمة الاحتمالية = ٠,٠٠٠)، البروتين الدهني منخفض الكثافة (القيمة الاحتمالية = ٤ • • , •) واليوريا (القيمة الاحتمالية = • • • , •). استنتجت الدراسة إن استخدام الفيتامينات المتعددة له تأثير إيجابي كبير على معايير الدم، وكريات الدم البيضاء، وكريات الدم الحمراء، وحجم الدم الوسطى، ومحتواه، ومستوى الفوسفاتيز القلوية، والبيليرويين المباشر، واليوريا، والكوليسترول الضار. إن استخدام الفيتامينات المتعددة له تأثير معنوي في حفض مستوى الكوليسترول، والدهون الثلاثية، والكوليسترول الجيد. ولذلك توصى هذه الدر اسة باضافة الفيتامينات المتعددة لتحفين النمو والحفاظ على صحة بداري التسمين.

الكلمات المفتاحية: الفيتامينات المتعددة، المعلمات الدموية، مستويات الدهون، وظائف الكبد و الكلي، دجاج التسمين