Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Kerbala
College of Veterinary
Medicine

Supplementation of Folic Acid to Lohmann hens Dite to Optimize Eggs Quality and Health Status

Thesis Submitted to the Council of the Faculty of Veterinary Medicine / University of Kerbala in Partial Fulfillment of the Requirement for the Master Degree of Science in Veterinary Medicine / public health

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1444 A.H **2022 A.D**

{بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ}

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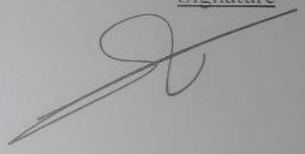

Declaration

I hereby declare that this thesis is my origin work except for equations and citations which have been fully acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University of Kerbala or other institutions.

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Dedication

Imam Mahdi, may God hasten his reappearance Oh hope of the weak on earth.

....My lovely mother ,she has always loved me unconditionally and whose good example has taught me to work hard for the things that I aspire to achieve.

....My spiritual teacher and Holy Father (Dr. amir Alshemmary) thanks dear brother for any thing.

....My husband (Dr. Alaa Hussein),I am greatly thankful for your tolerance and your kind support to successful Complete my research.

....My beloved sisters and My husband mother ,who have been constant source of help and encouragement .

....Our presence in my life is a Grace:

My children and My brother's children thank you for patience.

I owe a lot to you.

Abeer…

Acknowledgments

I thank and appreciate Alla Almighty firstly and finally who guided me to set up this search.

I would like to express my sincere thanks and gratitude to public health department specially my advisor, Dr. Yasser Jamal Jameel. He taught me a lot and helped to build up my academic background with his broad knowledge, His strong support and patience are what made this dissertation possible.

Also, I would like to thank (Dr. afrah kamel and Dr.sadiq safaa) for helping me with this research.

I also thank Al-Waeli Company for allowing me to conduct the scientific experiment in their poultry halls

Abeer……

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abbreviations	Meaning
µm	**micrometer**
cm	**centimeter**
DHF	dihydrofolate
DHFR	dihydrofolate reductase
dTMP	deoxythymidine monophosphate
dUMP	deoxyuridine monophosphate (dUMP)
ELISA	Enzyme-linked immunosorbent assay
FA	folic acid
FCR	feed conversion ratio
FOLR	folate receptor
Hcy	homocysteine
HPLC	High-performance liquid chromatography
HU	**Haugh unit**
LDL	low density lipoprotein
mm	**millimeter**
MS	methionine synthase
nadph	Nicotinamide adenine dinucleotide phosphate
ng/ml	**Nanogram per milliliter**
PABA	para-aminobenzoic acid
PCFT	Polar Capital Global Financials Trust
PLP	pyridoxal phosphate
ppm	Part per million
RFC	reduced folate carrier
SHMT	hydroxymethyltransferase
THF	Tetrahydrofolate
TS	thymidylate synthase

Abstract

This study was carried out to investigate the effect of supplementation of folic acid enriched diet on folic acid concentration of egg , serum , egg quality, immune response and intestinal morphology of laying hens .Five thousand lohmann classic brown laying hen's type were use in this experiment.

At the first period hens were fed on basal diet according to Lohmann Classic Brown nutrition requirement guide. It was contain a calculated amount from folic acid. This amount was coming from row diet premix. The Second period of the experiment all hens were fed same basal diet enriched with folic acid with 1mg/kg of feed.

Egg was collected and blood samples were collected from the Lehman's at the end of the first and second periods for measuring folic acid concentration for egg and serum by using HPLC and ELISA. Also immune response were tested by using ELISA. Egg quality were measuring by calculating of egg weight, albumin height, albumin diameter, yolk height, yolk diameter, yolk color, shell thickness and Haugh unit.

Duodenum, jejunum and ileum intestine sample were collected at the end of the first and second period for measuring villi height and crypt depth.

The results showed a significant increase of folic acid concentration of serum and egg after supplementation of 1 mg /kg feed of folic acid. ELISA serum folic acid concentration was recorded 145.4 ng/mL after supplementation of folic acid as Compare before supplementation with folic acid was recorded 79 ng/mL. On the other hand HPLC folic acid concentration after supplementation of folic acid to the diet was recorded 64.9 ppm as compare before supplementation with folic acid that was recorded 32 ppm. Egg quality was enhance after supplementation of folic acid. Egg weight, albumin height diameter, yolk height, yolk diameter, yolk color and Haugh unit were enhanced significantly ($p \leq 0.05$) after supplementation with folic acid, and egg shall thickness show no significant change between the two period.

Villi height and crept depth showed a significant difference ($p \leq 0.05$) in second periods after supplementation of folic acid compare to first period before folic

supplementation. The highest enhancement of villi hight and crypt depth was observed in duodenum and jejunum. However, Villi height and crept depth illium show no significant deference between the two periods.

The highest folic egg concentration will produce folic acid egg called egg folate this will help pregnant woman to reduce spinal cord defect in fetus. A rich laying hens diet may be enhance of egg quality, immune response to hens that consumes this diet. Gut health state may enhanced consuming of diet enrich with folic acid. Enhancing of villi hight and crypt depth lead to enhance of gut health state. This enhancement may be consuming of diet rich with folic acid.

Chapter One: Introduction

1. Introduction

Folic acid is a water-soluble Vitamin B9 which is the synthetic form folate that is found in supplements and added to fortified feeds. Folate is a generic term for both naturally occurring folate found in feeds and folic acid. Folates are vitamins that cannot be synthesized by animals (Jing *et al*., 2009).

Also humans cannot synthesize folate therefore folate has to be supplied through the diet to meet their daily requirements. The remaining amounts of the vitamin leave the body through the urine. Folic acid may also called Polyglutamyl,folacin,Pteroyl monoglutamate,Folate and vitamin B_9(Dietrich *et al*., 2005 and Ulrich and Potter., 2006).

Folic acid is essential for the normal growth and maintenance of all cells because it acts as a coenzyme for normal DNA and RNA synthesis (Duthie *et al*., 2002).And it is required in the biosynthesis of amino acids by participated in one-carbon transfer reactions which is required within the cell for purine and pyrimidine biosynthesis (DNA and RNA) and inter conversion of amino acids such as serine and glycine and for the synthesis of methionine from homocysteine (Bagley and Shane., 2005).

Folic acid have antioxidant activity to be involved in these effects of folic acid on health against reactive oxygen species (ROS) (Sarna *et al* .,2012).

Folic acid also maintenance of nervous system's integrity and decrease of neurological and neuropsychiatric disorders and reduction of the risk of neural tube defects (Gilbody *et al*., 2007; De Wals *et al*., 2007).

Folate deficiency during pregnancy may also increase the risk of preterm delivery, infant low birth weight, and fetal growth retardation so that increase folic acid will decrease neurological and neuropsychiatric disorders and cardiac diseases (YajnikandDeshmukh., 2008).

Folate has more health benefits in serum of pregnant women including their prevention of neural tube defects (NTD) in child during the first trimester (Smithells *et al*., 1976).Besides its possibility of reducing infertility also folic acid may also improve pregnancy reduce their risk of miscarriage (Grodnitskaya, and Kurtser., 2012).

It is possible to significantly increase folate concentration of eggs through fortification of the laying hen diet with synthetic crystalline Folic acid so that eggs

can be changed to one of the rich sources of natural folate (House *et al*., 2002; Hebert *et al*., 2005; Hoey *et al*., 2008; Dickson *et al*., 2010 and Tactacan *et al*., 2011).

Folate requirement recommended by NRC for laying poultry is very low (0.25 mg/kg diet). Thus, to meet the real requirement of folate in industrial poultries (Bagheri *et al*.2019).

FA supplementation improved feed efficiency over the entire production cycle of laying hens under a long term production condition (Dickson *et al*., 2010). These results were confirmed in another study which was conducted by Islam *et al*., (2009) who observed that increasing dietary methionine and FA in diet improved feed conversion ratio (FCR) and improved shell color and its thickness.

Egg shell color is an important parameter for determining egg quality. The eggshell color of eggs produced for human consumption consumer preference (Johnston *et al*., 2011). With colorful eggs potentially being considered more favorable by consumers (Ayim-Akonor and Akonor., 2014).

Aim of the study:

1- Folic acid concentration: Estimate the folic acid concentration in serum and eggs by using ELISA and HPLC.

2- Egg quality: egg weight, albumin height, albumin diameter, yolk diameter, yolk height, yolk color, egg shell thickness and Haugh unit.

3- Immune response: evaluate the antibody titer against Newcastle disease ND and Avian Influenza after 10 days of vaccinated of two periods before and after supplementation of folic acid.

4- Intestine morphology: villi height (VH) and crypt depth (CD).

Chapter Two: Review of the Related Literature

2. Review of the Related Literature

2-1-Folic acid

Folic acid is one of the B vitamins which is converted into folate by the body. It is used as a dietary supplement and in feed fortification and it is more stable during feed processing and storage (Choi *et al*., 2014).

Folate is required for the body to make DNA and RNA and metabolize amino acids necessary for cell division (Chudler and Johnson., 2019).Humans cannot make folate so which make folic acid important in our diet and it is occurs naturally in many feeds. The recommended adult daily intake of folate is 400 micrograms from feeds or dietary supplements. (Sobczyńska-Malefora and Harrington., 2018).

Folic acid has offering digestible proteins, enzyme, vitamins and co-factors as live enzyme factory (Protease, amylase and lipase) which help improving digestion, metabolism and utilization of nutrients for enhancing absorption and digestion of carbohydrates, protein and fats lead to increase feed conversion efficiency. Another hand folic acid increase beneficial bacteria which helping in synthesis of vitamins (Biotin, B1, B2, and K) and mineral metabolism which responsible for growth properties and metabolism (Kim, 2020).

2-1-1 Structure of folic acid:

The term folate represents the group of B vitamins (Vitamin B9) which have similar biological activity to folic acid. There are three major components in the parent structure of folic acid: a pteridine ring that can be either oxidized or reduced, coupled with para-aminobenzoic acid (PABA) through a methylene bridge, which is bound to glutamic acid or polyglutamate by a γ-peptide link (Tjong *et al*., 2021). Structure of an oxidized form of folate is illustrated in Figure (2-1), which can be converted to Dihydrofolic (DHF) after being reduced at the double bond present at the N-8 position. Further reduction at N-5 double bond leads to the formation of THF and in this state the N-5 of the pteridine moiety and N-10 of the PABA group may act as acceptors of single carbon units (Shams, 2022).

Folic acid is contain a fully-reduced pteridine ring together with additional glutamic acid molecules (polyglutamate) linked by γ-peptide bond (Cieślik and Cieślik., 2018).

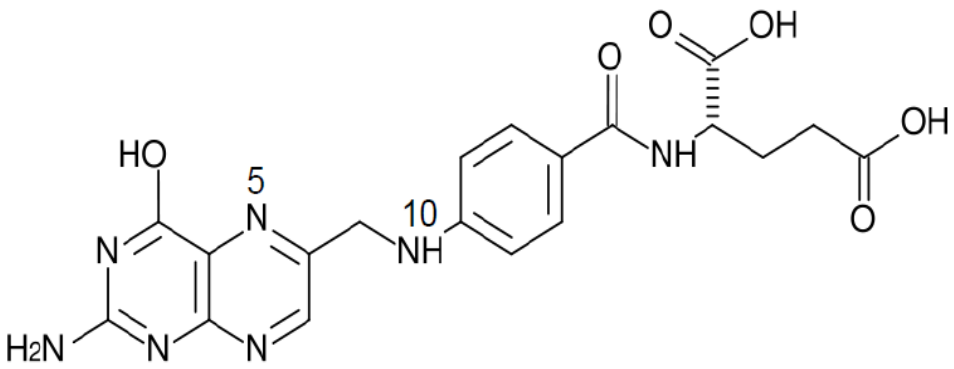

Figure (2-1): chemical structure of folic acid (Welch ,1983).

2-1-2 Absorption of folic acid:

Folic acid absorption is an active process that occurs primarily in the duodenum and jejunum. Due to prescreen of γ-glutamylcarboxy conjugase which is convert polyglutamated to monoglutamyl forms that can easily travel throw the brush border membrane of the duodenum and jejunum. The monoglutamate forms are transported across the enterocytes through the action of specific transporters: folate receptor (FOLR), reduced folate carrier (RFC), and proton-coupled folate transporter (PCFT). Once inside the enterocytes, synthetic FA is reduced to tetrahydrofolate (THF) by dihydrofolate reductase (DHFR) to gain a metabolic activity similar to other folate species (Visentin *et al*., 2014).

The absorption of the monoglutamated folate is facilitated by a pH-dependent folate transporter which transports both oxidized and reduced folates across the intestinal cell membrane (Shulpekova *et al*., 2021).

Absorbed folates are then delivered via the hepatic portal system to the liver where they are taken up by specialized transporters.

The folates that enter the liver have three potential destinations:

A- Folates can be converted to polyglutamate storage forms.

B- They can be secreted into the bile at the hepatic canalicular membrane.

C- Folate monoglutamates formed by the hydrolysis of stored polyglutamates in the hepatocytes can enter the hepatic vein and ultimately reach the systemic circulation where they can accumulate and meet the one-carbon requirements of peripheral tissues (Jing *et al*., 2010).

Folic acid has to be reduced and methylated into 5-methylTHF before it can enter the circulation, folate monoglutamates acid from FA that are not in the 5-methylTHF form are also transformed to 5-methylTHF during their passage through the liver. Therefore monoglutamated 5-methylTHF constitutes the only folate derivative appearing in the circulation after the ingestion of normal feed. When high doses of FA or other folate forms are consumed a part is absorbed by passive diffusion and appears in the peripheral circulation unchanged (Abbasi *et al*., 2018).

Thirty to forty percent of 5-methylTHF in the blood is associated with low affinity binding proteins. In most cases circulating 5-methylTHF is associated with albumin. While less frequently 5-methylTHF can attach to other proteins like α2 macroglobulin and transferrin (Bertoia *et al*., 2014).

Blood also contains a less abundant, high-affinity folate binding protein homologous to the cellular folate binding protein also known as the folate receptor as shown in figure (2-2) (Kamen and Smith., 2004).

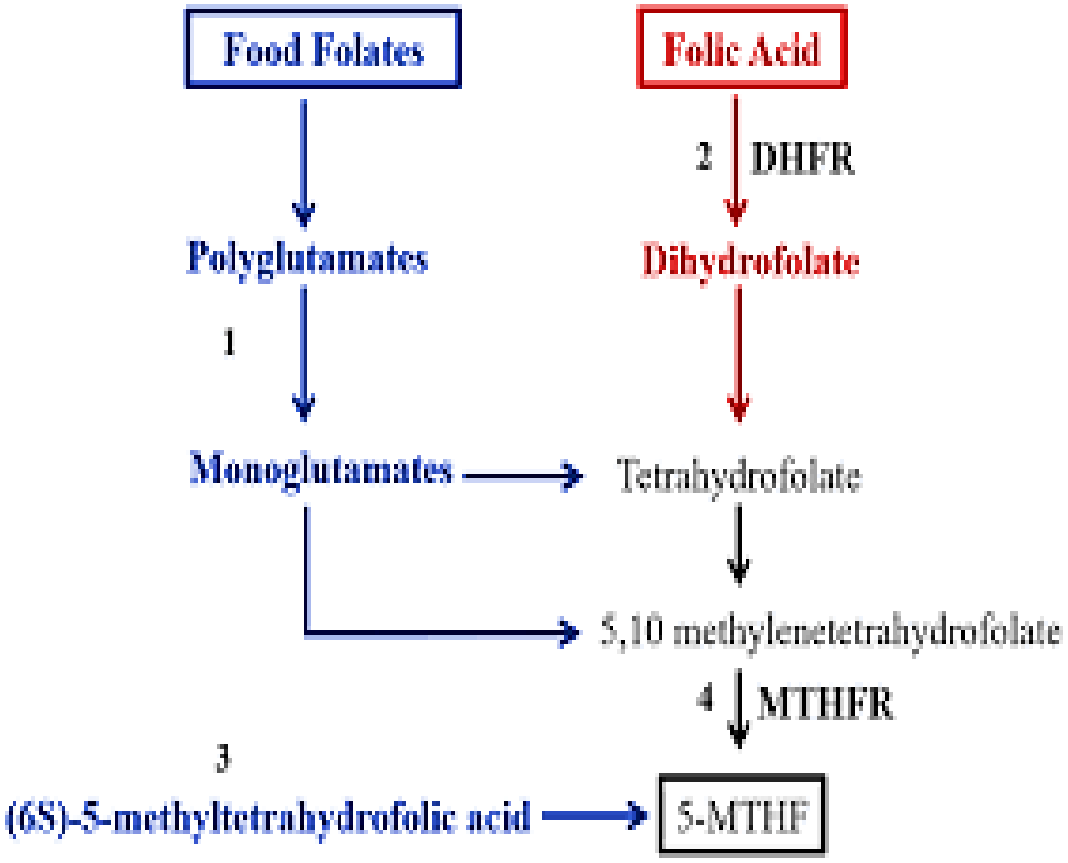

Figure (2-2) mechanism of absorption of folic acid (Cochrane *et al*.,2020)

2-1-3 Folic acid Metabolism:

The activation of the folate coenzyme is catalyzed by serine hydroxymethyltransferase (SHMT). This enzyme requires the amino acid serine to combine with pyridoxal phosphate (PLP) or vitamin B6 in order to transfer a hydroxymethyl group to THF to generate 5, 10-methyleneTHF and glycine (Perry *et al*., 2007).

The generated 5, 10-methyleneTHF from the SHMT reaction is a central compound in the folate cycle (Bagley and Shane, 2005). It either provides the supply of one-carbon groups for the formation of thymidylate for pyrimidine synthesis or it can be reduced to 5-methylTHF for use in the remethylation of homocysteine (Hcy) to methionine. In the synthesis of pyrimidine nucleotides, thymidylate synthase (TS) catalyzes the transfer of the one-carbon group (methylene) from 5, 10-methyleneTHF to the 5‟-position of deoxyuridine monophosphate (dUMP) and its reduction to a methyl group to generate deoxythymidine monophosphate (Födinger *et al* ., 2000).

The5,10 methylenetetrahydrofolate reductase (5,10-MTHFR) catalyzes the NADPH-dependent reduction of 5, 10-methyleneTHF to 5-methylTHF (Bagley and Shane, 2005).

The 5-methylTHF then provides methyl groups for the formation of methionine from Hcy in the methionine synthase (MS) catalyzed reaction. This reaction links folate metabolism to Hcy metabolism and finally allows THF to re-enter the pool of reduced folates (Škovierová *et al*., 2016).

The unsubstituted THF released from the methionine synthase catalyzed reaction is either utilized for purine nucleotide synthesis or is converted to polyglutamated forms of folates. In the synthesis of purine nucleotides, THF reacts with formate (via 10-formylTHF synthetase) to produce 10-formylTHF.The metabolism of folates into the polyglutamated form is required for the biological activity of folate as the polyglutamates are much more effective substrates for folate-dependent enzymes than are the monoglutamate derivatives . Additionally the conversion of folates to polyglutamates of chain length greater than 3 is also required for effective retention of folate by tissues(Zheng and Cantley., 2019).

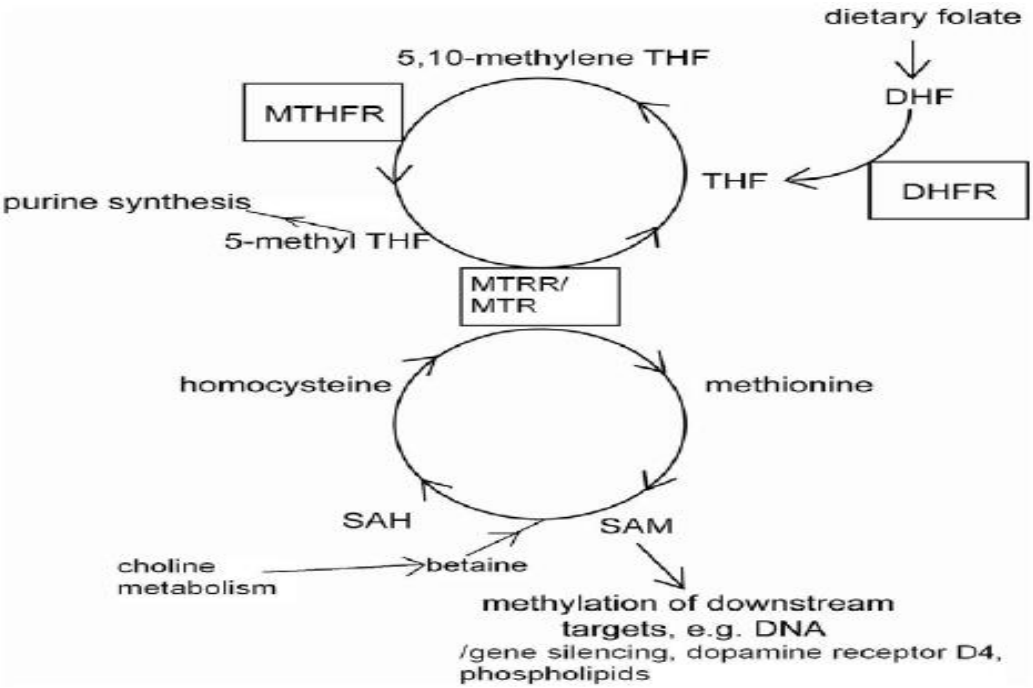

Fig (2-3): Simplified schematic of the folic acid metabolic cycle. (Spellicy, *et al*.,2012)

2-1-4 Folic acid deficiency in human:

Folate is required for a variety of bodily functions. The significance of folate in the prevention of Neural tube defects NTDs in infants sparked first interest in its health benefits (Imbard *et al*.,2013).

Neural tube defects arise when the neural tube fails to shut properly allowing amniotic fluid to enter the developing brain and spinal cord. Neural tube defects are caused by a disruption in the embryonic neurulation process. This starts at 21 days after conception and lasts for around 28 days. As a result, neurulation continues even before a woman realizes she is pregnant (Shookhoff, and Gallicano., 2010).

Neural tube defects (NTDs) are thought to be caused by a decrease in maternal and fetal folate supply and metabolism with the impacted pathways being dTMP or methionine synthesis (Stover, 2004).

Proposed mechanisms include the accumulation of Hcy , decreased rates of DNA synthesis due to impaired dTMP synthesis and hypomethylation of genomic DNA. Many genes that code for folate-dependent enzymes have been investigated for mutations that might cause NTDs (Blom *et al*., 2006).

The discovery of a definite link between poor folate consumption and the risk of cardiovascular disease piqued people's curiosity even more. Because a variety of pathways for Hcy-mediated vascular injury have been proposed. Practically all interventional investigations have focused on the link between Hcy levels and the risk of cardiovascular disease. Folate was solely thought to be useful for lowering Hcy levels. It was only a few years later that a favorable impact on vessels was linked to folate alone independent of Hcy (Andreotti *et al*., 2000).

Vascular endothelial dysfunction is a well-known essential event in the pathophysiology of atherosclerosis and a widely used surrogate end point in cardiovascular disease. This is due to folate's capacity to reduce the blood concentration of the possibly atherogenic thiol Hcy. which has been linked to cardiovascular disease etiology. In asymptomatic hyperhomocystenemia patients folic acid treatment was shown to ameliorate endothelium dysfunction. As well as in

individuals with existing coronary heart disease who are hyperhomocysteinemic (Widmer and Lerman., 2014).

patients with coronary artery disease without a placebo group in which the patients were their own controls .They discovered that folate supplementation corrected endothelial dysfunction by stimulating nitric oxide (NO) generation and inhibiting lipoprotein oxidation by FA in participants with no significant changes in Hcy levels. FA has also been shown to protect human low density lipoprotein (LDL) from oxidative changes (Yi *et al*., 2014 and Stanhewicz, and Kenney., 2017).

The advantages of folate to one's health go well beyond these two critical disorders. Ailments such as cancer are now recognized to be influenced by either low folate levels or variations in the genes that code for folate transporters and folate-dependent enzymes (Bailey *et al*., 2015).

The molecular processes behind the link between folate deficit and cancer risk are unknown however two theories have been proposed:

(1) Increased uracil misincorporation into DNA as a result of reduced dTMP synthesis.

(2) Decreased DNA methylation as a result of reduced SAM synthesis (Blount *et al*., 1997).

Because the sole difference between uracil and thymidine is a single methyl group Uracil is mistakenly integrated into DNA instead of thymidine. However uracil is promptly eliminated by DNA repair enzymes during DNA synthesis resulting in a single-strand break in the DNA molecule. This cycle of DNA breaking and repair continues if folate levels are consistently low leading to DNA double-strand breaks, chromosomal abnormalities, and malignant transformation (Poole and Logan., 2005 and Hazra, *et al*.,2010).

In response to experimental folate shortage, uracil misincorporation, genome-wide DNA strand breaks, and chromosomal instability have been seen in a variety of cell culture types, including human lymphocytes, human colonocytes, and Chinese hamster ovary cells. Furthermore, human cells cultured in low-folate environments

had more gene-specific DNA strand breaks in the p53 tumor suppressor gene. As well as a three-fold increase in micronuclei frequency (Hazra., *et al*.,2010; McGlynn *et al*.,2013).

Folic acid as antioxidant activities are comparable to those of vitamin C and E which are commonly accepted as the effective water and lipid soluble antioxidants. Although natural folates cannot be really considered as feed antioxidants they may act as effective antioxidants in vivo (Gliszczyńska-Świgło., 2007).

2-2-Folic acid in laying hen:

2-2-1 Absorption and metabolism of dietary folic acid in the laying hen:

The availability of nutrients derived from the meal is determined by the rate of absorption in the epithelial cells of the intestine. Folic acid is absorbed from the stomach by a membrane-bound folate transport mechanism that takes both oxidized and reduced forms of monoglutamated folate. In a variety of model systems. This mechanism has been demonstrated to be saturable and so constitutes a possible control point for blood folate concentration (Visentin *et al*.,2014; Alpers 2016).

Reduced folate carrier (RFC) has long been thought to be the molecular entity of the carrier-mediated intestinal folate transport mechanism before proton-coupled folate transporter (PCFT) was discovered (Zhao *et al*., 2011).However its significance in intestinal folate absorption has been questioned since RFC-mediated transport works best at near-neutral pH, but the intestinal folate transport system works best at acid pH (Zhao *et al*., 2009).

Polar Capital Global Financials Trust (PCFT) was recently discovered to be a proton-dependent high-affinity FA transporter with similar features to the intestinal folate transport system (Zhao and Goldman 2013).

As FA passes the mucosal cells of the intestine, a series of metabolic reactions occurs since it is not a natural folate metabolite. This is necessary for the conversion of dietary FAs into physiologically active forms. Folate-dependent enzymes are a set of enzymes engaged in this bioconversion,absorbed FA is reduced subsequently to

DHF and THF in the intestinal cells and hepatocytes by dihydrofolate reductase DHFR enzyme.(Alpers, 2016).

Tetrahydrofolate combine with a hydroxymethyl group from serine to produce 5, 10-methyleneTHF and glycine in a reaction catalyzed by SHMT, a PLP-dependent enzyme. The generated 5, 10-methyleneTHF is then reduced even more by 5, 10-MTHFR to 5-methylTHF (Ferrazzi *et al*., 2020).

The physiologically transportable form of folate which is secreted and reabsorbed into the small intestine with the bile (enterohepatic cycle) before being transferred by a folate binding protein to the yolk. Therefore the efficient conversion of dietary supplied FA into its biologically active form. In particular the 5-methylTHF is a prerequisite for its eventual transfer and deposition into eggs (Hebert *et al*., 2005).

Observed the same pattern in blood folate concentration which showed saturation nature when laying hens were fed diets high in FA. So due to the saturation of blood which serves as the precursor for egg folate deposition. Identification of the points which has an important role in folate absorption from alimentary system such as enzymes (polyglutamylfolate deconjugation) and intestinal pH is essential.Demonstrated a FA transport system in the entire intestine of hens. Maximum uptake rate of FAwas observed at acidic pH 6.0 and was increased in the duodenum and jejunum and decreased in the ileum and ceca (Tactacan *et al*., 2011).

2-2-2 Deposition of the folate in the egg:

Effective way of enhancing feed with natural folate is through the enrichment of folate in eggs (Dickson *et al*., 2010).

Five methyl tetrahydrofolate (5-MTHF) concentration in non-enriched eggs was very low (2.3 mg/egg) opposite to egg that hens was supplied by folic acid which show (17.5, 16.7, 28.2 and 15.3) mg/egg reported by(House *et al*.,2002; Hebert *et al*.,2005; Dickson *et al* .,2010 and Tactacan *et al*., 2011).

Conducted dose-response studies to determine the optimal dietary FA levels required for maximal egg folate deposition and found a linear increase in egg folate

concentrations when crystalline FA was added from 0 - 4 mg/kg in the laying hen diet.

However, at higher levels of FA supplementation, the efficiency of FA deposition in eggs dropped (House *et al*. (2002).

Circulating folate in the blood serves as the precursor pool for egg folate deposition, the observed saturation in blood folate concentration may be regulating the saturation in egg folate concentration. Therefore by identifying the control points and understanding the mechanisms at which they regulate the blood plasma folate levels better insights on ways to further increase the level of egg folate concentration may be elucidated Through supplementation of 4 mg FA/kg of diet, and fed for at least 3 weeks (Hebert *et al*., 2005).

The level of folate concentration in the egg can be increased by about 2 to 2.5 fold as compared with birds fed the basal diet only. More importantly the majority of the supplemented FA is converted to the natural form of folate. in the total folate composition of the egg more than 80% is in the form of 5-methylTHF and less than 10% is FA(Dickson *et al*.,2010).

When stored for 4 weeks at 4 ºC folate-enriched eggs experienced no change in folate levels relative to freshly collected eggs. These conditions correspond with the typical storage of commercial eggs and thus indicate that storage losses for folate-enriched egg should not be of any concern. Relative to green leafy vegetables, folates in animal products including eggs are also generally more stable during cooking (Altic *et al*.,2016).

More than 95% of folates in egg are located in the yolk most of the eggs folic acid are derived from dietary FA and those that originate from feed ingredients, including 5-methylTHF or other natural derivatives of folate that are ultimately converted to 5-methylTHF. Circulating blood 5-methylTHF from the diet and from folate stores within various tissues of the laying hen is transferred to specific sites within the egg (Bagheri *et al*., 2019).

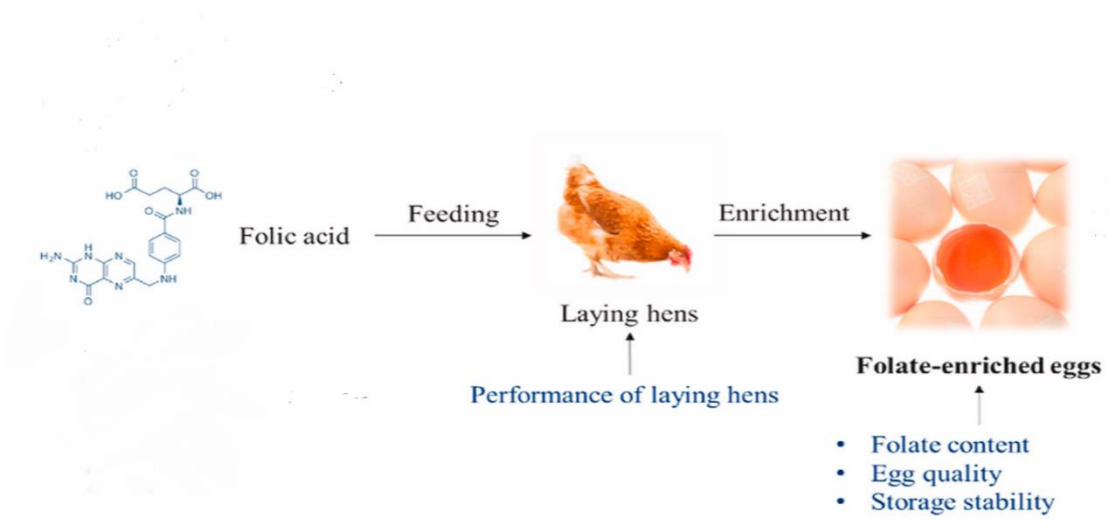

Figure (2-4): Simplified schematic of deposition of the folate in the egg (Gu *et al*., 2022).

2-2-3 Acceptability and cost

The latest evidence suggests that egg consumption up to six times per week is not associated with an increased risk of heart disease due to the miss understanding about fear that egg cholesterol may raise their blood cholesterol levels (Djoussé and Gaziano ,. 2008).

The egg industry has been very responsive in seeking new technology in exploiting eggs beyond their traditional feed value. Several specialty eggs are now being marketed routinely. Eggs enriched with omega-3 and selenium, both enhanced through the hens diet, are now marketed routinely in many parts of the world (Bhat *et al*., 2013).As much as both were able to find a niche in the specialty egg market it is likely that folate-enriched eggs will also be able to create a market of its own (Altic *et al*., 2016).

The cost of FA per 1000 kg of feed is approximately $ 9.14 based on a price of $ 228.50 per 100 gm of crystalline FA (Sigma-Aldrich Canada Ltd.). Therefore the cost of average egg weight of 60 gm, require only 0.09 cents per egg of FA supplementation (Tactacan *et al*. (2011).

2-3 Effect of folic acid on Immun-response:

The various antibodies produced by plasma cells are classified by isotype, each of which differs in function and antigen responses primarily due to structure variability. Five major antibody classes have been identified in placental mammals: IgA, IgD, IgE, IgG and IgM. This classification is based on differences in amino acid sequence in the constant region (Fc) of the antibody heavy chains. IgG and IgA are further grouped into subclasses (e.g., in human IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) based on additional small differences in the amino acid heavy chain sequences. Based on differences in the amino acid sequence in the constant region of the light chain, immunoglobulins can be further sub-classified by Estimation of the type of light chain (kappa light chain or lambda light chain) (Bhattacharya, 2008).

Cell-mediated immunity is especially affected by folate deficiency: the blastogenic response of T lymphocytes to certain mitogens is decreased in folate-deficient humans and animals, and the thymus is preferentially altered. The effects of folic acid deficiency upon humoral immunity have been more thoroughly investigated in animals than in humans, and the antibody responses to several antigens have been shown to decrease. Conversely, the phagocytic and bactericidal capacities of polymorphonuclear cells have been studied mainly in folate-deficient humans (Saeed *et al*., 2016).

Folic acid plays a crucial role in DNA and protein synthesis, suggesting that every mechanism in which cell proliferation intervenes may be altered (Wang *et al*., 2019).

The folic acid have been suggested to stimulate of immune system resulting in increase of Y-interferon production, higher production of immunoglobulin and stimulation macrophage and lymphocyte activity. Effect strives on many cell type including the adaptive and innate response such as dendritic cells, B- cell, epithelial cells, monocytes/macrophage, NK cells T-cell, involving T-cells with regulatory properties (Cianciulli *et al*., 2016 and Khan *et al*., 2020).

Also folic acid Its play important role for enhancing the three primary defense system in body defenses against pathogens (the intestinal microbiota) as regeneration immune function and epithelial cells (Maggini *et al*., 2018).

Folic acid has been demonstrated that there are three different pathways of immune system for enhancing macrophage activity that increases the ability to intestinal microbiota, increased resistance to infection and increased production of antibodies as immunoglobulin G and M **(**Tourkochristou *et al*., 2021).

Folic acid shown to stimulation of immune system as result result from increase of T-lymphocyte, serum protein levels and phagocytosis cells moreover, its stimulate immune cells to secrete cytokine Furthermore, it's have immunomodulatory effect are related to interact with gut epithelial cells and then stimulated the anti-inflammatory cytokines productions (Mikkelsen and Apostolopoulos., 2019).

Folic acid deficiency also inhibits the activity of CD8+ T cells and reduces the proliferative responses of lymphocytes and natural killer cell activity.In turn this inhibition is associated with decreased resistance to infections (Kunisawa *et al*.,2012).

Folic acid deficiency leads to various impairments of immunity, such as lymphoid atrophy and reduced numbers of lymphocytes. Supplementation bolsters these weakened immune responses (Mansouri *et al*., 2016).

Evidence has long indicated that in adequate vitamin intake disrupts host immunity. Accumulating evidence has revealed molecular and cellular mechanisms underlying myriad functions of vitamins in innate and acquired immune responses. Growing interest exists in the physiologic role of folic acid as an essential mediator in maintaining a healthy and functional immune system (Gombart *et al*., 2020).

2-4 Effect of folic acid of the laying hens intestine morphology:

Folic acid is on the feed vitamins which stimulate the growth, activity of microflora in gastrointestinal tract, composition, and improve hosts health and well-being by stimulate growth and activity of beneficial microorganism in gastrointestinal microflora (Rossi *et al*., 2011).

Studies has been found that folic acid increase oligosaccharide resulting from presence of beneficial intestinal microflora in colon which include increasing Ca absorption, shortening gastrointestinal transit time, increasing fecal weight, increasing the numbers of probiotics in the colon and lowering blood lipid levels that confers beneficial effects to humans (Rowland *et al*.,2018).

Folic acid have been reported that restricted the amount of bacteria-generated toxic metabolites that lead to reduce the growth of pathogenic bacteria colonies and this help in resistance of the different diseases like (*Clostridium*, *Enterococcus, Enterobacteriacea, Bacteroides* etc.) and this improve the immunological state of the bird **(**Olgun.,2017).

Folic acid have a significant increase in effect on villis depth and height that Which lead to betterment of the intestinal mucosa resulting from increase the absorption of nutrient due to an increased villi height **(**Li *et al*., 2020).

However nutrients absorption may be influenced as result of villi high reduction. Therefore poor absorption of nutrient, resulting from shortening of the villi and reduction of crypt depth which contribute to increased energy and protein using and-accordingly-result in a deterioration of the production parameters (Dailey,2014).

The advantages of folic acid increase growth rate and improve productivity have been reported enhance digestibility and utilization of nutrients, reduce stress after transportation, stimulation of immune responses, inhibition of organism and antibiotics therapy of vaccination by (Lauridsen *et al*., 2021) .

2-5 Effect of folic acid of the laying hens in egg quality:

The prevue results demonstrate that laying hens have the capacity to convert high doses of FA added to their feed into natural folates in their eggs. For optimal enhancement of egg folate concentration.(Hoey,*et al*., 2008 and Tactacan *et al*., 2011) which showed that it is feasible to produce eggs with an enriched total folate concentration by the addition of FA to the feed of laying hens.

Hen eggs are among the most commonly eaten feeds worldwide, as they have a high nutritional value, cheap costs, and are widely employed in international cuisines.

They consist of two parts: The egg white, which mainly consists of 85% water and 10% proteins (ovalbumin being the most abundant one) approximately, and the egg yolk, which is composed of almost 22% lipids (Cherian *et al*., 2002 and Fredriksson *et al*., 2006).

Folic acid also increase in the egg weight by increase in albumen and egg yolk which are directly related to egg weight (Abaş *et al*., 2008 and Eseceli *et al*., 2010).As well as the increase of proportion the folic acid that eaten, it can lead to an increase in the proportion of albumin in the blood and serum, which is a good indicator in laying hens because this increase can lead to an increase in the albumin amount and weight of the egg (Munyaka, *et al*., 2012;Chilom *et al*., 2018 and Sun *et al*., 2019).

Albumin height and Albumin diameter may be increased due to the increase in the albumin amount in the egg. Since that folate play important role in the protein synthesis in the body. Folate has an essential role in one-carbon metabolism and is a strong anti-proliferative agent. Folate increases DNA stability, being crucial for DNA synthesis and repair, the methylation cycle, and preventing oxidation of DNA by free radicals (Abbasi *et al*., 2018).

Folic acid play an important role in increase of albumin due to the folic acid helps in increase the proportion of estrogen. Estrogen plays a key role to increase the proportion of egg albums which leads to an increase in the height and diameter of the egg albums (Wallock-Montelius, *et al*., 2007).also increase egg weight so that adequate folic acid with the hen impairs the oviducts response to estrogen and ability to form albumen (Gaskins *et al*., 2012).

Li *et al*. (2020) found that after supplementation of folic acid the concentration of protoporphyrin 9 in the eggshell and eggshell gland of hens laying darker colored eggs was higher than that in hens laying eggs of a lighter color. In addition folic acid used with magnesium which is increase egg shale strength and the folic acid.

Benkova *et al* (2009) showed that fed laying hens with diets supplemented with FA indicated higher yolk color .

The Pigments that impart a yellow or orange color to egg yolk belong to a carotenoids group called xanthophylls. The most important xanthophylls for egg yolk coloration are zeaxanthin and lutein (Zaheer,. 2017).Lutein and zeaxanthin constitute the main carotenoids in egg yolk. Lutein and zeaxanthin are absorbed by the mucosa of the small intestine via passive diffusion, incorporated into chylomicrons and transported via the lymphatic system to the liver. Finally, in the hepatocytes, lutein is incorporated into low-density and high-density lipoproteins and transported to target tissues (Reboul, 2019).

The eggshell color is an important measure of egg quality for brown-egg laying hens because consumers have low preferences on light and pale brown-colored eggs. It is suggested that impaired eggshell coloration in brown eggs is often associated with an increase in age, stress and respiratory disease (Samiullah *et al*., 2015). Therefore, appropriate management practices to reduce stress and improve health of laying hens are required to prevent the production of poor eggshell color as hens become aged. Additionally, various nutritional strategies should be developed and implemented to increase brown eggshell coloration because dietary regimens may be easier to be adapted in a practical circumstance (Pitargue *et al*., 2017).

On the basis eggshell pigment properties such as photoactive antimicrobial defense and high affinity to eggshell protein. It is believed protoporphyrin 9 may provide a microbial barrier and enhance eggshell thickness. Whereas eggshell biliverdin may facilitate the development of embryos owing to its anti-oxidative potential (Fargallo *et al*., 2014). Protection against UV radiation (Maurer *et al*., 2011). And high permeability (Morales., 2020). In addition it has been reported that the biliverdin concentration of eggshells can reflect the physiological status of females during laying. Collectively these observations would thus tend to indicate that sexual signaling and the physiological and mechanical properties of shell pigments could to varying extents explain the function of eggshell color in avian eggs (Hargitai *et al*., 2016).

The main brown-colored pigment in eggshells is protoporphyrin 9 (PP9) produced from the hen’s eggshell gland. The PP9 in eggshells was derived from aging or spent erythrocytes during heme metabolism. It can be expected, therefore, that

dietary factors affecting heme metabolism such as PP9 synthesis may modify eggshell coloration of brown-egg laying he. (Samiullah., *et al* .,2015).

Folic acid or folates may promote porphyrin formation after application of ALA or ALA esters: by enhancing their uptake in tissues and by interacting positively with the ratelimiting enzyme PBGD, It seems that folic acid acts by facilitating the passage of ALA or MAL into cells (Ma *et al*., 2006).

Protoporphyrin IX is initially synthesized in the eggshell gland. It has, however, also been proposed that protoporphyrin IX is derived from free or aging erythrocytes.In a destruction process during which the heme of erythrocytes is degraded (Wang *et al*., 2009). However given the lack of evidence for an associated metabolic pathway. This hypothesis needs to be further assessed.

Haugh unit is one of the inner egg quality parameters. Haugh unit is standard method used for determining interior egg quality while being more sensitive than other usual methods (Keener *et al*, 2006).

Folic acid helps in increase the proportion of estrogen. Estrogen plays a key role to increase the proportion of egg albums, which leads to an increase in the height and diameter of the egg white (Wallock-Montelius, *et al*., 2007).

Chapter Three: Methodology

3. Methodology:

3-1 Experiment design:

Five thousand laying hens Lohmann Classic Brown type were reared for table egg production at AL –Diwaniya province, Albudayr city, private layer hen's house. This study was carried out for three months divided into two periods as in figure(3-1).

The first period extended from 11/12/2021 to 25/1/2022 (before supplementation folic acid) and the age of the hens were 39 to 45 weeks old. All hens were fed basal diet according to Lohmann Classic Brown nutrition requirement guide. Basal ration contain a calculated amount from folic acid.This amount found in raw material ingredient also, included in Alwafi production premix of vitamins and minerals (80 mg/100 kg premix).

Second period extended from 25/1/2022 to10/3/2022 (after supplementation of folic acid).All hens were fed same basal ration administrated 1mg /kg folic acid (Province supplier in Erbil city, Iraq).This products was manufacture by Brgan. Co. The Netherland (500mg/kg) of product folic acid was provided to basal diet. The basal diet ingredient is shown in appendix VII

As a routines all hens were received live attenuated Newcastle disease vaccine strain clan (VAXXON® clan) from vaccination international BV, the Netherland every month. Hens received the vaccine by spry method on 10 days before the end of the first and second periods.

3-2 Collection of the blood samples:

At the end of the first and second periods (10 days after vaccination) blood samples were collected from twenty different hens. Five ml (5 ml) of blood was drawn from the wing vein. And sterile Medical sterile syringes of 5 ml were used. The blood was placed in special gel tube not containing an anticoagulant.

The serum was separated by a centrifuge at a speed of 3000 r / min for 5 minutes. The separated serum put in an Eppendorf's tubes and kept in freeze at -20 ° C until the completion of the measurements for calculating plasma folic acid and to measure anti-body titer against ND and AI viruses by using ELISA.

Experiment design scheme:

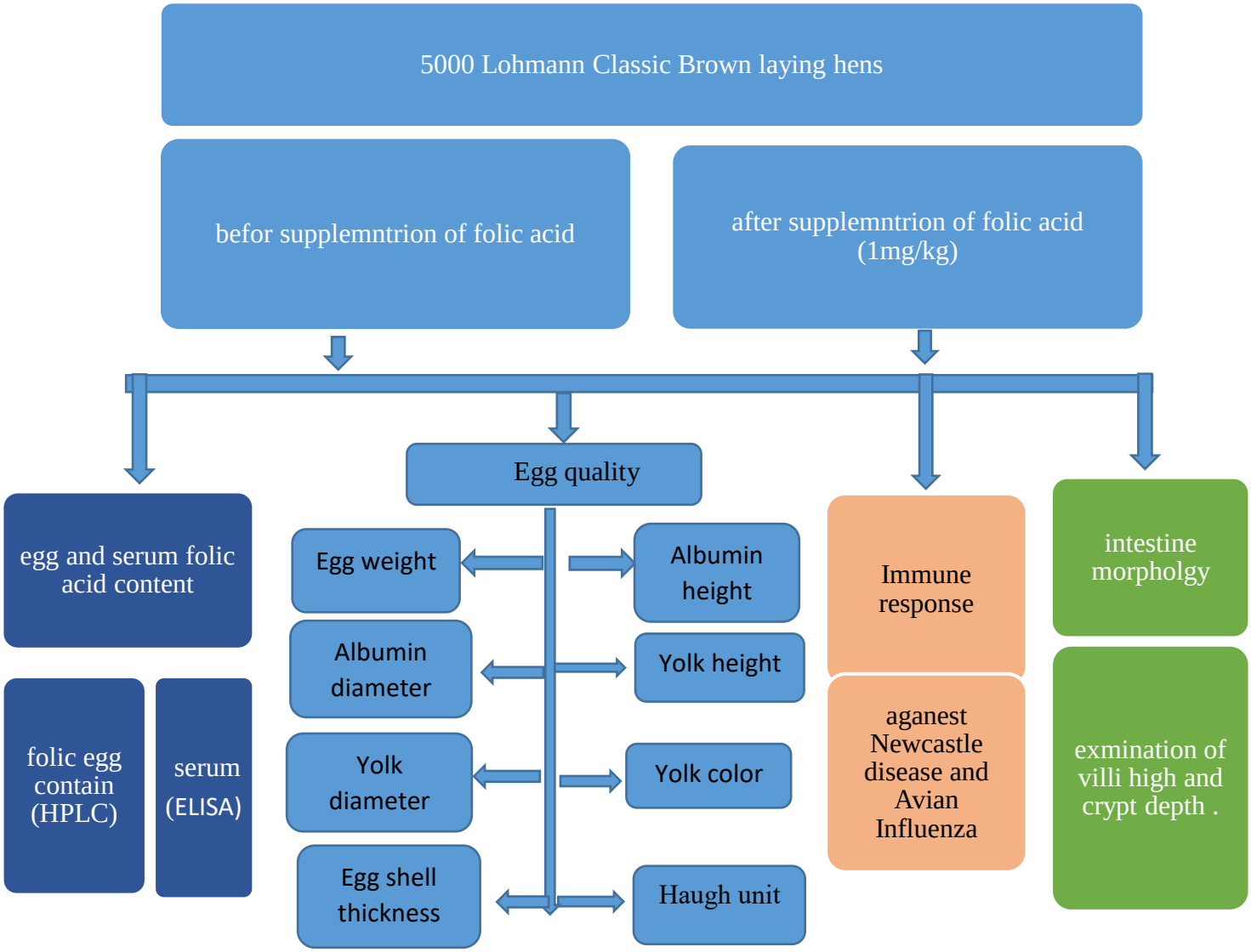

Figure 3.1: Experiment design diagram

3.3 Vaccination programs:

All birds were vaccinated with commercial Newcastle Disease attenuated vaccine NOBILIS® + CLONE 30 from MSD Animal Health Company at 15/1/2022 on (45) weeks of age by spray method for ND. We dissolve 1000 doses/L of distal water and set the nozzle to produce course spray by using (aerosol generators).The immune test was performed on the birds ten days after giving the vaccine on day 25/1/2022 to measure the titer of antibodies. The same program was given six weeks after the first vaccination on day1/3/2022 then the immune response was examined after ten days. And it was on 11/3/2022 to measure the titer of antibodies.vaccine program is shown in appendix VI

3.4 Organs collection for histological section:

Intestine section was collected at the end of the first and second period of the experiment; Hens were sacrificed by anesthesia using chloroform. The hens were dissected to remove samples (duodenum, jejunum and ileum). All organs were preserved in formalin at a concentration of 10% in clean plastic containers after numbering them until perform the histological section.

3.5 Instruments and equipment:

Instruments and equipment used in this study shown in table (3-1)

Table (3-1): Instruments and equipment:

Instruments and equipment	Sources
Centrifuge	Japan
Cooler box	China
Deposable syringe (5) ml	China
Disposable gloves	China
Eppendorf tubes and tips	China
Graduated glass pipettes size (2, 5, 10) cc	Silber ® -brand/Germany
Medical cotton	Turkey
Multi-channel pipette type-12	Transferpette ® -BRAND /Germany
Refrigerator	Beko ® Turkey
Sensitive electrical balance	Mettler, Switzerland
Single channel pipette (micropipette 1-50) microliter)	Transferpette ® -BRAND/ Germany
Sterile glass tube without anticoagulant	Venoject ® Terumo / Belgium
tripod micrometer	Ames Co USA
Test tube rack (stainless steel)	Germany

3.6 Laboratory chemicals and reagents:

Laboratory chemicals and reagents used in this study shown in table (3-1).

Table (3-2): Laboratory chemicals and reagents

chemicals and reagents	Sources
Absolut ethanol	haymankimia Uk
Avian Influenza antibody ELISA kit	green spring USA
Eosin Stain	Himedia Lab India
folic acid Hplc kit	Sykam Germany
Folic acid ELISA kit	Elabscience USA
Formalin	Chemanol SA
Hematoxylin Stain	Himedia Lab India
Newcastle antibody ELISA kit	green spring USA
Paraffin wax	Citotest China
Xylol	Alph chemika India

3.7 Estimation of folic acid:

3.7.1- Serum folic acid concentration (ELISA):

Serum folic acid contain was detected by use Enzyme Linked Immunosorbent Assay kit as shown in **appendix I.**

3.7.2 Egg folic acid concentration (HPLC):

Estimation egg folic acid was detected by use (HPLC) as shown in **appendix II.**

3.8 Estimation of Egg quality:

3.8.1. Estimation of Egg weight:

Measurement of egg weight by using an electronic balance.

3.8.2. Measurement of albumin height and albumin diameter:

Albumen height was measured by using a tripod micrometer and albumen diameter was measured by using venia caliper.

Figure (3-2): tripod micrometer

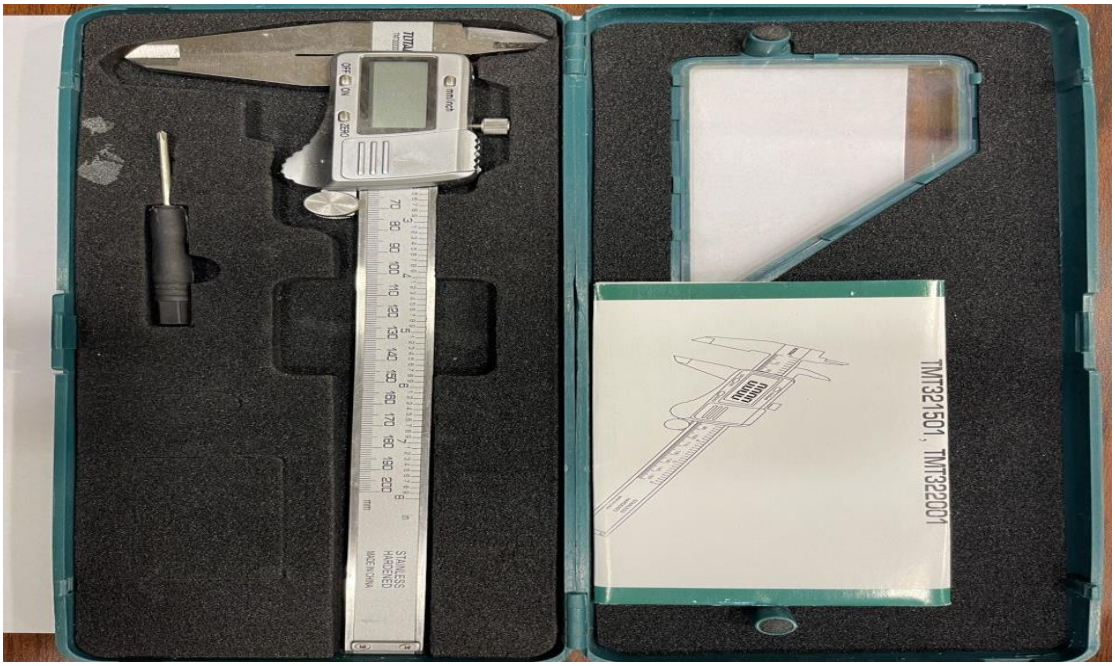

Figure (3-3):Venia caliper

3.8.3 Estimation of yolk height and yolk diameter:

The yolk diameter was measured with vernier caliper and yolk height was measured by using tripod micrometer after the eggs were broken out on a flat mirror.

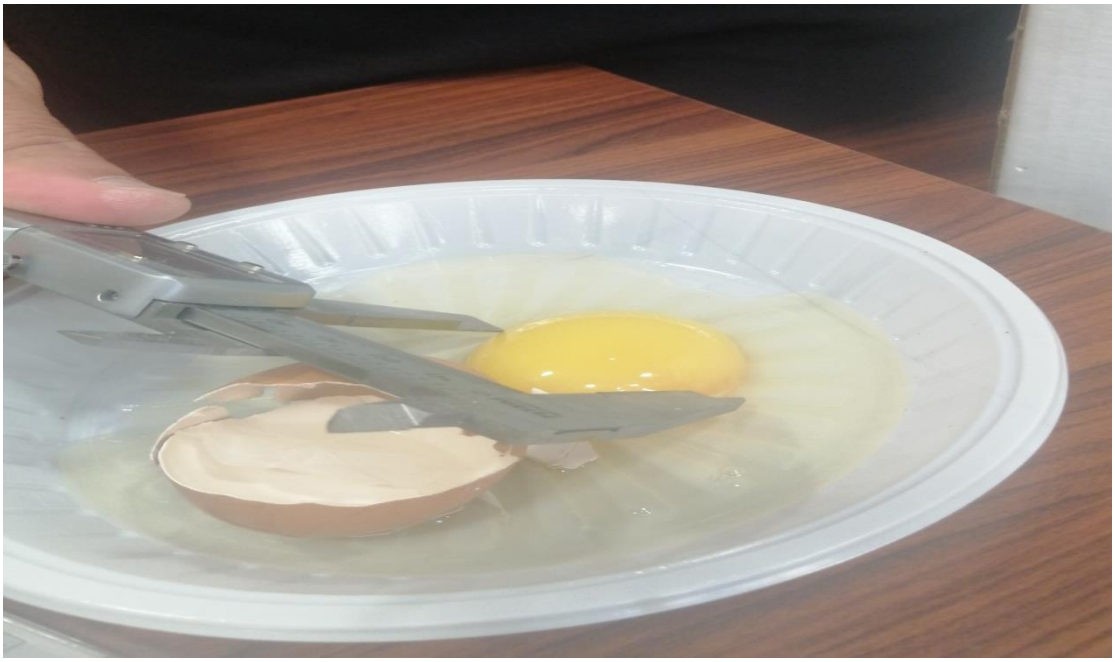

Figure (3-4): Estimation of yolk height and yolk diameter

3.8.4 Estimation of yolk color:

Yolk color (YC) was measured by Roch Color Scale which has 15 color gradation from very pale to deep yellow (North and Bell, 1990).

Figure (3-5): Estimation of yolk color

Figure (3-6): Roch Color Scale

3.8.5 Estimation of egg shell thickness:

Shell thickness was measured using gauge by taking the thickness from the narrow side (sharp region), the middle side (equatorial region) and the broad-end side (blunt region) of eggs. Finally, average shell thickness was calculated as average of these three measurements.

3.8.6 Estimation of Haugh unit:

The Haugh unit (HU) values were calculated from the egg weight (EW) and albumen height (AH) using the following formula as Estimation in (Silversides and Budgell, 2004).

$HU = 100 * \log10 (h - 1.7w^{0.37} + 7.6)$

Where: - HU = Haugh unit,

H = Albumen height in mille meter

W = Weight of eggs in grams.

3.9 Serological test: Enzyme Linked Immunosorbent Assay (ELISA) for IgG Ab titer:

3.9.1 ND Immune response:

Antibody titers against Newcastle Disease Virus in laying hens serum samples were detected before and after administrated of folic acid by using Enzyme Linked Immunosorbent Assay for each strain and the two periods, as shown in **appendix III.**

3.9.2 Avian Influenza immune response:

Antibody titers against Avian Influenza virus of laying hens serum samples were detected before and after administrated of folic acid by using Enzyme Linked Immunosorbent Assay kit as shown in **appendix IV.**

3.10 Histological examination:

Tissue sample parts were cut by rotary microtome and loaded onto glass slides and put on a hot plate at a temperature of 37 ° C until dry and the textile slides were then stained by (H & E) according to the method mentioned in (Bancroft et al.,2013).

Appendix V.

After the staining was completed the slide was examined by using a light microscope and a graduated lens was used to measure the villi height and crypt depth under magnified to X 10.

3.11 Statistical analysis:

Paired t-test was used to determine the amount of differences between the measurement of data before and after supplementation of folic acid to basal diet by using of SPSS.

Chapter Four: Results and Analysis

4. Results and Analysis

4.1. Effect of supplementation of folic acid on serum and egg folic concentration:

Table (4-1).showed that there was a significant (P≤ 0.05) increase in the serum folic acid after supplementation of folic acid as compare with before supplementation of folic acid. Serum folic acid registered 79.3 ng/ml before and 145.4 ng/ml after supplementation of folic acid.

Table (4-1).showed that there was a significant (P≤ 0.05) increase in the egg folic acid concentration after supplementation of folic acid as Compare with before supplementation of folic acid. Egg folic acid concentration registered 32.8 ppm and 64.9 ppm after supplementation of folic acid.

Table (4-1) Effect of supplementation of folic acid on Serum folic acid and Egg folic acid concentration (mean ±SE).

Parameter	Before supplementation of folic acid	after supplementation of folic acid
Serum folic acid ng/ml	79.3±0.57 B	145.4±1.39 A
Egg folic acid (ppm)	32.8±0.2 B	64.9±0.24 A

Deferent capital letters in the same row showed a significant difference (P≤ 0.05)

4.2. Effect of supplementation of folic acid on egg quality:

4.2.1. Effect of supplementation of folic acid on egg weight, Albumin height, Albumin diameter, yolk height, yolk diameter, yolk color, egg shell thickness and Haugh unit :

Table (4-2). Effect of supplementation of folic acid on egg quality(mean ±SE).

Parameter	Before supplementation of folic acid	After supplementation of folic acid
Egg weight(gm)	61.74±0.17 B	64.23±0.13 A
Albumin height (mm)	7.55±0.15 B	9.94±0.14 A
Albumin diameter(cm)	6.84±3.45 B	7.51±1.42 A
Yolk height (mm)	15.12±0.75 B	17.31±0.12 A
Yolk diameter(mm)	40±0.05 B	41.99±0.06 A
Yolk color	5.01±0.01 B	5.95±0.11 A
Shell thickness(mm)	0.317±0.002A	0.314±0.001 A
Haugh unit	84.78±0.74 B	98.527 ±0.2 A

Deferent capital letters in the same row showed a significant difference ($P \leq 0.05$).

Egg weigh showed that there was a significant ($P \leq 0.05$) increase after supplementation of folic acid as compare with before supplementation folic acid. Egg weight registered 61.74 gm before and 64.23 gm after supplementation of folic acid.

Albumin height showed that there was a significant ($P \leq 0.05$) increase after supplementation of folic acid as compare with before supplementation of folic acid. Albumin height registered 7.55 mm before and 9.94mm after supplementation of folic acid.

Also, **Albumin diameter** showed that there was a significant ($P \leq 0.05$) increase after supplementation of folic acid as compare with before supplementation of folic acid. Albumin diameter registered 6.84 cm before and 7.51 cm after supplementation of folic acid.

Yolk height showed that there was a significant (P≤ 0.05) increase after supplementation of folic acid as compare with before supplementation of folic acid. Yolk height registered 15.12mm before and 17.31mm after supplementation of folic acid.

However, **yolk diameter** showed that there was a significant (P≤ 0.05) increase after supplementation of folic acid as compare with before supplementation of folic acid. **Yolk diameter** registered 40 mm before and 41.99 mm after supplementation of folic acid.

Yolk color showed that there was a significant (P≤ 0.05) increase after supplementation of folic acid as compare with before supplementation of folic acid. **Yolk color** registered 5.01 before and 5.95 after supplementation of folic acid.

Egg shell thickness showed there was no significant difference (P> 0.05) after supplementation of folic acid as compare with before supplementing of folic acid. **Egg shell thickness** registered 0.317 mm before and 0.314 mm after supplementation of folic acid.

Haugh unit showed that there was a significant (P≤ 0.05) increase after supplementation of folic acid as compare with before supplementation of folic acid. **Haugh unit** registered 84.78 before and 98.527 after supplementation of folic acid.

4.3. Effect of supplementation of folic acid on intestinal morphology:

4.3.1 Effect of supplementation of folic acid on intestinal villi height (duodenum, jejunum and ileum):

The result showed that there was a significant (P≤ 0.05) increase in the intestinal **duodenum Villi height** after supplementation of folic acid as compare with before supplementation of folic acid. **Intestinal duodenum villi height** registered 991 µm before and 1207.7 µm after supplementation of folic acid.

The result showed that there was a significant (P≤ 0.05) increase in the intestinal **jejunum villi height** after supplementation of folic acid as compare with before supplementation of folic acid. **Intestinal jejunum villi height** registered 858 µm before and 997.5 µm after supplementation of folic acid.

While, showed there was no significant difference (P> 0.05) in the **intestinal ileum villi height** after supplementation of folic acid as compare with before supplementing of folic acid. **Intestinal ileum villi height** registered 676.1 µm before and 751.7 µm after supplementation of folic acid.

Table (4-3). Effect after supplementation of folic acid on intestinal villi height (mean ±SE)

Parameter	before supplementation of folic acid	after supplementation of folic acid
duodenum villi height (µm)	991±0.34 B	1207.7±0.33 A
jejunum villi height (µm)	858±0.51 B	997.5±0.76 A
ileum villi height (µm)	676.1±0.6 A	751.7±0.49 A

Deferent capital letters in the same row showed a significant difference (P≤ 0.05)

4.3.2 Effect of supplementation of folic acid on intestinal crypt depth (duodenum, jejunum and ileum):

The result showed that there was a significant (P≤ 0.05) increase in the intestinal **duodenum crypt depth** after supplementation of folic acid as compare with before supplementation of folic acid. **Intestinal duodenum crypt depth** registered 94.7 µm before and 164.2 µm after supplementation of folic acid.

Also, showed that there was a significant (P≤ 0.05) increase in the intestinal **jejunum crypt depth** after supplementation of folic acid as compare with before supplementation of folic acid. **Intestinal jejunum crypt depth** registered 81.59 µm before and 102 µm after supplementation of folic acid.

While, showed there was no significant difference (P> 0.05) in the **intestinal ileum crypt depth** after supplementation of folic acid as compare with before supplementing of folic acid. **Intestinal ileum crypt depth** registered 77.19 µm before and 79.2 µm after supplementation of folic acid.

Table (4-4). Effect of supplementation of folic acid on intestinal crypt depth (mean ±SE).

Parameter	Before supplementation of folic acid	After supplementation of folic acid
duodenum Crypt depth (µm)	94.7±0.26 B	164.2±0.32 A
jejunum Crypt depth (µm)	81.59±0.3 B	102±0.24 A
ileum Crypt depth (µm)	77.19±0.07 A	79.2±0.06 A

Deferent capital letters in the same row showed a significant difference ($P \leq 0.05$)

4.4. Effect of dietary supplement of folic acid on the immune response:

4.4.1. Effect of folic acid on the anti-body titer against Newcastle disease and Avian Influenza:

Table (4-5) showed that there was a significant increase ($P \leq 0.05$) in the Newcastle serum AB titer after supplementation of folic acid as compare with before supplementation of folic acid. Antibody titer registered 16900 ug/mL before and 19523 ug/mL after supplementation of folic acid.

Table (4-5) showed there was no significant difference ($P > 0.05$) in the Avian Influenza AB titer after supplementation of folic acid as compare with before supplementing of folic acid. Antibody titer registered 20204 ug/mL before and 19960 ug/mL after supplementation of folic acid.

Table (4-5) Effect of supplementation of folic acid on the anti-body titer of Newcastle disease and Avian Influenza

Parameter	Before supplementation of folic acid	After supplementation of folic acid
ND titer (ug/mL)	16900±200 B	19523±220 A
AI titer (ug/mL)	20204±102 A	19960±183 A

Deferent capital letters in the same row showed a significant difference ($P \leq 0.05$)

4.5 Effect of supplementation of folic acid on intestinal villi histology:

4.5.1 Effect of supplementation of folic acid on duodenum:

Figure (4-1) showed a significant increase in duodenum villi height and crypt depth after administrated folic acid group as compare with before supplementation of folic acid.

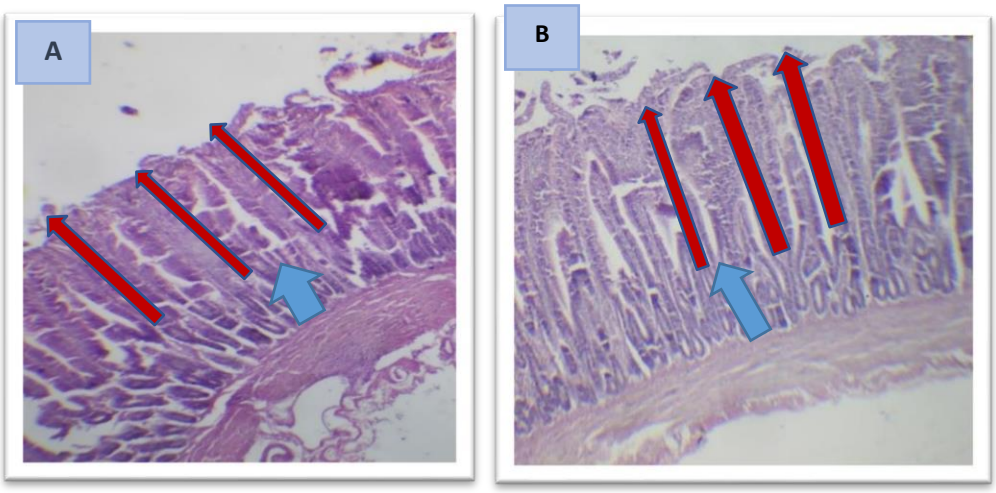

Figure (4-1): Photomicrograph of duodenum in intestine chicken, (A) before supplementation of folic acid, (B) after supplementation with folic acid (H&E X10)

The red arrow showed villus height, the blue arrow showed crypt depth.

4.5.2 Effect of supplementation of folic acid on jejunum:

Figure (4-2) showed a significant increase in jejunum villi height and crypt depth after administrated folic acid group as compare with before supplementation of folic acid.

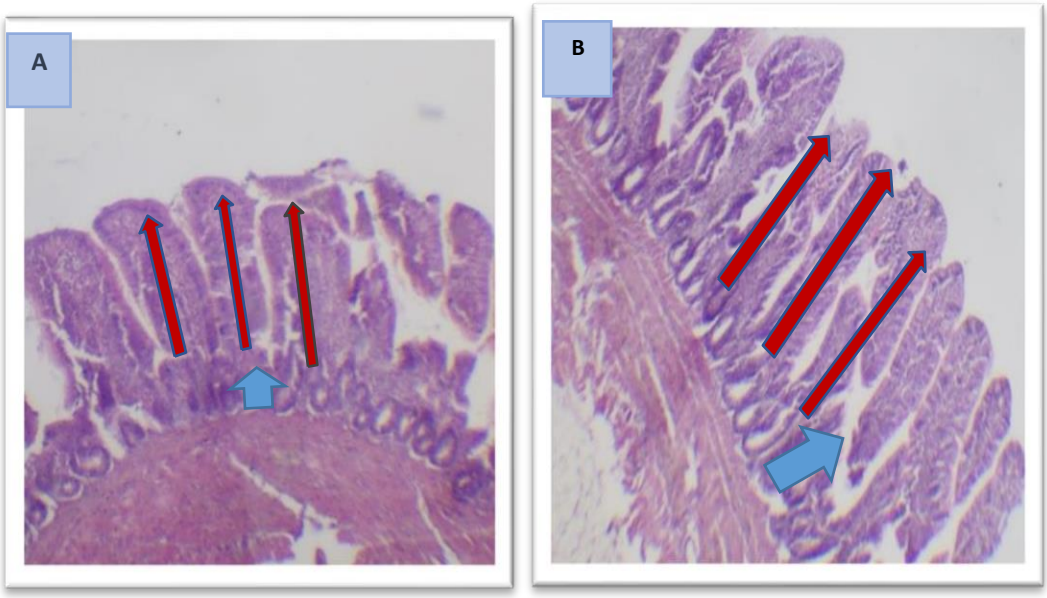

Figure (4-2): Photomicrograph of Jejunum in intestine chicken, (A) before supplementation of folic acid, (B) after supplementation with folic acid (H&E X10)

The red arrow showed villus height, the blue arrow showed crypt depth.

4.5.3 Effect of supplementation of folic acid on ileum:

Figure (4-3) showed a no significant difference in ileum villi height and crypt depth after administrated folic acid as compare to before supplementation of folic acid

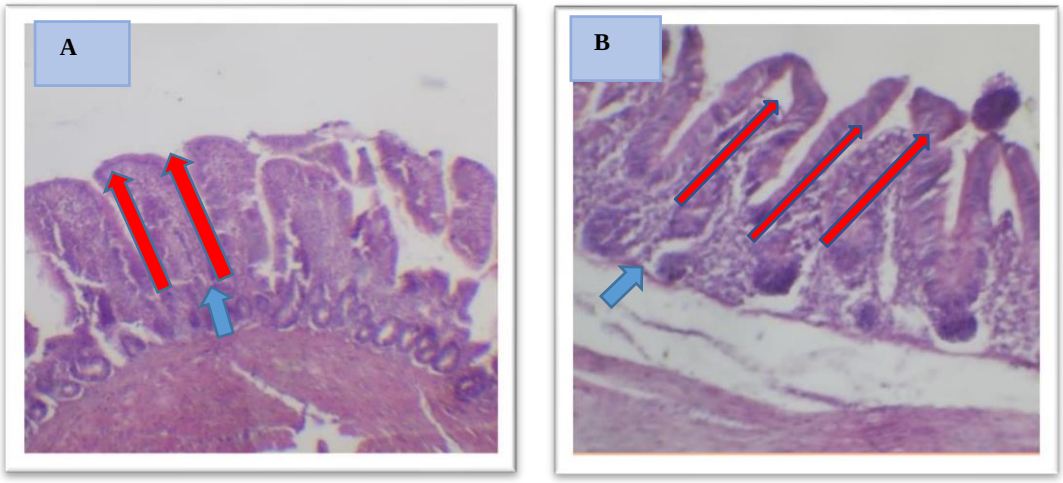

Figure (4-3):Photomicrograph of ileum in intestine chicken, (A) before supplementation of folic acid, (B) after supplementation with folic acid (H&E X10)

The red arrow showed villus height, the blue arrow showed crypt depth.

Chapter Five: Discussion

5. Discussion:

5.1. Effect of supplementation of folic acid on in serum and egg folic acid concentration:

Our results of the current study were showed a significant increase (P≤0.05) in the serum and egg folic acid concentration after supplementation of 1 mg /kg folic acid as compare with before FA supplementation. The results after supplementation folic acid were 145.4ng/ml for serum and 64.9 ng/ml for egg as showed in table (4-1). The improvement of serum folic acid may be due to prescreens of γ-glutamylcarboxy conjugase which is convert polyglutamated to monoglutamyl forms that can easily travel throw the brush border membrane of the duodenum and jejunum. (House *et al*., 2002; Hoey *et al*., 2008 and Bailey *et al*., 2015).

Absorbed folates are then delivered via the hepatic portal system to the liver by activetransport where they are taken up by specialized transporters. Folates that enter the liver have three potential destinations (Vergel *et al*., 1990 and Tactacan *et al*., 2011).As well as, the increase in serum folic acid may be due to the ability of folic acid to dissolve in water, which leads to its easy absorption by the villi of the intestine. As the intestinal villi have the ability to absorb water very efficiently and this increase folic acid Concentration in serum which observed when comparing the results before giving folic acid with after giving folic acid. This results are agreement with (Milman., 2012 ; Kiela and Ghishan., 2016 and Bobrowski-Khoury *et al*., 2022).

The results of the current study showed a significant increase (P≤0.05) of egg folic acid concentration after administrated folic acid as compare with before administrated folic acid. The enrichment of egg folic acid concentration may be due to increase in folic acid in the blood and in the body as a result of the increase in folic acid supplementation in the fed enriched folic acid diet provided to laying hens, which contributed to improvement proportion of folic acid in the egg by passive diffusion and this is consistent with (House *et al*., 2002 and Altic *et al*., 2016).

In most cases circulating 5-Methyltetrahydrofolate (5-methylTHF) is associated with albumin. While less frequently 5-methylTHF can attach to other proteins like α2 macroglobulin and transferrin. Thirty to forty percent of 5-methylTHF in the blood is associated with low affinity binding proteins. (Bertoia *et al*., 2014).Also the amount of nutrients inside the egg depends on the amount of vitamins present in the hens, and

therefore any increase in the proteins and vitamins of the hens leads to an increase in the percentage of nutrients inside the egg and improving the quality of the eggs due to the blood serves as the precursor pool for egg yolk deposition and hens albumin and the protein albumin is transferred from inside the body of the chicken to the inside of the egg and this agree with (Miller and White., 1986; Wang *et al*., 2007 and Réhault-Godbert *et al*., 2019).

5.2. Effect of folic acid on egg equality:

Our results of the current study showed a significant increase ($P \leq 0.05$) in the Egg weight, albumin height and diameter, yolk height and diameter, yolk color and Haugh unit after supplementation of 1mg/kg folic acid as Compare with before FA supplementation. Results agrees with (Arzeni *et al*.,2015; Bagheri *et al*., 2019; Czarnowska-Kujawska *et al*., 2021 and Gu *et al*., 2022).

On the other hand egg shell thickness showed no significant difference ($P>0.05$) after supplementation of 1mg /kg folic acid as Compare with before FA supplementation. And this agree with (Gu *et al*., 2022).

The improvement of the egg weight. may be occur due to increase the amount of albumen and egg yolk in the egg which are directly related to egg weight (Abaş *et al*., 2008).

(House *et al*., 2002) reported that the supplementation of high level of folic acid had no effect on egg weight .While recent study was agreement with (Abaş *et al*., (2008) and Eseceli *et al*., (2010) found in there study that the increase in the egg weight is directly related to albumen weight and egg yolk egg weight.

The increase of proportion the folic acid that a person eats lead to an increase in the proportion of albumin in the blood and serum. This is a good indicator in laying hens because increasing the amount of albumin will increase the deposition of albumin in eggs and this agree with (Munyaka *et al*., 2012; Chilom *et al*., 2018 and Sun *et al*., 2019).

Furthermore it was found that folic acid help in increase the level of estrogen which play a key role in increasing the proportion of egg albumin. This may leads to increase in the height and diameter of the egg white (Wallock-Montelius *et al*., 2007).

That may lead to increase egg weight therefore, an adequate folic acid with the hens diet impairs the oviducts response to estrogen and ability to form albumen (Saleh et al., 2021)

Our study showed a significant increase (P≤0.05) in the Albumin height and Albumin diameter after supplementation of 1 mg /kg folic acid as Compare with before FA supplementation. This result may be occur due to the increase in the albumin amount in the egg. Since that folate play important role in the protein synthesis in the body. Folate has an essential role in one-carbon metabolism and is a strong anti-proliferative agent. Folate increases DNA stability,being crucial for DNA synthesis, repair the methylation cycle, and preventing oxidation of DNA by free radicals which led to increase in protein formation. And this agree with (Abbasi *et al*., 2018).On the other hand studies have also found that if there is a deficiency in folic acid it will lead to imbalances in the DNA precursor pool. And uracil may be misincorporated into DNA. Subsequent misincorporation and repair may lead to double strand breaks and chromosomal damage .This leads to a defect in the protein synthesis process (Duthie *et al*., 2002).

Our study showed a significant increase (P≤0.05) in the egg yolk height and diameter after sublimation of 1 mg /kg folic acid as Compare with before FA supplementation.Our result may be occur due to the ability of folic acid to increase follicle stimulating hormone (FSH) secretion from the pituitary gland which play an important role in follicular growth and maturation, high level of FSH hormone increases the size of the yolk by increasing of blood flow and nutrient were received (Ma *et al*., 2006).Liu *et al*., (2018) found that in aging hens the size of egg yolk is decreased Due to the decrease in the ability of chickens to produce FSH.

Our result showed that there was a significant increase (P≤0.05) in the egg yolk color after folic acid supplementation as compare with before folic acid supplementation. This result is agreement with (Czarnowska-Kujawska *et al*., 2021 and Gu *et al*., 2022).

The yellow or orange color in egg yolk is due to group called carotenoids. The most important and most available for egg yolk coloration are zeaxanthin and lutein. Which are a powerful antioxidants that defend against unstable molecules called free radicals (Zaheer, K. 2017).The capacity of folic acid to act as an antioxidant may explain the rise in egg yolk color. Which discovered that folic acid's antioxidant effect

is mediated by many pathways including a decrease in plasma Homocysteine (HCY) concentrations, which may boost total antioxidant capacity (TAC) and minimize ROS production which lead to rise serum carotenoids consecration that lead to increase in the deposition of carotenoids in the egg yolk and increase yolk color (Zaheer 2017 and Asbaghi *et al*., 2021).

Wang *et al*., (2020) showed there was a significant increase in intestine absorption due to increase intestine villi and height. And the increasmant of villi height can effect on the absorption of carotenoids in small intestine villi and this may case increase carotenoids percentage in the body and increase serum carotenoids which led to increase egg yolk color.

Our result showed there was a no significant difference in the egg shall thickness after supplementation folic acid as compare with before folic acid. This result disagree with the results was found by (Islam *et al*. 2009).

Our study showed a significant increase ($P \leq 0.05$) in Haugh unit after 1mg /kg folic acid supplementation as compared with before supplementation of folic acid this result agree with (Wallock-Montelius, *et al*., 2007 and Krishnan Rajalekshmy,2010).

The improvement of Haugh unit may be occur due to increase in estrogen which plays a key role to increase the proportion of egg albumin which leads to an increase in the height and diameter of the egg white albumin that led to increase in haugh unit .Moreover, the increase in folic acid leads to an increase in the production of proteins in the body, which is reflected in the process of deposition of proteins in the egg, and thus an increase in the Haugh unit which increase depends on the increase in the proportion of proteins. And this agreement with (Wallock-Montelius, *et al*., 2007).

5.3. Effect of folic acid supplementation on immune response:

The improvement of immune response was showed that Newcastle Disease (ND) were improve significantly ($P \leq 0.05$) after supplementation of folic acid as compare with before supplementation of folic acid (table 4-5).

This improvement of the immune response against ND disease may be due to the ability of folic acid act to enhance the body health by promoting mucin secretion which improve barrier function and competitive exclusion of pathogenic anti-body by

competition on receptor sites and availability nutrient. This agree with (Rossi *et al*., 2011).

Folic acid has multiple roles and has received considerable attention in animal studies including neurotransmission regulation and gene expression and has a protective role in the immune system (Kunisawa *et al*., 2012 and Mikkelsen & Apostolopoulos., 2019), while the deficiency of folic acid leads to anemia, granulocytopenia, and lymphocytopenia (Antony,2007) .

Wintergerst *et al*.,(2007) found that he percentages of T cells were not influenced by dietary FA supplementation in the blood even though previous studies have demonstrated the ability of FA to enhance T cell proliferation and this disagree with our study.Accordingly folate deficiency was shown to reduce the proportion of T cells in circulation moreover impairing their proliferation in vitro, and the indicated that folate correction of this condition was faster in vitro as opposed to in vivo conditions (Dhur *et al*., 1991).Folic acid is also involved in T cell and mitogen regulation, which is essential for immunity and growth. Furthermore, folic acid influences the methylation cycle, and DNA and RNA biosynthesis and cell proliferation which means folic acid causes a rise in the body's synthesis of cells, particularly immunological cells (James, *et al*., 1994.).Dietary FA has beneficial effects on the level of IgG in these hens possibly through enhancement of the level or the activities of the B type lymphocytes (Klasing, 2007). Moreover dietary FA supplementation may modulate some immune responses in young laying hens, enhancing the level of biochemical constituents as well as the generation of immunoglobulins necessary for immune responses in response to bacterial and viral infections (Munyaka, *et al*.,2012) .

Hollingsworth *et al*., (2008) reported that the utero exposure to the methyl donor might affect the expression of the critical gene that plays a central role in immunity.

On the hand our study showed that there is no significant deference ($P>0.05$) of avian influenza after supplementation of folic acid when compere to before supplementation of folic acid.

5.4. Effect of folic acid on intestine morphology:

Our result showed that there was a significant ($p \leq 0.05$) increase in villi high and depth in duodenum and jejunum after supplementation of folic acid as compared with before supplementation of folic acid. On the other hand ileum has no significant ($P > 0.05$) difference in villi high and crypt depth after supplementation of folic acid as compared with before supplementation of folic acid.

The result showed an increase in the villi height and crypt depth after folic acid supplementation as compare with before folic acid supplementation. This rustle may be occur due to the ability of folic acid to reduce apoptosis in the cells and tissue. (Wang *et al*., 2021) Also the intestinal villi may be increase in high due to the ability of folic acid to increase proliferation of villi cells (Hwang *et al*., 2018).

Besides of, folic acid plays an important role in increasing the expression of mRNA in the cells. It can cause an increase in the growth and proliferation of cells and thus an increase in the length of the villi and this is was observe on after folic acid supplementation as compare with before folic acid supplementation (Li *et al*., 2020).

Folic acid absorption is an active process that occurs primarily in the duodenum and jejunum, due to prescreens of γ-glutamylcarboxy conjugase which is convert polyglutamated to monoglutamyl forms that can easily travel throw the brush border membrane of the duodenum and jejunum and this agree with (Visentin *et al*., 2014).

Moreover, folic acid has indispensable for one-carbon transfer reactions, including nucleic acid synthesis, amino acid metabolism, and methylation. Therefore, plays a crucial role in protein deposition, tissue synthesis and cell proliferation which may explains the elongation of villi height and crypt depth in duodenum and jejunum as compared to ileum and this result agree with (Liu and Ward., 2010)

Therefore, additional supplementation with folic acid may maintain the balance of intestinal epithelial cell renewal to keep the intestinal morphology and function (Wang *et al*., 2021).

Also, the intestine is not only an organ for digestion and absorption, but also an important immune organ. Improving intestinal morphology and integrity is the main way to improve the production in hen.

Chapter Six
Conclusions and Recommendations

6.1Conclusions:

From the current study, we conclude:

1- Adding 1mg /kg folic acid to lohmann hen's diet will increase of serum folic acid concentration and increase egg folic acid concentration.

2- Rich laying hens diet with folic acid may be enhance of egg quality, immune response of hens that consumes this diet.

3- Gut health status may enhanced consuming of diet enrich with folic acid. Enhancing of villi hight and crypt depth lead to enhance of gut health status

6.2Recommendations:

1-Use a different dose of folic acid in another type of laying hens.

2-Sudy the apoptotic factor before and after supplementation of folic acid.

3-Study the relationship between folic acid and serum proteins.

4-study the effect of product the folic egg on pregnant woman and their effect on CNS of fetus

5- study the effect of folic acid on laying hens diet on mucin secretion and gen expression.

6- -study the effect of folic acid broiler performance

7- Histological examination on ova duct and oogenesis.

8-study the effect of folic acid on digestibility of nitrogenous compound in the diet.

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Appendix

Appendix I

Calculate serum folic acid:

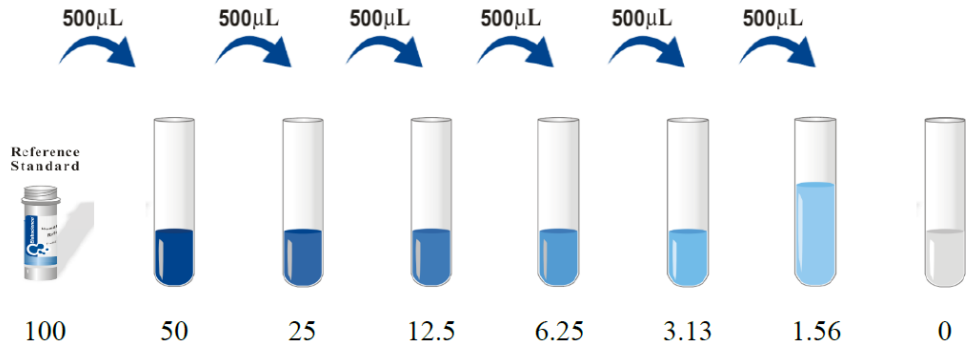
Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.

2. Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃water bathand mix it gently until the crystals have completely dissolved.

3. Standard working solution: Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard andSample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 100ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 100、50、25、12.5、6.25、3.13、1.56、0 ng/mL

Dilution method: Take 7 EP tubes, add 500uL of Reference Standard and Sample Diluent to each tube. Pipette 500uL of the 100ng/mL working solution to the first tube and mix up to produce a 50ng/mL working solution. Pipette 500uL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference. Note: the last tube is regarded as a blank. Don’t pipette solution into it from the former tube.



4. Biotinylated Detection Ab working solution: Calculate the required amount before the experiment (50μL/well). In preparation, slightly more than calculatedshould be

prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution withBiotinylated Detection Ab Diluent.

5. Concentrated HRP Conjugate working solution: Calculate the required amount before the experiment (100 μL/well). In preparation,slightly more than calculatedshould be prepared. Dilute the 100×Concentrated HRP Conjugate to 1×workingsolutionwithConcentrated HRP Conjugate Diluent.

Assay procedure

1. Add the Standard working solution to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (50 uL for each well). Add the samples to the other wells (50 uL for each well). Immediately add 50μLof Biotinylated Detection Ab working solutionto each well.Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37℃.Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Aspirate or decant the solution from each well，add 350 uL of wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.

3. Add 100 μL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.

4. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 2.

5. Add 90 μL of Substrate Reagent to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min.

6. Add 50 μL of Stop Solution to each well. Note: Adding the stop solution should be done in the same order as the substrate solution.

7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Calculation of results:

Average the duplicate readings for each standard and samples. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with anappropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Appendix II

Egg folic acid concentration:

The sample (5 g) was taken into a 100 mL screw-capped amber glass bottle, in which 50 mL 0.1M phosphate buffer (pH 7.0) was added. Then, the bottle was mixed with a vortex mixer for 15 min for an effective mixing of the sample with buffer. To it, 1 g pancreatic was added and allowed to dissolve for 5 min, followed by the addition of 6 mL 10% L-ascorbic acid solution. To mix well, the matrix solution was mixed with a vortex mixer for 5 min. Then, the sample bottles were kept in a shaking incubator at 37°C for 2 h, followed by transferring to a water bath (70°C) for 20 min for deactivation of the enzyme. The sample was allowed to cool to room temperature. Later, it was transferred into a 100 mL amber-colored volumetric flask, which was filled to the mark with 0.1M phosphate buffer. The sample solution was blended well, and aliquots were taken into two 50 mL centrifuge tubes. The respective tubes were centrifuged for 10 min at 5000 rpm. The supernatant was filtered through a Whatman 0.45 um filter, and then transport to HPLC analysis.

Sample were analyzed by high performance liquid chromatography HPLC model (SYKAM) Germany . the mobile phase was MeOH : Phosphate buffer : = (80 : 20) , the column separation was (C18 – ODS (25cm * 4.6 mm) and the detector = UV – 280 nm at flow rate at 1.0 ml / min.

Appendix III

Newcastle Disease Virus Antibody titer:

Preparation

1) BringELISA reagents to the room temperature (20-25℃) for 30 min to get best results.

2) Sample dilution: use the sample diluent to dilute the sample at 40 times(for example: 5ul serum sample+195ulSample diluent solution), mix the diluted sample evenly can get better result.

3) Washing solution preparation: Dilute the10×concentrated washing buffer with deionized water at10 times.(eg.10ml 10×concentrated washing buffer + 90mldeionized water), if there is crystallization in the 10×concentrated washing buffer, it is normal, dissolve it at 37℃.

Test procedure

1. Adding sample: Take out the required coated plates according to sample quantity (Can be detached)and record the sample position on a worksheet. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negative and positive control serum to its well accordingly, 100μL/well. Others are sample wells, addthediluted sample,100 μl/well.

2. Incubation: cover withAdhesive Foil after adding sample,incubate at37℃for30 min.

3. Remove adhesive foil. Pour the liquid out of the wells, add Washing solution into each well fully, be static for about 10s, pour out directly. Repeat 3 times, at last time pat to dry on absorbent paper.

4. Add 100μL enzyme conjugate into each well.

5. Cover with adhesive foil andincubate at37℃for30 min.

6. Repeat step 3.

7. Add100μL substrate into each well, mix properly,Color for 10 min at37℃in the dark.

8. Add 50μL stop solution into each well, shake evenly for 10s, and determine the result.

9. Read OD value of each well with ELISA Reader at double-wave length: 450/630nm.

Appendix IV

Avian influenza antibody test

Preparation

Return washing solution to room temperature before use, if there is salty crystals, shake to make the crystals dissolve, then use distilled water or deionied water to dilute it at 10 times. The diluted washing solution can store for 1 week at 4 ℃.

At serum dilution plate, dilute serum at 1:100 with sample dilution.

Test procedure:

1) Take pre-coated microplate (Can unseal for several time use as per sample quantity), add 100μL diluted serum to a well, meanwhile set 1 well for Negative control, 2 wells for Positive control separately. Add 100 μL Negative/Positive control to its wells. Shake softly (do not spill),incubate at 37℃ for 30 min.

2) Pour the liquid out of the wells, add 250μL diluted washing solution to each well, pour out. Repeat 4-6 times, then pat to dry on absorbent paper.

3) Add 100 μL Enzyme Conjugate to each well, andincubate at 37℃ for 30 min.

4) Repeat the step 2(washing). Remember pat to dry on absorbent paper at last.

5) Add 100μL substrate solution to each well, mix properly,react for 10 min at 37℃ in dark.

6) Add 50 μL stop solution in each well, and measure the result within 10 min.

Results.

Read the OD value with microplate-reader at 450nm.

Appendix V

Histological study Histological Technique (E and H) stain:

The intestine (duodenum, jejunum and ileum) of each animal were quickly removed and n prepared for histological study according to (Suvarna *et al*., 2018) aid of the light microscope as the following steps:

* Fixation:

The specimen fixated in the formalin 10 % for 24 – 48 hours.

* Washing and dehydration :

After fixation the specimens washed with water to remove the fixative in order to avoid the interaction between the fixative and staining materials used later. By dehydration the water had been completely extracted from fragments by bathing them successively in a graded series of of ethanol and water (70 %, 80 %, 90 %, and 100 % ethanol).

* Clearing:

Bathing the dehydrated fragments in solvent (xylene) for 30–60 minutes, this step was repeated 3 times. As the tissues clearing, they generally became transparent.

* Infiltration and embedding:

Once the tissue fragments were impregnated with the solvent, they were placed in melted paraffin in an oven, typically at 52 C. The heat causes the solvent to evaporate, and the space within the tissues becomes filled with paraffin.

* Sectioning:

After the oven, the specimen let at room temperature to be solid and removed from their containers in order to sectioning they were put in the rotarymicrotome and were sliced by the microtome, a steel blade into sections 5 micrometers thick . The sections were floated on water bath (50 – 55 C), then transferred into glass slides and left to dry.

* Staining:

The histological sections of the studied organs were stained with Hematoxylin - Eosin stain and the steps was

1- Dewax in Xylene for 5 min

2- Absolute Alcohol – 2 mins

3- Absolute Alcohol – 2 mins

4- Alcohol 90 % – 2 mins

5- Alcohol 80 % – 2 mins

6- Alcohol 70 % – 2 mins

7- Hematoxylin – 2 mins

8- Tab water 8 min

9- Eosin 45 sec

10-Alcohol 70 % – 2 mins

11-Alcohol 80 % – 2 mins

12- Alcohol 90 % – 2 mins

13-Absolut Alcohol – 2 mins

14- Absolut Alcohol – 2 mins

15-Xylene for 5 min

16-Xylene for 5 min

17-Covered with cover slid by use D.P.X

After that the slide is examined by using light microscope.

Appendix VI

Vaccine program

Day	Vaccine name	Disease	Dose	Route	Company Name
1	Nobilis® Rismavac + CA126	Merck disease	0.2 ml	S/C inj.	MSD Animal Health Co.
1	NOBILIS® H9N2+ND P	Influenza+ ND	0.25 ml	S/C inj.	MSD Animal Health Co.
1	CEVAC® TRANSMUNE IBD	Gumboro Disease	0.25 ml	S/C inj.	CEVAC Co.
1	Nobilis® MA5 + Clone 30	IB+ND live vaccine	1000 doses	Spray	MSD Animal Health Co.
12	Nobilis® MA5 + Clone 30	IB+ND live vaccine	1000 doses	Spray	MSD Animal Health Co.
14	Nobilis® Gumboro D78	Gumboro Disease	1000 doses	Drinking water	MSD Animal Health Co.
22	NOBILIS® ND CLONE 30	ND	1000 doses	Spray	MSD Animal Health Co.
35	Nobilis® MA5 + Clone 30	IB+ND live vaccine	1000 doses	Spray	MSD Animal Health Co.
38	Nobilis® IB + ND	IB+ND inactivated vaccine	0.5 ml	S/C inj.	MSD Animal Health Co.
38	NOBILIS® H9N2	Influenza	0.5 ml	S/C inj.	MSD Animal Health Co.
45	Nobilis® SG 9R	chicken typhoid	0.2 ml	S/C inj.	MSD Animal Health Co.
49	Nobilis IB 4-91	IB	2500 doses	Spray	MSD Animal Health Co.
49	NOBILIS® ND CLONE 30	ND	1000 doses	Spray	MSD Animal Health Co.

55	Nobilis® ILT	Infectious Laryngotracheitis	1000 doses	Eye drops	MSD Animal Health Co.
55	Nobilis® AE + Pox	Avian Encephalomyelitis+Pox	1000 doses	Wing web	MSD Animal Health Co.
63	NOBILIS® ND CLONE 30	ND	1000 doses	Spray	MSD Animal Health Co.
77	Nobilis® MA5 + Clone 30	IB+ND live vaccine	1000 doses	Spray	MSD Animal Health Co.
80	Nobilis® IB+ND+EDS	IB+ND+EDS	0.5 ml	S/C inj.	MSD Animal Health Co.
88	NOBILIS® ND CLONE 30	ND	1000 doses	Spray	MSD Animal Health Co.
90	Nobilis® SG 9R	chicken typhoid	0.2 ml	S/C inj.	MSD Animal Health Co.
100	NOBILIS® ND CLONE 30	ND	1000 doses	Spray	MSD Animal Health Co.
120	NOBILIS® ND CLONE 30	ND	1000 doses	Spray	MSD Animal Health Co.

Appendix VII

Ingredients of the diet

Ingredients	Contents
Corn	392
Soy-bean meal	266
Wheat	200
Soy-bean oil	12
Limestone	100
Dicalcium/phosphate	2
Mycofix	1
Salt	1
Sodium bicarbonate	1
Premix al-wafi	25
Total weight	1000
Nutrients	
Protein%	18
Energy Kcal/kg	2700
Av-Lysine	0.91
Av-Methionine	0.47
Av-Therionine	0.58
TSAA	0.72
calcium	3.54
Av-Phosphorus	0.4
Sodium	0.21
Chloride	0.23

Electrolytes MEQ/Kg	238
Crude fat%	3.06
Crude fiber%	2.49

الخلاصة

أجريت هذه الدراسة لمعرفة تأثير اضافة حمض الفوليك كمكملات للنظام الغذائي على محتوى حمض الفوليك في البيض ومصل الدم وجودة البيض والاستجابة المناعية والتشكل المعوي للدجاج البياض. .

في الفترة الأولى ، تم تغذية الدجاج على النظام الغذائي الأساسي وفقًا لدليل متطلبات التغذية البني الكلاسيكي من Lohmann. كانت تحتوي على كمية محسوبة من حمض الفوليك. و الذي كان قادمًا من المواد التي يصنع منها العلف . الفترة الثانية من التجربة تم تغذية جميع الدجاجات بنفس النظام الغذائي الأساسي بلاضافة الى حمض الفوليك بنسبة 1 ملجم / كجم من العلف.

تم جمع عينات الدم و البيض في نهاية الفترتين الأولى والثانية لقياس تركيز حمض الفوليك للبيض والمصل باستخدام تقنية HPLC and ELISA. كما تم اختبار الاستجابة المناعية باستخدام Eliza. تم قياس جودة البيض بحساب وزن البيضة ، ارتفاع الألبومين ، قطر الألبومين ، ارتفاع الصفار ، قطر الصفار ، سمك القشرة.

تم جمع عينات من اجزاء الامعاء الدقيقة في نهاية الفترة الأولى والثانية لقياس ارتفاع الزغابات وعمقها .

أظهرت النتائج زيادة معنوية في تركيز حمض الفوليك في مصل الدم والبيض بعد إضافة 1 ملجم / كجم من حمض الفوليك الى العلف .

سجل تركيز حمض الفوليك في مصل الدم بتقنية إليزا 145.4 نانوغرام / مل بعد إضافة حمض الفوليك قبل المكملات التي سجلت 79 نانوغرام / مل.

من ناحية أخرى سجل تركيز حمض الفوليك بتقنية HPLC بعد إضافة حمض الفوليك 64.9 جزء في المليون على أنه مركب قبل المكملات التي تم تسجيلها 32 جزء في المليون

حسن من جودة البيض و وزن البيض و قطر ارتفاع الالبومين و ارتفاع الصفار الصفار وقطر الصفار ووحدة هوف معنوياً بمقدار ($p\leq0.05$) بعد إضافة حمض الفوليك بلمقارنة مع قبل اضافته

أظهر ارتفاع الزغبات وعمقها فرقًا معنويًا ($p\leq0.05$) في الفترات الثانية من التجربة بعد اضافة حمض الفوليك بلمقارنة مع الفترة الأولى قبل الفوليك. و قد لوحظ ان أعلى نتائج في ارتفاع الزغابات وعمق الزغابة في الاثني عشر والصائم.

نستنتج إن إضافة 1 ملجم / كجم من حمض الفوليك إلى علف الدجاج اللومان قد يؤدي إلى زيادة محتوى حمض الفوليك في الدم ثم زيادة محتوى حمض الفوليك في البيض.

أعلى محتوى من بيض الفوليك ينتج بيض حمض الفوليك يسمى بيضة الفوليك وهذا سيساعد المرأة الحامل على تقليل تشوه الحبل الشوكي لدى الجنين.

قد يؤدي اتباع نظام غذائي غني بحمض الفوليك الى علف للدجاج البياض إلى تحسين جودة البيض والاستجابة المناعية للدجاج الذي يستهلك هذا النظام الغذائي. قد تعزز الحالة الصحية للأمعاء من تناول النظام الغذائي

المخصب بحمض الفوليك. يؤدي تحسين ارتفاع الزغابات وعمق الزغابة إلى تحسين الحالة الصحية للأمعاء. قد يكون هذا التحسين مستهلكًا لنظام غذائي غني بحمض الفوليك.

الخلاصة

جامعة كربلاء

كلية الطب البيطري

فرع الصحة العامة

إضافة حمض الفوليك إلى النظام الغذائي لدجاج من نوع لوهمان لتحسين الصفات النوعية للبيض والحالة الصحية

رسالة مقدمة الى مجلس كلية الطب البيطري / جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في اختصاص علوم الطب البيطري/الصحة العامة

كتبت بواسطة

عبير كريم سلطان

بإشراف

الأستاذ الدكتور

ياسر جمال جميل

التاريخ الهجري

1444

التاريخ الميلادي

2022