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Effect of threonine and zinc alone or in combination on immunity and intestinal morphology of broiler chickens

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***Abstract*---**Broiler chickens' immune response may be affected by the body health; Therefore, this Study was aimed to evaluated the was aimed to investigate the effect of threonine(Thr.) from the jefo Co.Canada and zinc (Zn.) from the Zinpro Co.USA as an important factor that regulates intestinal amino acid metabolism. The experiment period was five weeks started from 7/12/2021 to 11/1/2022 carried out in special felid. A total of 200 straight run one-day broiler chickens (Ross 308) were divided randomly to four groups (50/group) with 2 replicates (25 birds/pen). The control group (CON.) fed basal diet without any additives. The second group was fed the basal diets with high level calculated (%0.9) Threonine. The third group was fed the basal diets with level calculated (% 0.1) Zinc. The fourth group was fed the basal diets with (% 0.9) Threonine and was fed the basal diets with (% 0.1) Zinc. Feed and water provided add libitum to the end of the study. Blood samples were collected at 21 days of the study for immunological parameter the results showed that improvement of immunity response IgG titer against Newcastle Disease ND and Infectious bronchitis IB were improved enhance significantly ($P \leq 0.05$) in the (Thr-Zn), (Threonine), and (Zinc) respectively of groups compare with the (Control) in the twenty one age days. On the other hand , Intestinal samples were collected at 35 days of the study were observed by take tissue sample from duodenum, jejunum and ileum the histological changes in the small intestinal. The histological changes for depth of the in intestine crypts, and villus height show highly difference ($P \leq 0.05$)in the (Thr-Zn) ,(Thr) and (Zn) groups compare with control groups. In conclusion, adding a mixture of Threonine and Zinc to their base diet can improve the immune response, gut health status broiler chickens of Ross(308).

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***Keywords*---**broiler, immune response, intestinal morphology.

Introduction

Nutrition plays a major role of gainful chickens production and on an average it accounts for about 80–90% of the total cost of production (NAFIS, 2017). Chickens require precise levels of dietary nutrients particularly amino acids and energy (Kim *et al.*, 2007). Additionally, optimal dietary amino acid contents (Webel *et al.*, 1996), are prerequisite to support gut functions. A healthy gut is, therefore, necessary for profitable poultry production. Villi height and crypt depth are important indices for gut health measurement. Longer villi and shorter crypts are usually considered as markers of a healthy and well functioning gut (Qaisrani *et al.*, 2015). The immune status of the broiler chickens improves, with an improvement in the function of immune response (Corzo *et al.*, 2007; Zhang *et al.,* 2016).

Threonine (Thr), the third limiting amino acid after lysine and methionine in corn-soy diets, is an indispensable amino acid for broiler chickens (Berres *et al.*, 2007). Threonine has a major role in intestinal development and well-functioning (Stoll, 2006), because intestinal mucin is mainly made of Thr (Faure *et al.,* 2005). Dietary total Thr level between 0.70 and 0.93% can support optimum gut morphology (Schaart *et al.*, 2005; Zaefarian *et al.*, 2008). The maintenance of intestinal barrier and mucin synthesis also requires Thr. (Wang *et al.,* 2010) since mucus of the digestive tract contains about 40% protein that is majorly Thr. (Carlstedt *et al.*, 1993). Mucin is a glycoprotein in nature, which plays a vital role in protecting the intestine from acidic chyme, pathogens and digestive enzymes as well as maintains the intestinal integrity (Horn *et al.*, 2009). In gut health. The influence of Thr on gut health, immunity in broiler chickens (Qaisrani et al., 2018). Dietary Thr, above the requirements, promotes the growth of immune organs, stimulates the synthesis of immunoglobulins, improves immune response and alleviates the immune stress caused by Infectious bronchitis (IBD) and Newcastle disease virus (NDV) in broiler chickens (Trevisi *et al.*, 2015).

The zinc role of gut health is pivotal in broiler from hatch to the point of harvest (Shannon and Hill., 2019). Zinc is an important dietary factor which regulates intestinal amino acid and protein metabolism in animals (Wang *et al.*, 2009). Many studies have investigated the importance of Zn in gastrointestinal functionality and health. Zinc deficiency has been reported to negatively affect gut integrity by compromising the intestinal permeability (Crane *et al.*, 2007; Zhang and Guo, 2009; Li *et al.*, 2015), epithelial tissue integrity (Vallee and Falchuk, 1993), and the structure and function of the intestinal barriers (Rodriguez *et al.*, 1996; Lambert *et al.*, 2004). (De Grande *et al.*, 2020) found supplementation with Zn amino acid increased villus length and villi length to crypt depth ratio. Accordingly, the aim of the current study combined effects of Thr and Zn are suggested on the intestinal absorption and digestion , according to Rodríguez-Yoldi *et al.* (1993).

The aims of this study are to identify the following

The current study is aimed to study the effect of threonine and zinc alone or in combination their effect on gut health of broiler chickens by estimation of **:**-

1. To study of threonine and zinc alone or in combination on crypt width and villi height of broiler chickens.
2. To study of threonine and zinc alone or in combination on immune response against Newcastle disease and Infectious bronchitis in broiler chickens

Literature review

Threonine

Threonine has a major role in intestinal development and well-functioning (Stoll, 2006), because intestinal mucin is mainly made of Thr (Faure *et al.,* 2005). Dietary total Thr level between 0.70 and 0.93% can support optimum gut morphology (Schaart *et al.,* 2005; Zaefarian *et al.*, 2008). Mucin is a glycoprotein in nature, which plays a vital role in protecting the intestine from acidic chyme, pathogens and digestive enzymes as well as maintains the intestinal integrity (Horn *et al.*, 2009). In gastrointestinal tract, mucin acts as a filtering agent for nutrients and affects their digestion and absorption (Smirnov *et al.*, 2006). Threonine is “furthermore” involved in different metabolic processes such as protein synthesis and uric acid formation (Eftekhari *et al.*, 2015). Diets deficient in Thr may compromise immunoglobulin production because Thr is an integral part of immunoglobulin in broilers (Azzam and El-Gogary, 2015). Threonine is considered as the second limiting amino acid (Estalkhzir *et al.*, 2013) and, therefore, its supplementation is assumed to result in improved carcass characteristics. It is believed that Thr enhances (Estalkhzir *et al.*, 2013; Khan *et al.*, 2006). The present review describes the response of modern-day broilers to dietary Thr levels, and its effects on gut morphology, immunity and carcass characteristics.

Previous studies have demonstrated that dietary Thr supplementation can improve intestinal morphology and barrier function, increase intestinal goblet cell density and expression of mucin-2 (MUC2) mRNA, reequilibrate the gut microbiota, and enhance antioxidant ability (Faure *et al.*, 2006; Law *et al.,* 2007; Hamard *et al.,* 2010; Wang *et al.*, 2010; Azzam *et al.,* 2011, 2012). Similarly, extra Thr inclusion can promote the growth of immune organs, stimulate the synthesis of immunoglobulins—including immunoglobulin A (IgA), immunoglobulin G (IgG) and secretory immunoglobulin A (SIgA)—improve immune response, and alleviate the immune stress induced by Escherichia coli challenge or Newcastle disease virus (Bhargava *et al.*, 1971; Li *et al.*, 1999; Wang *et al.*, 2006; Corzo *et al.,* 2007; Kadam *et al.,* 2008; Azzam *et al.*, 2012; Ren *et al.,* 2014; Trevisi *et al.*, 2015) despite previous studies have also shown that dietary Thr content did not affect the production of antibodies, innate or adaptive immune responses of broiler chickens (Kidd *et al.*, 1997). Under commercial conditions, a higher level of Thr that exceeds the current NRC recommendation (1994) is required to achieve maximum immune function and health status for poultry (Corzo *et al.*, 2007;

Azzam *et al.,* 2011, 2012; Star et al., 2012). However, studies regarding an intake of dietary Thr in excess of current NRC (1994) recommendation on the immunity, oxidative status, and intestinal health of broiler chickens at an early age are rare. The Thr requirement recommended by NRC (1994) for broilers during 0 to 3 wk is 8 g/kg, and the maximum level of extra L-Thr supplementation for poultry is 3 g/kg according to the regulation issued by Ministry of Agriculture of China (2009). We selected 3 g/kg as the highest level for the extra L-Thr supplementation and hypothesized that extra Thr inclusion would exert a beneficial effect on broiler chickens at the early age. This study was therefore conducted to investigate the effects of dietary L- Thr supplementation on the growth performance, immune function, antioxidant capacity, intestinal integrity, and barrier function of broilers at an early age (Suryasa et al., 2021).

Zinc

The immune response of broiler chickens may be modified by the level of zinc in the diet. Organic zinc supplementation had a positive effect on the immunological capacity of broilers by improving the levels of immunoglobulins IgA, IgM, and IgG (Feng *et al.*, 2010), and may also improve the cellular response (Moghaddam *et al.*, 2009). However, for this type of response, higher levels of supplementation may be required from organic [120 mg/kg (0 to 3 wk) and 90 mg/kg (4 to 6 wk)] (Feng *et al.*, 2010) or inorganic (80 ppm to 5 wk), (Gajula *et al.*, 2011) sources. Zinc is absorbed with an efficiency from 15 to 60% (McDowell *et al.*, 2003) and the Zn plasma concentration varies according to its presence in tissues, being distributed among bone, muscle, liver, and pancreas (Georgievskii *et al.*, 1982). Depending upon their chemical nature and complexation constants, organic chelators may affect the bioavailability of zinc (Baker *et al.*, 1995). The binding of zinc to low-molecular-weight ligands (amino acids) or chelators (e.g., EDTA) that can be absorbed also has a positive effect on zinc absorption because the solubility of zinc is increased [NRC]. The increase in the efficiency of Zn retention can be obtained with lower Zn supplementation in the diet, with consequent reduction of Zn concentration in the excreta (Mohanna *et al.*, 1999). Zinc excretion can also be reduced with the use of organic Zn in the diet (Burrell *et al.*, 2004). The bone deposition of Zn increases linearly through the increment of Zn intake (Bao *et al.*, 2007). According to (Ao *et al.*, 2009), dietary organic Zn led to higher accumulation of Zn in the tibia than Zn sulfate. Footpad integrity in broiler chickens is directly related to litter moisture (Bilgili *et al.*, 2009), as an excess of moisture encourages the development of footpad lesions (Zhao *et al.*, 2010). observed that 80 ppm of organic Zn in the diet improved footpad integrity (Rossi *et al.*, 2007). also observed higher tissue resistance with the organic source. This is due to the fact that Zn is involved in maintaining tissue integrity and is required for synthesis of structural proteins, such as collagen and keratin. Its deficiency can lead to severe dermatitis, especially on the bird's footpad (Georgievskii *et al.*, 1982). The aim of this study was to evaluate diets with different levels of organic or inorganic Zn for broiler chickens exposed to immunological, nutritional, and environmental challenges and its influence on humoral immunity, Zn concentration in the tibia and carcass, footpad integrity, and performance.

Zn hydroxide at 100 mg/kg level showed the best result on increasing villus height, crypt depth and villus height to crypt depth ratio. (De Grande *et al.,* 2020). found supplementation with ZnAA increased villus length and villus length to crypt depth ratio. (Ma *et al.,* 2011). revealed that Zn-Gly didn't significantly increase the length of the villus, which is consistent with our results; however, it was not consistent regarding villus depth and reported that adding 90 mg/kg Zn-Gly increased the length of a villus in the duodenum, which is consistent with the present study. (Levkut *et al.*, 2017). found Zn-Gly more effective than zinc methionine on intestinal villi as it increased villi length, which does not consistent with the results of the present study. Zn can affect the morphology of the small intestine and increase its absorption capacity and growth performance (Feng *et al.*, 2010). In addition, Zn is essential for cell proliferation and differentiation, particularly the regulation of DNA synthesis and mitosis division (Beyersmann and Haase 2001). Zn deficiency is also associated with a decrease in the villus height (Southon *et al.* 1986). On the other hand, 42- day-old broiler chickens treated with Zn-Gly (90 mg/kg) showed an increase in their villus height. Average villi surface area of the duodenum has shown a similar pattern and Zn supplementation can affect the villi height and surface (Lonnerdal, 2000). The used organic sources of Zn in poultry diets are more absorbed compared with the inorganic sources. The difference in Zn absorption between organic and inorganic sources can affect the growth of intestinal villi (Levkut *et al.* 2017).

Materials and Methods

Managment of Broiler chickens

This study was carried out in a special field, from 7/12/2021 to 11/1/2022. Broiler Chickens were obtained from commercial hatchery from Kerbala province (hatchery Al-Baz). Feed and water was given ad *libitum*. All brioler chickens received starter diet from (1-10 days) and grower diet from (11-35 days). The starter and grower diet of the experiment were prepared as crumble were met the NRC requirements (NRC, 1994).

Ingredients/gram	Starter%(1-10 days) kg	Grower %(11-35 days)kg
Corn oil	1.5	2.5
Soybean	33.5	33
Corn	57.5	60
Flour	5	2
Provimi Premix	2.5(starter premix 3088)	2.5(finisher premix 3110)
Calculation composition	100%	100%
Crude protein CP%	21	20.27
Crude fiber CF%	2.77	2.74
Calcium Ca%	0.961	0.919
AV-phosphorus	0.42	0.371
ME poultry kcal/kg	2800	3100
AV-methionine	0.47	0.42
AV-TSAA	0.74	0.68
AV-threonine	0.63	0.61

AV-Lysine	1.18	0.98
Electrolytes	263	241.12

The feed additives used in the experiment

A. Threonine (Thr) : use used threonine synthetic source from Jefo Co.Canada. Website https://panjiva.com/Jefo-Nutrition-Inc/44332272
B. Zinc (Zn) : ues used zinc from the Zinpro Co.USA. Website https://www.zinpro.com/products/

Preparation of poultry farm

After cleaning the walls, ceiling and floor by clean water and disinfectant. All windows were opened and all ventilation exhausted vans were switched for ensuring removal of toxic gases completely before chicks admittance, Wateres and feeders were cleaned with disinfectant, then disterbuted to the groups. All hall groups were provided with suitable litter (wood sawdust), lighting and ventilation were controlled according to recommendation. All chicks were reared accordind to Aviagen guide (Aviagen, 2020).

Vaccination programs

There is no drug therapy was used on the day of hatch. All birds were vaccinated with commercial Newcastle Disease and Infectious Bronchitis Disease attenuted vaccine NOBILIS® MA5 +CLONE 30 from MSD Animal Health Company at 10 days of age by spray method. We dissolve 1000 doses/ ½L of distal water and set the nozzle to produce course spray (aerosol generators). Then, all birds were vaccinated with nobilis® Gumboro D78 from MSD Animal Health Company by drinking water at 14 days of age . It is a live freeze-dried vaccine containing live Infectious Bursal Disease (Gumboro) virus strain D78 with stabilizers.Chickens were thirsty by withholding normal water supply from the chickens for 2 hours depending on the ambient temperature. We dissolved 1000 doses in 14 liters of water as the age of the chickens in days and set in the waterers for drinking.

Table (3-2) show vaccinated program

Age of chicks	Disease	Type of vaccine	Administration rout
10	Newcastle and Infectious bronchitis	NOBILIS® MA5 +CLONE 30	Spray method
14	Gumboro IBD	Nobilis® (Gumboro D78)	Via Drinking Water

Experimental design

A total of 200 straight run one-day broiler chickens (Ross 308) were divided randomly to four groups (50/group) with 2 replicates. Each replicate was subdivided to 25 birds/pen as show in (Table 3-3). The control group (Con) fed basal diet without any additives. The second group was fed the basal diets with level calculated (0.9%) dried Threonine. The third group was fed the basal diets with level calculated (0.1%) dried Zinc. The last was combination group (Thr-Zn) fed the basal diets with calculated (0.9%) dried Threonine and was fed the basal diets with level calculated (0.1%) dried Zinc.This experiment period was five weeks started from 7 /12/2021 to 11/1/2022. Feed and water provided to the end of the study.

Blood sampling

All blood samples were collected at day 35 of age from three birds from each replicate randomly were obtained from the wing vein in a test tube without anticoagulant. The tubes were allowed to clot at 18 ^{o}C temperature and centrifuged for 10 minute/ 3000 rpm. Serum was isolated, collected and stored in freeze (-18 ^{o}C) for analysis. Blood serum were used to determine ELISA ntibody titer against ND vaccine at the end of study.

Parameters studied

1. Histological changes: by studying the villi height, and crypt width.
2. Broiler chickens immune response: by estimating of antibody titer against ND vaccines at the end of the experiment.

Histologicl examination

Histological sampling for birds killed at 35 days, section from the middle of duodenum, juojenum and ileum (about 0.6 cm in length) were excised longitudinlly at the antimesentric attachment and gently flushed with NaCl (9 g·L-1). These samples were then fixed in a solution of formalin buffer (90 mL·L-1) for 12 to 24 h at 4 ^{o}C, then rinsed and stored in 70% ethanol at 4 ^{o}C until analysis, Villi and crypts were carefully individualized under a dissecting microscope. The preparation were then mounted between slides and coverslips, with addition of an aqueous agent for microscopy (Aquamount improved gun, VWR, West Chester, PA). Ten villi and 10 crypts of lieberkuhn from each segment of each bird were measured using an optical microscope. The sample of duodenum, juojenum and ileum of 2 birds from each line, representative of the population on the basis of BW, were rehydrated with PBS and stored at 4 ^{o} C until analysis.

Each sample was then embedded in embedding medium in liquid nitrogen, cut at – 20 ^{o} C into –um-thick cross-section using a cryostat, and placed on gelatine –treated glass slides. Three cross-sections were obtained from each sample for further and observation. A routine procedure was carried out using Meyer hemalun and eosin (Sigma Chemical Company). The preparation were then mounted between slides and coverslips with the addition of an aqueous agent for microscopy. The slides were examined using an optical microscope. Fitted with a video camera and the images were analysis software(FiJi version 2.0,).Two images of each section were captured for each sample with a final manification of 10 x .The thickness of the muscularis layer was measured on all section (Boroojeni *et al.*,2019).

Immunological tests

Serological test: Enzyme Linked Immunosorbent Assay (ELISA)

Antibody titers against Infectious brouchitis and Newcastle Disease Virus in broiler chicks the serum samples were measured at the end of study by using Enzyme Linked Immunosorbent Assay for each broiler strains and for different groups (Spalatin et al., 1973).

Statistical analysis

Data was analyzed as one-way ANOVA by using the general linear model (GLM) procedure with SPSS 22.0 software (Corp, 2011). Four treatments means were separated by using a "protected" Duncan`s analysis at level (P < 0.05).

Results

Study of threonine and zinc and their combination on Crypt width and villis height of broilers

Tables (4-5) showed Crypt width and villis height separately. The results showed significant differences (P≤0.05) compared with (the control group) Crypt width and villis height between (Thr) and (Zn) groups and combination groups (Thr+Zn) figure (4-1).

Table (4-1): showed of threonine and zinc and their combination on Crypt width and villis height of broilers

Group Intestine (µm)	Con	Thr	Zn	Thr+Zn
Duodenum				
Villus height (µm)	811.16±3.37 D	1203.48±2.41 B	1151.02±1.41 C	1547.40±23.37 A
Crypt width (µm)	198.68±0.67 D	258.65±2.55 B	235.57±2.80 C	393.81±1.42 A
Jejunum				
Villus height (µm)	596.30±19.24 D	880.26±1.60 B	766.93±9.64 C	1080.24±25.84 A
Crypt width (µm)	149.16±0.84 D	204.75±2.00 B	196.01±1.75 C	260.73±1.38 A
ileum				
Villus height (µm)	261.17±0.99 D	441.83±5.31 B	403.87±2.04 C	601.98±11.25 A
Crypt width (µm)	94.78±1.26 D	144.52±1.14 B	120.10±0.87 C	152.88±1.53 A

Different letters represent a significant difference at (P≤0.05) CON = fed basal diet without supplement. Thr= fed basal diet with 0.9% dried threonine. Zn = fed basal diet with 0.1% Zinc. Thr-zn = fed basal diet with 0.9% dried threonine and 0.1% Zinc.

Study of Threonine and Zinc on humeral immunity in broiler chicken against Newcastle disease and Infectious bronchitis

The figure results (figure 4-6) of the humeral immunity response were showed significantly differences (p≤0.05). The improvement of humeral immunity IgG titer against Newcastle Disease ND and Infectious bronchitis IB were improved significantly (P≤0.05) in the (Thr-Zn), (Threonine), and (Zinc) groups respectively compare with the (Control) in the ten and fourteenth age days.

Figure (4.2) the effect of threonine and zinc their combination on humeral immunity in broiler chickens at 21 days of the study (Mean± SE)

Group Vaccine	Con	Thr	Zn	Thr-Zn
ND	7800.00±334.664 C	8926.80±161.70 B	9842.20±323.329 B	12332.00±402.645 A
IBD	2878.00±61.106 C	3190.00±114.455 B	3428.00±98.152 B	3778.00±97.642 A

Different letters represent a significant difference at (P≤0.05)
CON = fed basal diet without supplement. Thr= fed basal diet with 0.9% dried threonine. Zn = fed basal diet with 0.1% Zinc. Thr-zn = fed basal diet with 0.9% dried threonine and 0.1% Zinc.

Discussion

Study of Threonine and Zinc and their combination on Crypt width and villis height of broilers

Our result in the table (4-1) found a significant differences (P≤ 0.05) compared with (the control group) the Crypt width and villis height in intestine for between in the (Thr+Zn) combination was recorded as mean ±SE according to duodenum (393.81±1.42) , (1547.40±23.37) and jejunum (260.73±1.38), (1080.24±25.84) and ileum (152.88±1.53),(601.98±11.25) respectively. compare with control group was recorded duodenum (198.68±0.67) , (811.16±3.37) and jejunum (149.16±0.84),(596.30±19.24) and ileum (94.78±1.26),(261.17±0.99) respectively.

In addition, the improvement in growth performance attributed to Amino acide (Thr) and zinc had an effect on improving the digestibility of dietary protein in the intestine (Rodríguez-Yoldi *et al.*, 1993). Mix Thr-Zn increase in villus height and Crypt width helped increase the surface area of absorption, thus improving the intestinal utilization of broiler chickens (Qaisrani et al., 2015). Damaged villi or epithelium gut may impair intestinal absorption, which may impair poultry performance (Xue *et al.,* 2018). That increasing the overall growth performance indicates that Threonine and Zn additives can improve the flavor and palatability of the feed (Wenk, 2003; Wang *et al.*, 2007). Several researchers have found that Thr and Zn can significantly increase villi height (Rodríguez-Yoldi *et al.* (1993). Therefore, the intestinal villi play an important role in enhancing the digestion and absorption of nutrients, and increasing the surface area of the small intestine, Intestinal villi are long and thin and the shape of the finger and the intestinal mucosa are shown anatomically animal changes in all groups (CON, Thr, Zn and Thr+Zn) figure (4-1).

increasing villus height, crypt depth and villus height to crypt depth ratio. (De Grande *et al.,* 2020). Threonine and Zinc can improve intestinal morphology and barrier function, increase intestinal goblet cell (Faure *et al.*, 2006). Mucins are a glycoprotein, that are the main component of mucous layers that cover intestinal epithelium. Mucin is secreted by goblet cells. The main protect the intestinal cells from digestive enzymes and acids (Kim & Ho 2010). Mucin can improve intestinal morphology and barrier function, increase intestinal goblet cell density , re equilibrate the gut microbiota, (Hamard *et al.,* 2010; Wang *et al.,* 2010; Azzam *et al.*, 2011, 2012). Additionally, Mucins act as a physical barrier against foreign pathogens Zaghari *et al.* (2011) who concluded that gut functionality seemed to be improved . Moreover, Rezaeipour *et al.* (2012) observed that the villi length and crypt depth were increased in duodenum, jejunum and ileum segments as a result The main function of mucus layer is to protect the epithelium from bacteria, acidic chyme and digestive enzymes. A healthy gut plays a key role in an ideal growth performance of broilers because it supports a better digestion and absorption of nutrients. Thus, increasing villus height, crypt depth and villus height to crypt depth ratio. (De Grande *et al.,* 2020) to enhance the digestive and absorptive capacity and antioxidant status in the intestine (Hong *et al.,* 2015) are led to improve BW, WG, FI and FCR and increasing Mucins and the activity of digestive enzymes and improve immune in broiler chickens.

Study of Threonine and Zinc and their combination on immunity response in broiler chicken against Newcastle disease and Infectious bronchitis

The figure results (figure 4-2) of the immunity response were showed significantly differences ($p \leq 0.05$). The improvement of humeral immunity IgG titer against Newcastle Disease ND and Infectious bronchitis IB were improved significantly ($P \leq 0.05$) in the Thr-Zn mean (12332.00) for Newcastle disease virus vaccination and (3778.00) for Infectious bronchitis virus vaccination , Threonine, and (Zinc) groups respectively compare with the (Control) with mean (7800.00) and (2878.00) for Newcastle and infectious bronchitis, respectively, in the twenty one age day.

However, all the chicks of this study recorded significantly ($P \leq 0.05$) increment in antibody titers against Newcastle Disease vaccine with progress the age, This increment in Ab appear obviously in the treatment groups as compared with the control at (21) days of age, Increasing Thr concentration in diet might enhance immune organs growth, stimulate the synthesis of antibodies and relieve immune stress caused by Newcastle disease (ND) virus (Azzam *et al.,* 2012; Trevisi *et al.*, 2015). It was reported that broilers fed diet containing 0.9% total Thr increase in ND antibody titer compared with those fed control diet. This results was with as (Fard *et al.*,2014) who was concluded that adding 0.9% of threonine to the diet can enhance some immune parameters of chickens to a certain extent, but the effect on Newcastle disease virus is weak. Intestinal morphology is influenced by threonine, especially in the doudenum , jejunum and ileum. Also immunoglobulin parameters (IgG) were significantly affected by the dose from zinc, who was concluded that adding (0.1%) of Zinc to the diet increased the production of broiler chickens antibodies (Kidd *et al.*, 1996).

The improvement of immune response was showed that IgG titer against Newcastle Disease ND Infectious bronchitis IB were improve significantly (P≤0.05) in the (Thr-Zn), (Thr), and (Zn) groups respectively compare with the control tables (4-6). This improvement may be due to Threonine and zinc act to enhance the body health by promoting mucin secretion which improve barrier function and competitive exclusion of pathogenic bacteria by competition on receptor sites and availability nutrient. Threonine and zinc on have been reported to exert an immunomodulatory effect by ability to interact with epithelial.

Vaccine efficacies have been investigated in large number of studies of chicken additive fed based on immunological-parameters focusing mainly on humeral immunity (Zhang *et al.*, 2009; Tohid *et al.*, 2010; Pourhossein *et al.*, 2015; Tu and Siegel, 2015; and Molee *et al.*, 2016). Increase of Antibody titer production a significantly against Newcastle disease and Infectious bronchitis IB compared to the control group in broiler diet attributed to Thr and Zn have immune-stimulatory and immune-modulatory effect (King and Seal, 1998). Immunity plays a major role in achieving maximum growth performance of broilers. The immune status of the broilers improves, with an improvement in the function of immune organs (Corzo *et al.,* 2007; Zhang *et al.,* 2016). , dietary Thr requirements are increased to sustain the maintenance necessities in the gut mucosa (Corzo *et al.,* 2003) and to enhance immunity (Bhargava *et al.*, 1971; Roudbaneh *et al.*, 2013).

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