Effect of Vitamin E on The Humoral Immune Response of Broiler Chicks Vaccinated With Infectious Bursal Disease Vaccine "IBDV"

A thesis
Submitted to the council of the college of Veterinary Medicine-University Of Basrah
In partial fulfillment of requirements for the Degree of Master of Science in Veterinary Medicine In Pathology (Poultry diseases)

By
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Supervisor Declaration

I certify that this thesis was prepared under My supervision at the department of Pathology/College of Veterinary Medicine/ University of Basrah as a partial fulfillment of the requirement for the degree of Master of science in Veterinary Pathology/Poultry Diseases.

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In the view of the available recommendation I forward this thesis for debate by examining committee.

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Abstract

A total of 210, one-day old commercial broiler chicks were divided into three equal groups (A,B,C). The birds were raised under the same condition. Feed and water were supplied ad libitum. At 14\textsuperscript{th} day of age, all groups were vaccinated with mild IBDV(Bursine\textsuperscript{®}-2/Fort Dodge/USA). Group A was supplemented with 100mgVE/kg of ration for 7 days before vaccination, while group B was supplemented with the same dose for 7 days after vaccination, whereas group C was acted as control.

Bursal index was measured to detect the effect of VE on treated birds. Also ELISA test, total protein, albumin, globulins, SGOT(ALT), SGPT(AST), and Glucose were determined to investigate the effect of VE on immune response.

The results showed that the treated groups A,B had a significant increase (p<0.05) than group C in bursal index, Ab titers, while the treatment groups A and B had a significant decrease (p<0.05) than group C in GOT and GPT.

The effect of VE supplementation on serum total protein, albumin, and globulins in group A and B, resulted in a significant differences (p<0.05) between treatments. Of this important result was the increment (p<0.05) of globulins of group B which was fed VE for 7 days after vaccination in comparison to that of both group A and control group.
Serum glucose level of group A resulted in a significant increase (p<0.05) of glucose in group A in comparison to that of the control group.

In conclusion the present study revealed that vitamin E has an important role as immune stimulater in broiler chicks especially when supplement at 100mg/kg of diet from 1st to 7th days of age before vaccination with IBD vaccine which resulted in stimulation of antibody production and increase bursa Fabricius weight as well as enhancement of immune activity.
Introduction

Vitamin E (VE) is known for its role as an antioxidant, protecting unsaturated bonds of cellular membrane phospholipids against free radical attack (Tappel, 1972). Vitamin E also has been shown to be a requirement for normal development and function of the immune system (Tengerdy et al., 1984).

In chickens, VE deficiency significantly reduced bursal growth and reduced the number of lymphocytes in the bursa and the thymus gland (Marsh et al., 1986). Additionally, VE deficiency reduced the proliferation response of T-cells to mitogen stimulation and was shown to specifically affect the differentiation of T cells (Chang et al., 1994).

Gore and Qureshi (1997) reported that VE enhanced cell-mediated and humoral immunity in broiler chicks. It altered the functional capacity of mononuclear leukocytes in young broilers as indicated by higher numbers of eliciting inflammatory exudate cells, a greater percentage of phagocytic macrophages, increased numbers of sheep red blood cell (SRBC) phagocytosed per macrophage, increased nitrite production by macrophages, and higher IgG antibody titers in response to a second SRBC injection, but did not affect spleen or bursal weights.

VE has been shown to enhance immunity to Escherichia coli infection (Tengerdy and Brown, 1977), coccidiosis
(Colnago *et al.*, 1984), infectious bursal disease (McIlroy *et al.*, 1993), and Newcastle disease (Franchini *et al.*, 1995) in chickens.

Environmental conditions in broiler houses tend to fluctuate and are often less than optimal. For example, ventilation may be reduced by producers in an effort to decrease heating costs during cold weather. Under these rearing conditions, broiler chicks are exposed to pathogens and other environmental stresses at an age when they are not fully immunocompetent (McCorkle and Glick, 1980).

Infectious bursal disease (IBD) is an acute and highly contagious viral disease affecting young chickens and is characterized by massive damage to the bursa of Fabricius (FB) and by immune-suppression (Lukert and sayf. 1997).

Considering the potential benefits of VE on the health of fast-growing broiler chickens, and the immune-modulator effects of VE on broiler chickens need to be investigated, therefore the main objectives of this study was:-

1. To investigate the effect of vitamin E in the humoral immune response of broiler chicks before and after vaccination with miled IBD(Bursine®-2) vaccine.
2. To determine the effect of vitamin E in enzymes activity.
3. Evaluation of its' effect in the development of bursa Fabricius during the first few days of life.
Literature Review

2-1 Vitamin E (VE)

VE is a fat-soluble vitamin that exists in eight different forms, each form has its own biological activity, which is the measure of potency or functional use in the body (Traber and Packer, 1995). Alpha-tocopherol (α-tocopherol) is the name of the most active form of VE in humans and animals. VE in supplements is usually sold as alphatocopherol acetate, a form of alpha-tocopherol that protects its ability to function as a powerful biological antioxidant (Traber and Arai, 1999). The synthetic form is labeled "D, L" while the natural form is labeled "D". The synthetic form is only half as active as the natural form (Farrell and Roberts, 1994).

VE is a generic term used for a group of chemically-similar compounds sharing the tocopherol and tocotrienol structures (Robert, 2001), which are lipid-soluble; hence, vitamin E is known as a fat-soluble vitamin. Vitamin E is an important anti-oxidant which protects against lipid peroxidation which could contribute to cell membrane weakness (Combs, 1992).

2-1-1 Vitamin E - Structures and Chemistry
2-1-1-1 Nomenclature

Vitamin E is the generic term used for all of the compounds in this group. The vitamin can exist as two types of structures: the tocopherol and tocotrienol structures (DeLuca, 1978).

2-1-1-2 Chemistry

Vitamin E is not a single molecule, but a family of 8 related molecules called tocopherols and tocotrienols. Moreover, each of the different tocopherols exists in eight stereoisomers. However, having described this molecular complexity, it turns out that dietary vitamin E is predominantly alpha and gamma-tocopherol. The structure of alpha-tocopherol is depicted below; note the long hydrocarbon chain similar to the tail of a fatty acid.

Tocopherols (Vitamin E) are equipped to perform a unique function. They can interrupt free radical chain reactions by capturing the free radical; this imparts to them their antioxidant properties. The free hydroxyl group on the aromatic
ring is responsible for the antioxidant properties. The hydrogen from this group is donated to the free radical, resulting in a relatively stable free radical form of the vitamin (DeLuca, 1978; Britgelius-Flohe et al., 2002).

2-1-2 Physiological Aspects of Vitamin E

2-1-2-1 Absorption

The process of absorption is passive and does not require the use of a protein carrier to bring in the vitamin (DeLuca, 1978). Absorption occurs in the small intestine and the vitamin can only be absorbed if it has been cleaved by esterases located in the stomach lining. It is then packaged into very low-density lipoproteins (VLDL) by the addition of lipid-like substances. VLDLs enter the lymphatics and are eventually released into the bloodstream. The alpha-tocotrienol appears to be better absorbed than the other tocopherol forms (Walter, 1989; Blatt et al., 2001).

2-1-2-2 Transportation

VE does not have a specific carrier protein in the bloodstream but it is transferred by hepatic and lymphatic mechanisms (Swann and Kendra, 1998). When it is first absorbed into the hepatic portal vein it is contained inside a lipid-like structure called a chylomicron (Combs, 1992). This structure is then converted hepatically to three distinct lipoprotein
structures: high-density lipoprotein (HDL), low-density lipoprotein, and very low-density lipoprotein (VLDLs). The alpha-tocotrienol is the form most rapidly secreted into the plasma after uptake. The transport process is an important aspect in the delivery of VE. Without the protective barrier of the lipoproteins (HDL, LDL, and VLDL), the vitamin would be exposed to the oxidative radicals circulating through the blood. Additionally, vitamin E will prevent the oxidation of LDL; oxidized LDL is thought to be a factor in atherogenesis, so adequate amounts of vitamin E will help protect against hardening of the arteries, heart attacks, and stroke (Swann and Kendra, 1998; Traber and Ari, 1999).

2-1-2-3 Tissue Uptake

Tissue uptake occurs by one of two ways: either by lipases digesting the lipoprotein constituents or by "receptor mediated uptake" by binding of the lipoprotein to a specific tissue receptor site. This allows for the vitamin to enter the tissue. VE enters a variety of different tissue types, with adipose and the adrenal gland having the highest levels. It is found primarily in mitochondria. It is thought to play a role there in either stabilizing ubiquinone, or in helping ubiquinone transfer electrons. The body's capacity for storage of the vitamin is worth noting. The vitamin can be stored in tissue for long
periods of time (years) due to its exceedingly slow turnover rate. Interestingly, the natural alpha-tocopherol form of the vitamin is stored up to six times longer than synthetic versions (Swann and Kendra, 1998; Van Metre and Cailan, 2001).

2-1-2-4 Metabolism

VE is considered to be metabolized after it has performed its antioxidant function. It is converted from a tocopherol to a tocopherylquinone. The elimination of this end product is primarily through the faeces but a small fraction is removed by urine (less than 1 percent) (Swann and Kendra, 1998).

In order for tocopherylquinone to be excreted, it first has to be converted to tocopherylhydroquinone, a partially reduced form. This form can then combine with glucuronic acid so that it can mix with bile. Bile is removed from the body through feces (Walter, 1989; Herrera and Barbas, 2001)

2-1-3 Sources of vitamin E from diet

Foods containing wheat are a good source of VE. These foods vary in their content of VE based on the particular source and processing involved. Wheat germ oil is the richest source of natural VE. If the wheat product is processed to make other foods such as margarine, the content of vitamin E is reduced due
to the methods involved in formulation and exposure to chemicals (acids and bases) that can destroy VE. Other dietary sources are nuts and seeds that are rich in oils, and vegetable oils derived from these sources (Robert, 2001).

2-2 Action of vitamin E on the immune response

VE donates electrons to free radicals, thereby making them stable. This prevents free radicals from binding to fatty acids, inhibiting oxidation reactions, and therefore maintaining cell membrane integrity (Halliwell and Gutteridge, 1991). According to Klasing (1998), in addition of being the first line of body defenses against the action of free radicals, protecting the host cells, VE modulates the immune response. It also decreases the synthesis of prostaglandins, leukotrienes, and cytokines, which regulate the inflammatory response, thereby reducing damage caused to the tissues by the inflammatory process. Abdukalykova, and Ruiz-Feria (2006) evaluated the benefits of VE supplementation in broiler diets, and observed that birds fed high level of VE improved feed conversion ratio as compared to the control group which fed lower level of VE. Broilers that received the higher VE level presented reductions carcass downgrading due to diseases, septicemia/toxemia, and inflammatory processes, respectively, relative to the control group. On the other hand, Erf, et al., (1998) supplemented broiler diets with increasing VE levels did not find effect of VE
supplementation on thymus T and spleen B lymphocytes and macrophages percentage. However, far-away from supplementation level, VE increased T-cell production as compared to birds that did not receive VE supplementation.

Boa-Amponsem et al., (2000) fed broiler diets containing different levels of VE, and challenged the birds with an intravenous solution of sheep red blood cells (SRBC). There was no effect of VE supplementation on anti-SRBC antibody titers, but the heterophil/lymphocyte ratio increased, indicating that VE improved the phagocytic capacity of the immune system, protecting the birds against the invasion of pathogenic microorganisms. Evaluating VE supplementation on the immune response of broilers, Leshchinsky & Klasing (2001) observed that VE lower levels promoted higher antibody titers at 7 days after the inoculation of SRBC as compared to higher levels of VE dietary addition.

2-3 Vitamin E and Immune Responses of Broiler

The commercial broiler of today appears to have compromised humoral immunocompetence, which manifests in poorer antibody production to diseases, higher mortality, and lower resistance to stressors (Qureshi and Havenstein, 1994; Cahaner et al., 1998). Such correlated responses have been attributed to the intense selection for rapid growth to market weight. In experimental White Rock populations divergently
selected for juvenile body weight (BW), humoral immune responses to SRBC were less in the high than the low weight line (Miller et al., 1992). The broiler industry is increasingly dependent on husbandry practices, including medicaments for controlling diseases to realize the objectives of selective breeding (Liu et al., 1995).

VE, unlike antibiotics and other chemicals, is a nutrient and is not known to cause any unacceptable side effects of welfare concern. It has been reported to enhance immune competence of chickens (Gore and Qureshi, 1997) and thereby enhance their immunity to several diseases including *Escherichia coli* infection, coccidiosis, infectious bursal disease, and Newcastle disease (Erf et al., 1998). Effects of VE appear to be influenced by several factors including age and dietary levels (Gore and Qureshi, 1997).

### 2-4 Infectious bursal disease (Gumboro)

Infectious bursal disease (IBD) is an acute and highly contagious viral disease affecting young chickens and is characterized by massive damage to the bursa of Fabricius (FB) and by immunosuppression (Lukert and Sayf, 1997). The infectious bursal disease virus (IBDV) belongs to the family *Birnaviridae* (Brown, 1986), and it consists of two segments of double-stranded RNA (Jackwood et al. 1984). There are two IBDV serotypes, but only one (serotype 1) is pathogenic for
domestic fowl. The expression of serotype 1 pathogenicity, however, varies. While some infected chickens will not even show any clinical symptoms of the disease, other chickens may die: in specific pathogen free (SPF) chickens, IBD mortality ranges between 30% and 70% (Nunoya et al. 1992).

Acute bursal disease cases were mainly reported from commercial broiler chicken feeding farms and laying broiler pullet farms, and were rare in non-commercial flocks (Juranova et al., 2001).

The basis for infectious bursal disease prevention is specific immunoprophylaxis. Inactivated oil vaccines and live a pathogenic or attenuated vaccines of various virulence levels are used. There are three types of live vaccines: mild, intermediate and highly virulent (Lukert and Sayf,1997). Intermediate vaccines can induce a higher level of immunogenicity in chickens than mild vaccines, but they may differ in virulence and some may even induce atrophy of the bursa of Fabricius in young chickens. Virulent vaccines are suitable in areas with a highly pathogenic IBDV (Mazariegos et al., 1990).

Lack of information on the characteristics of virulent strains makes the monitoring of incidence of the highly virulent IBD virus very difficult (Tsukamoto et al., 1995).

2-4-1 IBDV and Broiler Immunity

Infectious bursal disease virus (IBDV) is ubiquitous. Current control strategy involves protection of broilers against
IBD via passive and in many cases active immunity (Fussell, 1995).

2-4-1-1 Passive Immunity: Hen Hyperimmunization

The most popular strategy for IBDV control is hen hyperimmunization (Sharma and Rosenberger, 1987). Poultry integrators use live IBDV vaccines and two or more inactivated vaccines in replacement pullets and hens in order to hyperimmunize hens. Passive immunity to IBDV is then transferred to broiler progeny providing some level of early protection against field challenge. Some companies rely on passive immunity only for broiler protection and do not use any live vaccines in progeny (Sharma and Rosenberger, 1987).

2-4-1.2 Active Immunity: Broiler Vaccination

In addition to passive immunity, live IBD vaccines may be given in an effort to gain active immunity against IBDV (Giambrone, 1995; McMurray, 1995; Putnam, 1995). Live IBD vaccines are administered either in ovo or at hatching, and in the field through booster vaccinations. Live Delaware variant and classic combinations are often recommended (Miller Heins, 1995). There is much written on the timing of live IBD vaccine administration in broiler progeny, usually depending upon antibody titer levels as measured by ELISA or other techniques (Ather, 1993). It is difficult to arrive at an appropriate time to vaccinate because of the myriad of titer levels seen in progeny.
from different breeder flocks. Exclusive placements from individual breeder flocks are the exception rather than the rule (Ather, 1993).

2-4-3. IBD and VE.

In a field study in Northern Ireland, McIlroy et al. (1993) measured the profitability of increased vitamin E supplementation when broilers were under far less disease pressure. In this work, 43 flocks exhibited no clinical signs of any disease, but bursal samples showed subclinical IBD. The birds received either different levels of VE per ton of feed and/or the standard supplementation level (lowered level), with each treatment fed throughout the life of the birds. The researchers reported a significant percent improvement in feed efficiency and a significant percent increase in weight gains in the birds receiving the higher level of VE supplementation. Because of the improved performance, these flocks also produced more percent greater net income than the flocks receiving the standard supplementation level. In this work, the researchers also assessed the benefits of vitamin E in flocks that faced even less disease challenge. Indeed, the researchers found neither subclinical IBD nor clinical signs of other diseases in these broilers flocks.
VE has been reported to enhance immune competence of chickens (Gore and Qureshi, 1997) and thereby enhance their immunity to several diseases including infectious bursal disease (Erf et al., 1998).
Materials And Methods

3.1 Materials

3.1.1 Birds
A total of 210 one-day old commercial broiler chicks were obtained from a local hatchery (Fadik Hatchery) at Basrah city. They were divided into three equal groups as shown in Table 1.

3.1.2 Vitamin E (VE)
VE (MISAVITE100/Mission Pharmaceuticals Limited/India) as a capsule was used in this study.

3.1.3 Vaccines
Mild IBDV (Bursine®-2/Fort Dodge/USA). The vaccine was supplied in a separated vials. Each individual vial contained 1000 doses.

3.2 Methods
3.2.1 Experimental design:
The birds were raised under the same condition. Feed and water were supplied ad libitum. They were reared until the 35th day of age.
Table 1:- Experimental design.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>NO. OF BIRDS</th>
<th>TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vaccination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type of vaccine</td>
</tr>
<tr>
<td>A.</td>
<td>70</td>
<td>Mild IBD Vaccine</td>
</tr>
<tr>
<td>B.</td>
<td>70</td>
<td>Mild IBD Vaccine</td>
</tr>
<tr>
<td>C.</td>
<td>70</td>
<td>Mild IBD Vaccine</td>
</tr>
</tbody>
</table>

3.2.2 Vitamin E (VE) supplementation:
Each capsule contained 100 mg pure VE with excipients. Each capsule content was mixed with one kg of ration to obtained 100 mg /kg of feed and presented *ad libitum* to groups A, and B for 7 days before and after vaccination respectively as shown in the design of the study (Table1).

3.2.3 Vaccination:
Birds of all groups were vaccinated via drinking water with mild IBD (Bursine®-2/Fort Dodge/USA) vaccine at the 14th day of age. The vaccine was supplied in a separated vials. Each individual vial contained 1000 doses. Vaccination was conducted according to the standard method included in the
directions of the manufacturer. Sterile distilled water was used for this purpose. Drinking water was withdrawn for about 3hrs. The vial content of vaccine was dissolved in 3 liters of distilled water mixed with an about 7.5 gm powder skim milk (P.S.M.) at a ratio of 1:400 (w/v) and presented to the birds for about half an hour. The tissue culture infectious dose (TCID5) was TCID50100.

3.2.4. Blood collection

About 2-5 ml of blood has been collected from 10 birds of each group in a test tube. Blood samples were obtained at 28th day of age through wing vein puncture. The blood was centrifugated at 1500 rpm for one minute and the sera were kept at -20°C until the time of analysis (Aaron, et al; 2005).

3.2.5. Laboratory tests.

3.2.5-1 Estimation of Bursa weight: Body weight (Bursal index)

Ten birds of each group have been randomly taken, individually weighed and sacrificed at 21st day of age. Bursa of Fabricius of each individual bird was weighted after removing from the carcass. The bursal index were calculated by applying the formula mentioned below (Moraes et. al, 2004):

\[
\text{Mean of bursa weights} \\
\text{Bursal index} = \frac{\text{Mean of bursa weights}}{x} \times 100
\]
3.2.5-2. Indirect ELISA test (Enzyme Linked Immuno-Sorbent Assay).

SYNBIOTICS CORPORATION ProFlok® PLUS (USA), ELISA kit was used for detection of antibody titer against IBD vaccine after vaccination with mild IBD type vaccine. ELISA test procedures were performed according to Alam et al.,(2002):

3.2.5-3. Serum Proteins estimation.

3.2.5-3.1. Serum total Protein.

According to Wotton(1964), Biuret method has been used for measuring the total serum protein of the broiler chicks. The principles of this process is the reaction of carbamyl group of protein with cupric sulphate of biuret solution to make a purple complex. The technique which have been used for the determination of the serum protein was according to the guideline of the (RANDOX/UK) kit, as briefly listed below:

<table>
<thead>
<tr>
<th>Materials</th>
<th>Tested Sample</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>-</td>
<td>1 ml</td>
</tr>
<tr>
<td>Serum</td>
<td>0.2 ml</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
The solution was warmed in a water bath at 37°C for 10 min. and the result was read with a spectrophotometer at 540nm wavelength. Measurement of total serum protein has been performed according to the following formula:

\[
\text{Total protein gm/100 ml} = \frac{\text{Sample} - \text{Blank}}{(\text{Standard} - \text{Blank})} \times 6(\text{standard concentration})
\]

3.2.5-3.2. Serum albumin estimation.

A kit SPINREACT(SPAIN) has been used for measuring albumin in broiler sera.

Test principle

Albumin in the presence of bromcresol green at a slightly acidic pH, has been produced a color changed the indicator from yellow-green to the green blue. The intensity of the color formed was proportional to albumin concentration in sample.

Procedure:

<table>
<thead>
<tr>
<th>Components</th>
<th>Blank</th>
<th>Standard protein</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>2 ml</td>
<td>2 ml</td>
<td>2 ml</td>
</tr>
<tr>
<td>Standard protein</td>
<td>-</td>
<td>10 µl</td>
<td>-</td>
</tr>
<tr>
<td>Serum sample</td>
<td>-</td>
<td>-</td>
<td>10 µl</td>
</tr>
</tbody>
</table>
Test tubes were left for 5 minutes. Then they were recorded on wavelength 462 nm of computerized spectrophotometer and the following equation was applied

\[
\text{Sample} \times \text{Standard albumin concentration} = \text{albumin in gm/dL}
\]

Standard albumin concentration = 6 gm/dL

3.2.5-3-3. Serum immunoglobulins.

Serum immunoglobulins were calculated by indirect method according the following formula (Colse, 1986)

\[
\text{Serum globulin g/dl} = (\text{Serum total protein}) - (\text{Serum albumin})
\]

3.2.5-4. Serum Glucose determination.

According to Colse (1986), reduction method colorimatic had been used for measuring serum glucose in the serum of broiler chicks, which depending on protein structure by sodium hydroxide, then reduce phosphomolybdic to molybdenum blue which produce the blue color.

| Materials | Sample | Standard | Blank |
The materials were mixed well and centrifugated at 3000 rpm for 10 min. Depending on the directions of the manufacturer of the glucose kit (bioMerieux/France), the basic principles of procedures of this test was carried out as shown in the table below:

<table>
<thead>
<tr>
<th>Materials</th>
<th>Sample</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution</td>
<td>1 ml</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Serum</td>
<td>10µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>10µl</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>-</td>
<td>10µl</td>
</tr>
</tbody>
</table>

All test materials were mixed well in a test tube and incubated for 10 min at 37°C. The result was determined in spectrophotometer at 510 nm. The level of serum glucose were measured according to the following equations:

\[
\text{Glucose} \, \mu g/dl = \frac{\text{sample}}{\text{standard}} \times \text{Standard concentration (100)}
\]
3.2.5-5. Serum Glutamate Pyruvate Transaminase (GPT) and Glutamate Oxalate Transaminase (GOT) enzymes estimation:

A Tansaminases-kit (bioMerieux/France) was used for measurement of GPT & GOT. Coloric determination of GOT or GPT activity was evaluated according to the Reitman and Frankel method (1957). The principles of this method can be summarized by the following reactions:

**GOT**

GOT: Asparate + α keto glutarate $\rightleftharpoons$ oxalo acetate + glutamate

**GPT**

GPT: Alanine + α ketoglutarate $\rightleftharpoons$ pyruvate + glutamate

The pyruvate or oxaloacetate formed was measured in its derivative form, 2,4-dinitrophenylhydrazone. The basic principles of procedures of this test is shown in the table below:
Reagents:

<table>
<thead>
<tr>
<th>Reagent1: GOT substrate</th>
<th>Phosphate buffer pH 7.5</th>
<th>85 mmol/l</th>
<th>200 mmol/l</th>
<th>2 mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Asparate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a ketoglutarate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reagent2: GPT substrate</th>
<th>Phosphate buffer pH 7.5</th>
<th>95 mmol/l</th>
<th>200 mmol/l</th>
<th>2 mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alanine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a ketoglutarate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Reagent3: Color reagent | 2,4dinitrophenylhydrazin HCL | 1 mmol/l | 0.1 l/l |

<table>
<thead>
<tr>
<th>Reagent4: Standard</th>
<th>Pyruvate</th>
</tr>
</thead>
</table>

Procedure:

By using spectrophotometer, the wave length was 505nm (490-520nm), adjusted to zero by distilled water.

Standard curve: Pipette in to test tubes (ml):

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Reagent 1 or Reagent 2</td>
<td>1</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Reagent 4</td>
<td>-</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Reagent 3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

All reagents were mixed and the mixture was left for 20 minutes at room temperature.

| NaOH 0.4 N | 10 | 10 | 10 | 10 | 10 | 10 |
Mixed and waited for 5 minute and then measured.

<table>
<thead>
<tr>
<th></th>
<th>GOT units/ml</th>
<th>GPT units/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>215</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>126</td>
<td>-</td>
</tr>
</tbody>
</table>

**Measurement of enzymes:**
GOT and GPT was measured after setting up the following tubes for each serum:

<table>
<thead>
<tr>
<th></th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>1 ml</td>
<td>-</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>-</td>
<td>1 ml</td>
</tr>
<tr>
<td>Incubated for 5 minutes at 37°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>0.2 ml</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Mixed and incubated at 37°C for</td>
<td>Exactly</td>
<td>Exactly</td>
</tr>
<tr>
<td></td>
<td>1 hour</td>
<td>30 min.</td>
</tr>
<tr>
<td>Reagent 3</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Mixed and let stand for 20 minutes at room temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaOH 0.4 N</td>
<td>10 ml</td>
<td>10 ml</td>
</tr>
<tr>
<td>Mixed and waited for 5 minutes. Measured under room conditions identical to those used for the standard curve.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.2.5-6. **Statistical Analysis:**

The data were subjected to analysis of variance and significant differences at (P<0.5) and determined by ANOVA-one way; SPSS v.12.0(2004).
Results

4-1. Bursal index.

The average weights of bursa of Fabricus of ten samples from each group were taken after the recording of weights of each bird. For estimation of bursal index the ratio of average weight of bursa to the average weight of ten birds have been considered as detected in the following equation.

\[
\text{Bursal index} = \frac{\text{average bursa weights}}{\text{average body weights}} \times 100
\]

Table(2) Bursal index and antibody titer of group A and B fed VE before and after vaccination with mild IBD vaccine.

<table>
<thead>
<tr>
<th>Groups</th>
<th>VE supplementation/100mg/kg of diet</th>
<th>Means ± SD. Of Bursa index</th>
<th>Means ± SD. Of antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time</td>
<td>Duration</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Before</td>
<td>7 days</td>
<td>0.28±0.06 a</td>
</tr>
<tr>
<td>B</td>
<td>After</td>
<td>7 days</td>
<td>0.25±0.07 b</td>
</tr>
<tr>
<td>C</td>
<td>Control (without VE)</td>
<td></td>
<td>0.14±0.04 ab</td>
</tr>
</tbody>
</table>

a, b, ab and c Mean figures with different superscripts at vertical

Data in Table(2) displayed the effect of VE supplementation on bursa weight of chicks in group A and B which fed VE before and after vaccination with mild IBD
vaccine. Treatment resulted in an increase weight of bursa of both groups in comparison to that of control group C as declared by bursal index. These increment were statistically significantly at (p<0.05).

4-2. ELISA

Table (2) shows the effect of the antibody titers (Ab) tested by ELISA in chicks, resulted in an increase antibody titer in group A (5545±1.335) and group B (3809±1.336) as compared with control Group C (2285±1.523). These results were statistically significant at (p<0.05).
The titer of antibodies of group A is the largest titer which is higher than titers of group B and C as p<0.05.The titer of group B is significantly lower than tested group A and higher than the antibody titer of control group

**4-3. Total Serum Proteins**

Table (3) exhibited that the effect of VE supplementation on serum total protein, albumin, and globulins in group A and B before and after vaccination with mild strain of IBD vaccine respectively, resulted in a significant differences (p<0.05) between treatments. Of this important results is the increment (p<0.05) of globulins of group A (1.58±0.37) which was fed VE 7 days before vaccination in comparison to that of both group B (1.22±0.43) and control group C (0.75±0.46).
**Table(3):** Total serum protein, albumin, and globulin in group A, and B fed VE before and after vaccination with mild strain of IBD vaccine.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean total Protein ml/dl value± SD</th>
<th>Mean albumin Value ml/dl ± SD</th>
<th>Mean globulin ml/dl value± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.62±0.48a</td>
<td>3.04±0.58a</td>
<td>1.58±0.37a</td>
</tr>
<tr>
<td>B</td>
<td>4.96±0.53b</td>
<td>3.74±0.43b</td>
<td>1.22±0.43b</td>
</tr>
<tr>
<td>C</td>
<td>5.02±0.26ab</td>
<td>4.14±0.71ab</td>
<td>0.75±0.46ab</td>
</tr>
</tbody>
</table>

*(a,b,ab) mean figures with different superscripts at the vertical column were significantly differed at P<0.05 .Figures represent means ±SD of total protein, albumin and globulin of 10 samples for each treatment analyzed.

**Figure (3). Total serum protein, albumin, and globulins of group A, B, and C**

4-4. Serum Glutamate pyruvate (SGPT) and Serum Glutamate Oxalate(SGOT) Transaminase activities.
Table(4): SGOT and SGPT levels in group A and B fed VE before and after vaccination with mild IBD vaccine.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±SD SGOT value (Un/µl)</th>
<th>Mean±SD SGPT value (Un/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>39.80± 2.17 a</td>
<td>31.00±1.87 a</td>
</tr>
<tr>
<td>B</td>
<td>28.60± 2.19 b</td>
<td>33.80±1.30 b</td>
</tr>
<tr>
<td>C</td>
<td>45.80± 2.59 ab</td>
<td>36.00±2.24 ab</td>
</tr>
</tbody>
</table>

a, b, and ab Mean figures with different superscripts at vertical column were different significantly at (p<0.05)

Data presented in Table(4) demonstrated the effect of vitamin E supplementation on serum GPT and GOT in group A, and B which were fed 100mg/kg of diet of VE before and after vaccination with mild strain of IBD vaccine for 7 consecutive days. The result indicated that VE supplementation produced significant decrease (p<0.05) in the enzymes activity in comparison to the control group C.

Figure (4). SGOT and SGPT of group A, B, and C
4-5. Serum glucose.

Table(5) Serum glucose level of group A and B fed VE before and after vaccination with mild IBD vaccine.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean± SD. Glucose value m/100dlμ</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>203.6± 10.3 a</td>
</tr>
<tr>
<td>B</td>
<td>189.8± 10.9 b</td>
</tr>
<tr>
<td>C</td>
<td>193.8± 6.6 ab</td>
</tr>
</tbody>
</table>

a, b and ab Mean figures with different superscripts at vertical column were differed significantly at (p<0.05)

Table(5) expressed that effect of VE supplementation on glucose level in both groups A and B which were vaccinated with mild strain of IBD vaccine and fed VE before and after vaccination respectively, resulted in a significant increase (p<0.05) of glucose in group A in comparison to that of Group B and control group C.
Figure (5). Serum glucose of group A, B, and C
**Discussion**

VE is a biological fat soluble antioxidant (Halliwell and Gutteridge, 1991), which inhibits the oxidation of long chained unsaturated fatty acid of the cell membrane (McDowell, 1989; Hennekens, 1986). Vitamin E has been shown that it directly influence the immune system of birds under several experimental models (Kidd et al., 2001; Friedman et al., 1998). VE enhances the humoral immune response to antigen stimulation and increase resistance to infectious diseases (Tengerdy et al., 1972). Erf and Bottje (1996) found that; high dose of vitamin E increased the resistance of broiler against encountered diseases as well as weight gain and performance.

Data in Table (2) displayed the effect of VE supplementation on bursa of Fabricius weight of chicks in group A and B which fed VE before and after vaccination with mild IBD vaccine. Treatment resulted in an increase weight of bursa of Fabricus of both groups in comparison to that of control group C as shown by bursal index. These increment was statistically significant at (p<0.05).

The results of bursal index differences attributed to VE supplementation and type of vaccine used. Winterfield and Thacker (1978) tested immunogenicity and virulence of 8 intermediate and mild vaccination strains against IBD and found considerable differences among the strains. Two of these strains
were highly virulent, produced clinical symptoms, caused damage to BF and even death of birds. Similar results were reported by Naqi et al. (1980). Muskett et al. (1979) who studied the properties of two live IBD vaccines on susceptible chickens and observed major damage to the bursa of Fabricius caused by one of them. According to Edwards et al. (1982), immunosuppression may last up to 4 weeks following vaccination. Many reports investigated the effect of VE on B and T cells proliferation in bursa of fabricius and thymus respectively (Erf, et al., 1997). Boa-Amponsem et al., 1999, found the dietary VE at level 300 mg/kg were resulted in an increment in population of both T and B cells in thymus, and bursa of Fabricius respectively.

In the present study, the effect of VE on the specific antibody titer against IBD vaccine has been conducted. The result of effect of VE on level of antibody titers in group A and B which were vaccinated with mild (Bursine®-2) strain of IBD vaccine at 14th day of age and fed VE before and after vaccination respectively, and tested by ELISA has been represented in Table (3). The table showed that 100 mg/kg of diet VE for 7 consecutive days before and after vaccination with mild IBD vaccine resulted in a significant (p<0.05) increase of antibody titer in group A and B in comparison to the control group C. It is a highly interesting to notice that supplementation of VE before vaccination, as shown in the table
(2), resulted in highly increasing of antibody level of group A in comparison to that of group B and C. This result was in agreement with that of Leshchinsky & Klasing (2001) who reported that effect of VE on antibody production depends on antibody function and antigen immunogensity. Many different levels of VE were used, the authors found that the higher levels of VE either 100 or 200 mg/kg diet improved the production of antibody in treated groups in comparison to the control as well as those groups which received low level. The results of the present study were also in agreement with those of Leshchinsky & Klasing (2001). Albuquerque et al.,(2006) found that 120 mg VE/ kg diet resulted in an increase antibody titer of birds fed the vitamin before vaccination at 14th day of age with Newcastle disease. Abdukalykova and Ruiz-Feria (2000) found that birds fed 80 mg of vitamin E/kg of diet 6 days before vaccination with IBD vaccine produced higher antibody titer in comparison to that fed 40 mg of VE before vaccination also. Tengerdy and Nockels (1973) stated that higher VE level produced the higher levels of antibody titers. They concluded this fact through their feeding birds with a diet containing 132 mg/kg in comparison to the free VE control group.

The present study was also in agreement with that of Boa-Amponsem, et.al., (1999), who reported that 300 mg VE/kg diet showed higher antibody titer than that of 10 mg/kg and control group which produced the same Ab titer. Muir et al;
(2006), concluded that 100, and 250 mg of VE/kg diet resulted in an increase in antibody titer of birds vaccinated with killed *Salmonella typhimurium* vaccine in comparison to those fed 50, 2500 and 5000 mg of VE/kg diet. Excessive VE intake has a detrimental effect on antibody production in chicken and turkey as issued by Friedman, et al; (1998) and Muir et al; (2006).

Table (3) exhibited that the effect of VE supplementation on total serum protein, albumin, and globulins in group A and B before and after vaccination with mild strain of IBD vaccine respectively, resulted in a significant differences (p<0.05) between treatments. Of this important result is the increment (p<0.05) of globulins of group A (1.58±0.37) which was fed VE 7 days before vaccination in comparison to that of both group B (1.22±0.43) and control group C (0.75±0.46).

The result of effect of VE supplementation on stimulation of immunoglobulins in the present study was in agreement with that of Muir et al; (2006), who stated that 100, and 250 mg VE/kg diet fed for chicken vaccinated with killed *Salmonella typhimurium* vaccine resulted in an increase in the level of immunoglobulins IgG and IgM when compared with those fed 50, 2500 and 5000 mg VE/kg diet. Gore and Qureshi (1997) reported that low levels of VE (10, 20, and 30 IU) had no effects on immunoglobulins production and the result was not different from that of the free VE control group. Franchini et al. (1990) mentioned that 30, 90, 180, and 360 IU of VE/kg of
turkey diet did not produce significant effect on total serum protein.

No differences were found among the albumin levels between the control group and experimental groups of the present study. This result was in agreement with many researchers who reported that biochemical parameter such as total serum proteins are age-dependent (Selvarj et al., 2004; Krasnodebska-Depta and Andrej, 2000). Serum proteins (total protein, albumin, and globulin) may clearly differed genetically and with environmental conditions (Naziefy-Habibabadi, 1997; Simaraks, et al., 2004). Bozickovic (2000), presented that the serum protein parameters were clearly differed when birds encountered with nutritional, infectious, and immune exciters.

Data presented in Table(4) demonstrated the effect of vitamin E supplementation on serum GPT and GOT in group A, and B. The result indicated that VE supplementation produced significant (p<0.05) decrease in the enzymes activity in comparison to the control group C. This result was in agreement with that of Egbal (2001) who found that there was a significant increase in the level of SGOT and SGPT following avian influenza infection which confirmed the evidence of liver damage. The result was in agreement with that of Al-Afaleq, 1998; EL –Batrawi and Awad; 1993; Ley et. al.; 1983; Panigraphy et. al.; 1986 who stated that experimental infection of chicken with IBDV produced an decrease of GOT and GPT
activity. Kechrid *et al.;* 2007 also found that supplementation of vitamin E resulted in a decrease GOT and GPT production.

Table (5) expressed that effect of VE supplementation on glucose level in group A(203.6±10.3) which was vaccinated with mild strain of IBD vaccine and fed VE before vaccination, resulted in a significant increase (p<0.05) of glucose in group A only in comparison to that of control group C (193.8±6.6). This result was in agreement with that of Kechrid *et al.;* 2007 who found that addition of VE resulted in high concentration of blood glucose in rats.

This study showed an increase in level of glucose occurred by VE supplementation, this observation was in agreement with McIlory *et al.,* 1993 who showed an increment in body weight and blood glucose when fed broiler with 161 IU of VE per kg of diet in contrast to these got 43 IU of it.
Conclusions

The present study revealed that:

1- Vitamin E has an important role as immune stimulator when vaccinated against IBD in broiler chicks.

2- Vitamin E enhanced immunity when fed before vaccination with mild IBD (Bursine\textsuperscript{®}-2) vaccine.
Recommendations

1. Further studies on VE to demonstrate its' effect on vaccinations against other diseases.

2. It is recommended to study the pathological changes of VE on the immune organs.
References


Kechrid, Z; Derai, H; Layachi, N. (2007). The beneficial effect of vitamin E supplementation on zinc status, carbohydrate metabolism, transaminases and alkaline phosphatase activities
in alloxan-diabetic rats fed on zinc deficiency diet. Int J Diabetes & Metabolism. 15: 46-50


الخلاصة

استخدمت في هذه الدراسة مائتان وعشرة من أفراد فروع اللحم بعد يوم واحد، وقسمت إلى ثلاثة مجموعات متساوية تحت تزويدها في نفس الظروف مع توفير المياه والغذاء بصورة حرة.

في اليوم الرابع عشر لقحت المجموعات الثلاثة بلقاح مرض التهاب جراب فابرشي المناعي (Bursine®-2/Fort Dodge/USA).

المجموعة الأولى أعطيت فيتامين هـ بمعدل 0.1ملغم/كم من العليقة لمدة 7 أيام قبل الالتهاب، المجموعة الثانية أعطيت نفس الكمية لمدة 7 أيام بعد الالتهاب، أما المجموعة الثالثة استخدمت كمجموعة سليمة.

لمعرفة تأثير فيتامين هـ على الاستجابة المناعية فقد تم جمع الدم في اليوم الثامن والعشرين وأجريت الاختبارات مثل اختبار الاليازا واختبارات البروتينات الكلي في الدم وفحص الألبوبيرون والكليروبلوبين وقياس نشاط إنزيمات الكبد وكذلك نسبة الكلوكوز بالدم.

إن إعطاء فيتامين هـ أدى إلى زيادة وزن جراب فابرشي وزيادة إنتاج الأجسام المضادة في المجموعتين الأولى والثانية مقارنة مع مجموعة السيطرة عند استعمال جراب فابرشي كدليل للمناعة وكذلك في اختبار الأليزا. وهذه الزيادة كانت إحصائياً بفرق معنوي مقداره (p<0.05).

تأثر فيتامين هـ على البروتين الكلي في المصل والألبومين في المجموعتين الأولى والثانية نتج عنه فرق معنوي مقداره (p<0.05). في حين نجد أن هناك زيادة ملحوظة في مقدار الكليروبلوبين في المجموعة الثانية التي أعطيت فيتامين هـ مدة 7 أيام بعد الالتهاب مقارنة بالمجموعة الأولى والسيطرة.

فقد لوحظ أن هناك نقصان في فعالية الإنزيمات في المجموعتين الأولى والثانية إذا ما قورنت بمجموعة السيطرة.

أما مستويات الكلوكوز في المجموعة الأولى فكانت هناك زيادة معنوية مقدارها (p<0.05) مقارنة مع مجموعة السيطرة.

إن نتائج الدراسة الحالية كشفت أن فيتامين هـ دور مهم كمحفز للمناعة في دجاج اللحم عند استخدامه بجرعة 0.1ملغم/كم من العليقة من اليوم الأول إلى اليوم السابع من عمر قبل الالتهاب بلقاح الكمبورو مما يؤدي إلى تعزيز إنتاج الأجسام المضادة وزيادة وزن جراب فابرشي.
تأثير فيتامين هـ على الاستجابة المناعية الخلطية في أفراخ اللحم الملقة بلقاح التهاب جراب فبريشيا المعدي

رسالة مقدمة إلى
مجلس كلية الطب البيطري / جامعة البصرة
وهي جزء من متطلبات نيل درجة الماجستير في علوم الطب البيطري
فرع الأمراض (أمراض الدواجن)

من قبل
علي مجيد حسن

1430 هـ 2009 م