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Effect of supplementation of threonine, zinc and their combination on some production traits and intestinal morphology of broiler chickens

Thesis

Submitted to the Council of the College of Veterinary medicine at University of Kerbala in Partial Fulfillment of the Requirement for the Degree of Master in the Sciences of Veterinary Medicine in Public Health

By

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Supervised by

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بسم الله الرحمن الرحيم

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I hereby declare that this thesis is my original work except for question 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

To almighty Allah Creator of the heavens and the earth.

To my wounded homeland…. IRAQ

To the one who taught me giving, to the one I am so proud to carry his name, to my father.

To my love, merciful, To whom paradise is under her feet My Loveliest Mother .

To who Support me in my life….. my brother's

To who gives me her heart…..my wife

To The Flowers of my life…… my Son & daughters

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Table of abbreviation

Abbreviation	
Con	Control
Thr	Threonine
Zn	Zinc
BW	Body Weight
WG	Weight gain
FI	Feed intake
FCR	Feed conversion ratio
ELISA	Enzyme Linked Immunosorbent Assay
MT	Metallothioneins
SOD	Superoxide dismutase
CWR	Crypt width ratio
Ab	Antibody
ND	Newcastle disease
IB	Infectious bronchitis disease
VH	Villi height
CD	Crypt depth

Abstract

Broiler chickens' performance and immune response may be affected by the body health; Therefore, this study aimed to evaluate the effect of threonine or/ and zinc on productive, histological and immune response of broiler chickens. The experiment period was five weeks starting as of 7/12/2021 to 11/1/2022, carried out in private hall. A total of 200 straight-run one-day broiler chickens were divided randomly into four equal groups (50 birds/group) with 2 replicates (25 birds/pen). The First group (Con): fed basal diet without any additives, the second group (Thr): fed the basal diets with level (0.9%) threonine, the third group (Zn): fed the basal diets with level (0.1%) Zinc, the fourth group (Thr+Zn): fed the basal diets with (0.9%) threonine with (0.1%) zinc. Feed and water provided *ad libitum* to the end of the study. Blood samples were collected at aged 21 days of the chicks old for study the immune response. The results showed that improvement of immune response Ab titer against Newcastle Disease and Infectious bronchitis were enhanced significantly ($P \leq 0.05$) in the Thr+Zn, Thr., and Zn. as compare with the (Con) in the twenty-one days old. The growth performance of broiler chickens were varies greatly ($P \leq 0.05$) among Thr+Zn, Thr and Zn of feed intake, body weight, feed conversion ratio and body weight gain compared with Con.

On the other hand, Intestinal samples were collected at 35^{th} days of the study by taking tissue sample from duodenum, jejunum and ileum to observe the morphological changes in the small intestinal. The histological changes were showed in depth width, and villus height enhancement of gut health stutus were showed in the Thr+Zn, Thr and Zn groups significantly ($P \leq 0.05$) compare with the Con.

In conclusion, adding a mixture of threonine and zinc separately or in combintion to their basel diet can improve the immune response, gut health status, and growth performance broiler chickens.

Chapter one

1-Introduction

Nutrition plays a major role of gainful broiler chickens' production and on an average, it accounts for about 80–90% of the total cost of production (NAFIS, 2017). Modern broiler chickens can potentially attain 2 kg of body weight by consuming 3 kg of feed within 5 weeks (Choct, 2009). Chickens require precise levels of dietary nutrients particularly amino acids and energy (Kim *et al*., 2007). Additionally, optimal dietary amino acid contents are prerequisite to support gut health functions (Webel *et al*., 1996). A gut healthy plays a key role in an ideal growth performance of broilers because it supports a better digestion and absorption of nutrients (Qaisrani *et al*., 2015). A healthy gut is, therefore, necessary for profitable broiler chickens' production, Villi height and crypt width are important indices for gut health measurement, longer villi and shorter crypts are usually considered as markers of a gut health and well-functioning (Qaisrani *et al*., 2015) 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). 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 made up of Thr, serine and proline, mucin is a glycoprotein in nature, with Thr being the major (28 to 40%) component of mucin (Carlstedt *et al.*, 1993); (Eftekhari *et al.,* 2015). Mucin 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) and allows nutrients passing through a gut (Qaisrani et al., 2018). Dietary Thr, above the requirements, promotes the growth performance, stimulates the synthesis of immunoglobulins, improves immune response and alleviates the immune stress caused by Newcastle disease vaccine and Infectious bronchitis in broiler chickens (Trevisi *et al*., 2015).

Zinc (Zn) is the most commonly added trace mineral in broiler chickens' feeds and it is an essential nutritional trace element for all forms of life as it plays an important role in numerous biological processes (Faa *et al*., 2008; Ranaldi *et al*., 2013; Bonaventura *et al*., 2015). It is important for growth performance, immune response function, metabolism (Kietzmann and Braun 2006; Feng *et al*., 2010; Liu *et al*., 2011). Previous studies have shown that the effect of zinc from different sources, zinc organic (e.g., zinc amino acid) on production performance growth varies (Schlegel and Sauvant *et al.,* 2013). The bioavailability of zinc organic is higher than that of inorganic zinc. A number of researchers have used organic Zn (Burrell *et al.,* 2004), in broiler diets and found an increase in body weight and body weight gain.

The zinc play role of gut health is pivotal in broiler chickens' performance from hatch to the point of production (Shannon and Hill., 2019) zinc is an important dietary factor which regulates intestinal amino acid and protein metabolism in broiler chickens (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 health integrity by compromising the intestinal permeability (Crane *et al.*, 2007; Li *et al*., 2015) epithelial tissue integrity the structure and function of the intestinal barriers (Rodriguez *et al*., 1996; Lambert *et al*., 2004) de Grande *et al*., (2020) clear that Zn amino acid increased villus length and villi length to crypt width ratio. Accordingly, the aim of the current study combined effects of Thr and Zn are suggested on the intestinal absorption and digestion.

<u>Aims of the study</u>

The current study is aimed to study the effect of supplementation threonine, zinc and their combination on some production and intestinal morphology of broiler chickens by estimation of : -

1-Effect on body weight, feed intake, weight gain, and feed conversion ratio.

2- Effect on crypt width and villi height.

3- Effect on immune response against Newcastle disease and Infectious bronchitis.

Chapter two

Review of the Related literature

2.1 Threonine:

Nutrition plays an essential role of gainful broiler chickens' production and it accounts for about 80–90% of the total cost of production (NAFIS, 2017) modern broiler chickens can potentially attain 2 kg of body weight by consuming 3 kg of feed within 5 weeks (Choct, 2009) this higher growth rate, however, requires highly digestible protein and energy concentrated diets, which makes broiler feed more expensive. To minimize feed cost and increase its efficiency, Modern broiler chickens diets are formulated on digestible amino acids basis. There are about 500 types of essential molecules known as amino acids in any living organisms, and only 20 of them are genetically encoded (Walsh *et al*., 2013) Out of these 20 amino acids, 10 are classified as essential, meaning that they can’t be synthesized and must be supplied to the diets for a maximum growth performance.

Out of these 10 essential amino acids, lysine and methionine are considered as the first two limiting amino acids for broilers (Corzo *et al*., 2007). Threonine is ranks the third limiting amino acid and is very important for maintenance and synthesis of the protein in the body and contains 11.7% nitrogen (Kidd and Kerr, 1996). Threonine requirement of broilers depends on many factors including age of the birds, core ingredients in the diet and dietary crude protein (CP) level (Barkley and Wallis, 2001). National Research Council, (1994) recommended values for total dietary threonine were 0.70% for starter (0 to 21 days), 0.65% for grower (22 to 42 days), and 0.60% for finisher (43 to 56 days) periods, respectively.

Threonine contains both alpha and beta carbons in its structure and, It has 4 isomers (D, L, D-allo, and L-allo). In broilers, only L-form of threonine is utilized and transamination of alpha-keto as well as D-isomers to L-form does not occur. Catabolism of threonine results in production of pyruvate, glycine and acetyl-CoA that play a vital role in animal body metabolism including ketogenesis and glucogenesis (Baker, 1985).

Threonine has an essential major role in intestinal development and well-functioning (Stoll, 2006), because intestinal mucin is mainly made of threonine (Faure *et al.,* 2005). Dietary total threonine level between 0.60 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) its supplementation is assumed to result in improved carcass characteristics. It is believed that Thr supplementation enhances feed intake, body weight gain and ultimately carcass weight (Estalkhzir *et al*., 2013; Khan *et al*., 2006).

2.1.1 Influence of dietary threonine of poultry: -

2.1.1 Effects of threonine on growth performance

Growth performance is an important parameter to evaluate the effectiveness of feed offered to the broiler chickens. Different environmental conditions also influence growth performance of broiler chickens depending on the dietary Thr. level (Kidd *et al.*, 2003). These later authors reported that female broilers showed best growth performance at 0.60 to 0.67% of total dietary Thr. whereas for male broilers it was 0.63 to 0.68% during finishing (42 to 45 days) period (Corzo *et al*., 2003) found that ideal total dietary thr level was 0.69% for growth performance and 0.71% for feed conversion ratio (FCR) during finisher period (30 to 42 days) in broilers. In contrast, reported that total dietary Thr requirement was 0.74% for body weight gain and 0.71% for breast meat yield during grower and finisher (21 to 42 days) period (Kidd *et al.*, 2004). estimated that the requirement of the total thr was 0.66% from 3 to 6 weeks of age (Li, 2000). Threonine requirements for feed intake were 0.79% during starter and grower phase and 0.72% for finisher phase (Samadi and Liebert, 2006); (Ciftci and Ceylan *et al*., 2004) reported that ideal total dietary Thr levels for growth performance were 0.68 to 0.75% for starter (0 to 21 days) and 0.65 to 0.68% for grower (22 to 42 days) periods. This decrease in Thr requirement with increase in age may be due to varying dietary crude protein level (Rangel-Lugo *et al.,* 1994) in hygienic environmental conditions Thr. requirements might be under NRC recommended level Azzam and El-Gogary, (2015) executed a trial to investigate the effects of dietary Thr on growth performance in broilers from 1-14 days of age by feeding 0.89, 0.93 and 0.97% Thr, along with a control diet containing 0.65% Thr, the later authors found that the broiler chickens fed diets containing 0.97% of Thr showed 5.1% higher feed intake, 6.4% higher body weight gain and 1.4% better FCR compared with the broiler chickens fed control diet (Najafi *et al.,* 2017). Roudbaneh *et al.,* (2013) reported that Thr requirement under stress and unhygienic environmental conditions were increased to 0.81% to maintain growth performance including FCR.

Similarly, Kheiri and Alibeyghi, (2017) reported that the broiler chickens fed diets containing 0.90% Thr had 1.1% increased FI, 3.2% higher body weight gain and 1.7% better FCR (Feed conversion ratio) compared with those birds fed control diets. Min *et al.,* (2017) conducted a trial to investigate the effect of Thr on broiler chickens' growth performance from 1 to 42 days of age by using total 0.75, 0.94 and 1.12% Thr of the diet, along with a control diet containing total 0.62% Thr, the results revealed that broiler chickens fed diet containing 0.75% Thr had 1.6% higher FI, 6% higher average daily gain and 4.2% better FCR compared with the birds fed control diet. Chen *et al*., (2017) similarly, used 0.88 and 1.08% of total dietary Thr, along with a control diet containing 0.77% Thr to evaluate its effect on growth performance of broilers, birds fed with diet containing 0.88% dietary Thr had 0.6% higher body weight gain, 2.8% better FCR, and 1.0% reduced feed intake compared to those fed with the control diet. Valizade *et al*., (2016) investigated the effect of 0.675 and 0.843% of total dietary Thr on growth performance of broilers in comparison with a control diet containing 0.641% Thr. Diet containing 0.843% dietary Thr supported better growth performance in broilers, with a 4.7% better FCR and 1.0% increase in body weight gain, compared with those fed control diet.

The improved growth performance with higher level of Thr. may be due to provision of higher level of required Thr for an ideal growth performance. Comparing the effect of 0.74, 0.81, 0.88 and 0.96% total dietary Thr on growth performance, Eftekhari *et al.,* (2015) reported that diet containing 0.81% total dietary Thr supported a 5.1% better FCR compared with the birds fed NRC recommended (0.74% total dietary) Thr content. In another study, (Shirzadegan *et al.,* 2015) evaluated the effect of 0.74, 0.79, 0.81 and 0.84% of total dietary Thr on growth performance of broiler chickens. The study concluded that broiler chickens fed diets with 0.84% total Thr performed better and resulted in a 3.1% increase in feed intake, 10.5% higher body weight gain and 4.7% better FCR compared with those fed diets containing 0.74% total dietary threonine.

(Ospina-Rojas *et al.,* 2013) investigated the effect of 0.70% (control) and 0.77% total dietary Thr levels on broiler chickens' performance. The results indicated that broiler chickens fed with 0.77% dietary Thr had good performance with 1.3% higher body weight gain, 1.4% better FCR and a 0.2% reduction in feed intake compared with those receiving control diet Corzo *et al.,* (2007) evaluated the influence of feeding six levels, namely, 0.51 (control), 0.58, 0.65, 0.72, 0.79 and 0.86% of total dietary Thr on broiler chickens growth performance. This dose response study indicated that 0.86% dietary Thr was ideal for broiler chickens' growth performance that resulted in 103% higher body weight gain, 133.7% better FCR and 13.3% lower feed intake during 22–42 d compared with those fed the control diet. Ciftci and Ceylan, (2004) evaluated the effect of four levels (0.54 (control), 0.60, 0.66 and 0.72%) of total dietary Thr on growth performance of broilers. The 0.72% dietary Thr supported 23.2% increase in FI, 27.1% increases in body weight and 3% better FCR compared with the control diet. Data on the influence of different levels of dietary Thr on growth performance of broiler chickens are summarized.

This literature review highlights that broiler diets are usually in Thr and supplementation of dietary Thr, above (NRC) recommendations, in most of the studies, resulted in an improved growth performance.

2.1.2 Effects of threonine on gut health status.

The positive effects of Thr supplementation on growth performance in broiler chickens may be due to development of intestinal mucosa as well as in digestive enzymes function (Dozier *et al.,* 2001), a healthy gut plays a key role in an ideal growth performance of broilers because it supports a better digestion and absorption of nutrients. Villus height (the distance from the apex of the villus to the junction of the villus and crypt) and crypt depth (the distance from the villus junction to the basement membrane of the epithelial cells at the bottom of the crypt) 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) threonine is reported to have a key role in maintaining gut health in broilers. For example, Chen *et al.,* (2017) evaluated the effect of two levels (0.88 and 1.08%) of total dietary Thr on broiler (Arbor Acres plus) performance in a comparison with the strain recommended level (0.77%). Feeding higher level of Thr (1.08%) increased villus height (VH) by 18.3%, villus height to crypt width ratio (CWR) by 33%, and reduced crypt depth (CD) by 12.5% in the duodenum, jejunum compared with broiler chickens fed the strain recommended level of Thr. Similarly, Zhang *et al*., (2016) Studied the effect of two levels (0.49 and 0.90%) of dietary Thr. on broiler (Ross-308) gut health. It was observed that feeding diets containing higher level (0.90%) of Thr resulted in an increased VH by 57.6%, VCR 35.7% and a 18.9% decrease in jejunal CD compared with those fed 0.49% of Thr. Najafi *et al.,* (2017), Conducted a trial by feeding 0.89, 0.93 and 0.97% Thr of diet along with a control diet containing 0.65% of Thr. The duodenum of broilers fed 0.89% of Thr showed increased VH by 15%, VCR by 1.8% and reduced CD by 2% compared with those fed control diet. Improvement in gut morphology may be related to the involvement of Thr in mucin synthesis.

Mucin protects intestinal epithelium from acids, digestive enzymes, and works as a filtering fence against outside pathogens (Kim and Ho, 2010). Mucin is made up of Thr, serine and proline, with Thr being the major (28 to 40%) component of mucin (Carlstedt *et al*., 1993). Eftekhari *et al*., (2015) Fed an NRC recommended (0.74% total dietary Thr.) level and three higher levels (0.81, 0.88 and 0.96%) of total Thr. to evaluate its effect on gut morphology in broilers, it was observed that the broilers fed with 0.88% total dietary Thr had better gut health with a 0.08% greater VH, 0.4% increased CWR, and a 0.3% reduction in the jejunal CD compared with the recommended level.

After absorption, Thr is used for gut protein synthesis, which group together the inner side of the gut as mucin, and protects gut from anti-nutritional factors and pathogens (Lee *et al*., 2007), This may explain the better developed gut in broilers fed higher, than the recommended, levels of dietary Thr. Synthesis of de novo mucin and mucosal protein is faster in the presence of Thr in the lumen, which indicates the importance of Thr for proper functioning of the gut (Nichols and Bertolo, 2008). In portal drained viscera, protein synthesis requires higher amount of Thr, compared with other amino acids, that further highlights the importance of Thr for broilers (Schaart *et al*., 2005). Geyra *et al*., (2001) reported that development of CD was important to speed up gut maturation as well as for renewal of intestinal cells.

The increased intestinal mucin secretion (as a result of developed crypt, which contains mucin secreting goblet cell) as well as proliferation of enterocytes (as a result of Thr) also increases absorption of nutrients (Ospina-Rojas *et al*., 2013). The small intestine uses about 30 to 50% of Thr along with other amino acids (arginine, proline, isoleucine, valine, leucine, methionine, Lys, phenylalanine, glycine and serine) and does not spare it for other extra-intestinal tissues (Wu, 1998). For rapid turnover of intestinal tissues, and to increase surface area for nutrients absorption, therefore, higher than NRC recommended level of Thr is required for a better gut health.

2.1.3 Effect of threonine on immunity: -

Immunity plays a major role in achieving maximum growth performance of broiler chickens. The immune status of the broiler chickens improves, with an improvement in the function of immune organs (Corzo *et al.,* 2007; Zhang *et al.,* 2016). For example, a greater activity and an increase in weight of immune organs including thymus, bursa, and spleen can result in more antibodies production. The NRC recommendations for Thr normally established for healthy birds reared in ideal management conditions, whereas in commercial production systems birds are generally exposed to various types of stresses (Roudbaneh *et al*., 2013). Under unhygienic environmental conditions, 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). 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. 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 are capacity, intestinal integrity, and barrier function of broilers at an early age. (Corzo *et al*., 2007) similarly, described that total dietary Thr requirements to improve growth performance were 0.71 to 74% on new litter, whereas for used litter these were 0.73 to 0.78%. During finisher phase due to old litter and poor management conditions, immunity of birds is compromised. It has been reported that dietary Thr requirements increase in birds suffering from disease including *clostridial* infection (Star *et al*., 2012). Increasing Thr concentration in diet might enhance immune organs growth, stimulate the synthesis of antibodies and relieve immune stress caused by *Escherichia coli* challenge or Newcastle disease (ND) virus (Azzam *et al*., 2012; Trevisi *et al*., 2015). It was reported that broilers fed diet containing 0.67% total Thr had 21% increase in ND antibody titer compared with those fed control diet. Data on the effect of different levels of dietary Thr on broilers immunity is summarized. (Mandal *et al*., 2006). Compared the effect of feeding 0.96% (control), 1.02% and 1.12% total dietary Thr. on broiler chickens' immunity.

2.2 Zinc and its biological importance: -

Zinc is an essential nutritional trace element for all forms of life as it plays an important role in numerous biological processes (Faa *et al*., 2008; Ranaldi *et al.*, 2013; Bonaventura *et al*., 2015). Zinc not only contributes to the synthesis, stability, and catalytic activity of many proteins, but also influences nucleic acid metabolism and immunological responses (Stefanidou *et al*., 2006). Moreover, it plays an important role in wound healing and in restoring the integrity of damaged tissues (Batal *et al.,* 2001; Jahanian and Rasouli, 2015). Zinc also has antioxidant effects (Gammoh and Rink, 2017). As it is a cofactor of the Cu/Zn superoxide dismutase, which plays a crucial role in the protection of cells against oxygen radicals (Oteiza, 2012) zinc ensures normal growth, health and fertility, development of bones and feathers, and regulates appetite in broiler chickens (Shao *et al.,* 2014; Kwiecien *et al.,* 2017). Cellular zinc homeostasis is strictly regulated by uptake and elimination of zinc through specialized transporters and by sequestration of zinc by carrier proteins such as metallothioneins (Bonaventura *et al.,* 2015). Even minor changes in zinc homeostasis can lead to clinical consequences which are most distinct in tissues with a high cell turnover, such as the skin, the gastro-intestinal mucosa, and the immune system (Bonaventura *et al.*, 2015). Due to the absence of a specialized zinc storage system, a daily intake of zinc through the diet is necessary to ensure the homeostasis that allows zinc to maintain and support its numerous functions (Bonaventura *et al*., 2015).

2.2.1 Effect of zinc on growth performance of poultry: -

In broiler diets, zinc may be used either as organic zinc (e.g., Zn protein, Zn amino acid, or Zn picolinate) or inorganic zinc (e.g., ZnCl2, ZnSO4, or ZnO). The recommended zinc level in broiler diets by the National Research Council (NRC) is 40 mg/kg of diet, which can be supplemented via inorganic or organic forms.On the other hand, broiler production in tropical countries is generally suboptimal as indicated by the poor growth performance, suppressed immune function, respiratory disease incidence and high mortality rate (Jaiswal *et al.,* 2017). Apparently due to the high ambient temperature and relative humidity occurring in the regions, it had been reported that addition of zinc to the diet of broilers reared under heat stress improved the production performance and reduced the feed conversion ratio (FCR) (Kucuk *et* *al*., 2003). This indicates that zinc addition may be more important for broiler production in the tropics in order to minimize the negative effects associated with such high temperature condition.

There has been an increasing demand for broiler chickens' meat, and therefore, rearing fast-growing and well-muscled broiler breeds is more profitable. However, the genetic selection of broilers for muscle deposition and growth rate have caused growth and bone mineralization abnormalities. During the short life of broiler chickens their skeletal system undergoes intensive growth (Cook, 2000). The proper function of the skeletal system plays an essential role in poultry production, because it not only provides structural support for the bird but it is also an important mineral source for metabolic needs (Sahraei *et al*., 2012). Moreover, limb bones are essential for proper locomotor function. Bone deformities or even fractures resulting from insufficient adaptation of the skeleton to high body weight are observed. There are several causal factors that often operate in parallel or cyclically, exacerbating the effects of a negative impact on bone development. These factors include unstable and unbalanced dietary energy, protein and minerals, poisoning, hormonal disorders, and genetic predisposition (Cook, 2000). Because of male chickens have heavier body weights than females, the overloading or abnormal loading conditions of pelvic limb bones, resulting in structural damage and deformed lower legs, are more common in males than in females (Śliwa *et al.*, 1996; Tomaszewska *et al.,* 2017).

In broiler chickens, Zn deficiency results in insufficient bone mineralization, skeletal malformation, and reduction of weight gain (Sahraei *et al*., 2012). In addition, Zn-deficient diets reduce egg production and hatchability in layers and breeders (Kienholz *et al*., 1961). In addition, zinc is essential for neurogenesis, synaptogenesis, neuronal growth, and neurotransmission; it is stored in specific synaptic vesicles by a class of glutaminergic neurons and released as a neuro-modulator in an activity-dependent manner (Maret and Sandstead, 2006). A number of researchers have used organic Zn in broiler diets and found an increase in body weight and body weight gain (Burrell *et al*., 2004). Higher biological efficacy of ZnMet in terms of increase in the growth was observed if Zn is bound by fibre and phytates in basal diets (Jahanian *et al*., 2008a; 2008b). Ao *et al*., (2006) observed that a chelated Zn proteinate (Bioplex® zinc) increased body weight gain and feed intake with increasing levels (from 5 to 40 mg/kg diet), it study concluded that supplemental Zn required for optimal broiler growth rate during starting phase (1-21 days of age) was 9.8 mg/kg diet. Moreover, Jahanian *et al.*, (2008a), conducted an experimental trial to investigate the potential of dietary organic sources (Zn-lysine, Zn-methionine and Znacetate) on the broiler performance and carcass traits. The result showed a significant increase in the feed intake with organic Zn inclusion, while the overall feed conversion improved with 80 mg organic Zn/kg diet. Liu *et al.* (2013) stated that Zn proteinate had a significant beneficial effect on weight gain in broilers. Jahanian and Rasouli, (2015) Observed that partial substituting of inorganic Zn by ZnMet improved the weight gain of broiler chickens.

Many studies have been performed to examine the impact of different organic zinc sources on poultry feed intake and conversion efficiency. Hess *et al.,* (2001), added 40 mg/ kg zinc from three different sources (ZnMet, Zn-Lys or ZnMet plus Zn-Lys) to broiler diets, they found improved FCR when organic zinc-amino acid complexes were used in feed for female broilers. The better FCR was observed in earlier weeks (1.66 in average for zinc amino acid complexes vs. 1.73 for the control); this may be attributed to proper feathering, as reported by(Jahanian *et al.,* 2008a).

2.2.2 Effect of zinc on immunity

Lymphoid tissue in broilers is divided into two groups: the central lymphoid (thymus, bursa) and the peripheral lymphoid (spleen). The immunoglobulin parameters (IgA, IgG, and IgM) and complement parameters (C3 and C4) were not significantly affected by the dose or different forms of zinc. Mineral supplementation of diets with organic or inorganic sources is required to meet the nutritional requirements of broiler chickens, because diets based only in natural ingredients produce clear trace mineral deficiencies (Bao *et al*., 2007), zinc requirements have been estimated to be 37 mg/kg from organic sources (Ao *et al*., 2007), and 84 mg/kg from inorganic sources (Huang *et al*., 2007), the NRC states that Zn requirements are 40 mg/kg, regardless of the source. 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), zinc is absorbed with an efficiency from 15 to 60% (McDowell, 2003), zn plasma concentration varies according to its presence in tissues, being distributed among bone, muscle, liver, and pancreas (Georgievskii, 1982), depending upon their chemical nature and complexation constants, organic chelators may affect the bioavailability of zinc (Baker and Ammerman, 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), 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. Explained that zinc addition to broiler chickens diets did not affect the relative weight of lymphoid organs because zinc consumed by broilers was preferentially used to support the metabolic processes that support growth performance, whereas the use of zinc for the development of organs related to the immune system was minimized Cui *et al*., (2004) stated that zinc has an important role in the broiler immune system, which can be seen from the limited development of either lymphoid organs or the mature population of blood T lymphocytes when zinc deficiency occurs in broiler chikens. In broiler chickens, zinc-deficient birds have characteristic microscopic lesions in their lymphoid organs (Burns, 1983), lymphoid organs are part of the structure and function of the immune system in broilers that can protect the body from attack by microorganisms, and zinc is known to have an important role in the immune system of the animal because it is needed in the function, structure, and development of the immune system (Kidd *et al*., 1996), immunoglobulin is a protein that has antibody activity. These results were inconsistent with previous reports by Beach *et al.*, (1980) who showed that adding zinc to the zinc deficient diet increased the production of broiler chickens' antibodies. Furthermore, (Feng *et al.*, 2011), reported an improvement in immunoglobulin levels (IgA, IgM, and IgG) with the dietary replacement of 120 mg/kg of inorganic zinc (ZnSO4) with 90 or 120 mg/kg of organic zinc (zinc-glycinate).

In addition, the level of inorganic forms of zinc increased pancreas MT, Zinc has an important role in many physiological functions, which includes numerous metalloenzyme systems (Andrews, 2000), stated that MT is a cysteine-rich protein that has the ability to bind zinc and other heavy metals involved in stress response activities. Researchers have also demonstrated that MT is synthesized in tissues in response to dietary zinc and binds excess zinc, which has a negative impact on the body (Huang *et al*., 2007), moreover, other researchers, i.e., Sandoval *et al*., (1998) have reported that pancreas MT protein may represent a good indicator of the zinc status of broiler chickens. The optimum dose that produces the highest pancreatic MT was 90.63 mg/kg. Because the only available data were for the control and inorganic groups, the current study could compare only the effects of the inorganic zinc group and the control group.

The zinc level did not affect antioxidant activity (i.e., SOD in meat and the liver). Zinc is a cofactor for SOD and has an important function in antioxidant systems, i.e., as an inhibitor of the oxidation process by protecting proteins and enzymes and as an inhibitor of the formation of free radicals (Yuan *et al*., 2011), SOD is widely distributed and protecting various organs and tissues from peroxidation (Noor *et al*., 2002). Similarly, a study of Zago and Otezein, (2001) showed that zinc is the main component of Cu/zinc SOD, and SOD has cell defense functions against oxidative stress, which explains why zinc deficiency can lead to the increased production of free radicals (Ozturk *et al*., 2003), the insignificant effects of zinc addition on thymus, bursa of fabricius and spleen could have been a direct result of the nonsignificant effect of the zinc on feed consumption; thus, the supply of nutrients for the development of these organs was not altered (Bartlett *et al*., 2003).

2.2.3 Effect of zinc on gut health status

Zinc 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). Movement of cells from crypts to the villus tip is the cause of renewing, which makes them ready for absorption. Length in crescent of villus is associated with enzyme increscent suitable for digestion and absorption, which is caused by supplements with ZnAA (De Grande *et al*. 2020).

2.2.4 Effect of Zinc and threonine on meat quality of broiler chickens:

2.2.4.1 Carcass characteristics:

Information about the effect of zinc on carcass characteristics, such as dressing percentage, carcass yield and carcass composition of broilers. Rossi *et al*., (2007), stated that carcass and cut-up (drumstick, thigh, and breast) yield were not influenced by the addition of increasing levels of dietary organic zinc in broilers. Similarly, (Hess *et al*., (2001) showed that no significant difference was detected when birds supplemented with zinc (40 mg.kg) from zinc methionine, zinc lysine or a commercial mixture of zinc methionine and lysine for whole carcass, abdominal fat, or different cut-up yields. Collins and Moran, (1999) indicated that supplemental manganese and zinc did not alter processed carcass weights and abdominal fat percentage of diverse broiler strains. They did find significant differences in breast and fillet yields among two broiler strains.

2.2.4.2 Carcass quality

Quantifiable properties of meat, such as water holding capacity, shear force, drip loss, cook loss, pH, shelf life, collagen content, protein solubility, cohesiveness, and fat binding capacity, are indispensable for processors involved in the manufacture of value added meat products (Allen *et al*., 1998), raw meat used in further processed products is required to have excellent functional properties that will ensure a final product of exceptional quality and profitability. However, despite their importance, the poultry grading system used worldwide continues to be based on aesthetic attributes, such as conformation, presence or absence of carcass defects, bruises, missing parts, and skin tears, without taking into account the functional properties of meat consequently, this grading system has not been beneficial for the further processing industry (Barbut, 1996).

2.2.4.3 Effects of threonine on carcass characteristics of poultry: -

After slaughtering, except blood and feathers, body of eviscerated bird is called carcass. In broiler chickens, Thr requirement for carcass yield is variable, depending upon age, strain, sex of broiler chickens, CP content of feed, type and proportion of dietary ingredients used (Barkley and Wallis, 2001), The improved carcass characteristics may be due to increased amount of essential amino acids (Thr) in diet (Estalkhzir *et al.,* 2013). Al-Hayani., (2017) conducted a trial by feeding three different levels (0.3, 0.6 and 0.9%) of Thr in broilers diet. The authors concluded that the broiler chickens fed diet containing 0.90% of Thr resulted in an enhanced carcass weight by 3.7%, breast weight by 2.3%, whereas thigh weight was reduced by 1.1% Similarly, El-Faham *et al*., (2017), e xecuted a trial using two levels (0.77% and 0.87%) of Thr in broiler diet. The results revealed that the broiler chickens fed diets containing 0.87% of Thr showed 28% higher breast weight, 1.6% increased drumstick weight, whereas 9.6% reduced thigh weight compared with those fed diet containing 0.77% of Thr. Shirzadegan *et al.*, (2015) executed a trial to evaluate effect of different levels (0.74, 0.79, 0.81 and 0.84%) of total dietary Thr on broilers carcass characteristics threonine is involved in building muscle mass along with serine and improves gut morphology resulting in better absorption of nutrients, and ultimately improves carcass characteristics of broiler chickens. The effects of different levels of dietary Thr on carcass characteristics in broiler chickens are summarized.

Chapter three

Methodology

3.1 Experimental design

A total of 200 straight-run one-day broiler chickens Ross (308) were divided randomly into four groups (50 birds/group) with 2 replicates 25 birds/pen as in show (figure 3.1). The First group (Con) fed basal diet without any additives. The second group (Thr) was fed the basal diets with level (0.9%) threonine. The third group (Zn) was fed the basal diets with level (0.1%) zinc. The fourth group combination (Thr+Zn) fed the basal diets with (0.9%) threonine and was fed the basal diets with level (0.1%) zinc. This experiment period was five weeks started from 7 /12/2021 to 11/1/2022. Feed and water provided *ad libitum* to the end of the study. Figure (3.1) showed the experimental desigen.

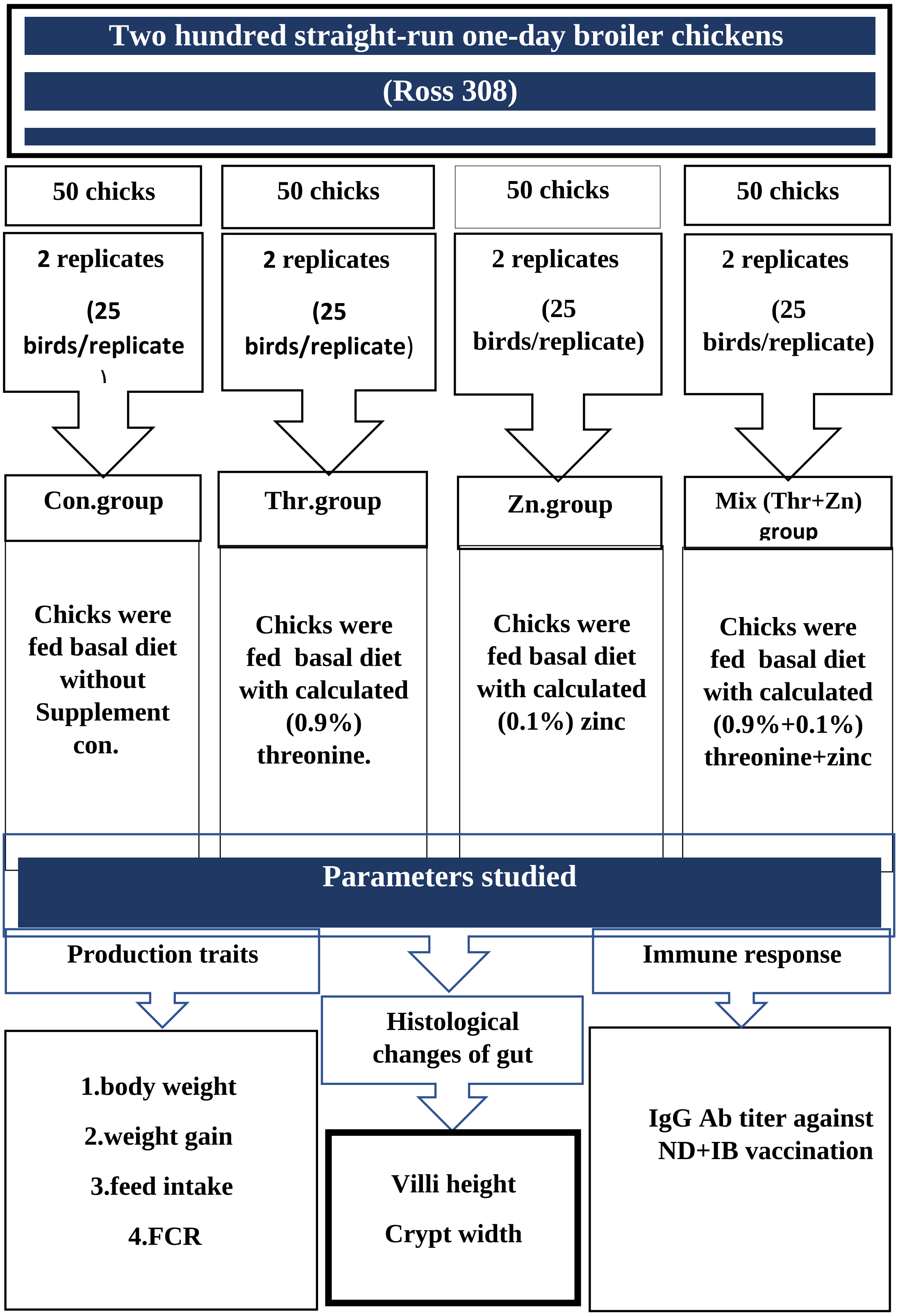

figure (3.1). Experimental design of the study

3.2 Feed additives used in the experiment:

A. Threonine (Thr) : we used synthetic threonine source from Jefo Co.Canada.

B. Zinc (Zn) : we used zinc from the Zinpro Co.USA.

3.3 Management of broiler chickens:

This study was carried out in a private hall, from 7/12/2021 to 11/1/2022. Broiler Chickens were obtained from commercial hatchery from Karbala province (hatchery Al-Baz). Feed and water was provided *ad libitum*. All broiler chickens fed starter phase diet from (1-10 days) and grower phase diet from (11-35 days). The starter and grower diet of the experiment were prepared according to NRC requirements **(NRC, 1994)**. Table (3.1).

Table (3.1): Ingredients and nutrients composition of Starter and Grower diets.

Ingredients/gram	Starter%(1-10 days)	Grower %(11-35 days)
Corn oil	1.5	2.5
Soybean	33.5	33
Corn	57.5	60
Flour wheat	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

3.4 Preparation of poultry hall:

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 chickens admittance, Wateres and feeders were cleaned with disinfectant, then disterbuted to the groups. All expermental 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).

3.5 Vaccination programs:

There is no drug therapy was used on the day of hatch. All birds were vaccinated with Newcastle Disease (ND) and Infectious bronchitis (IB) 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.

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

3.6 Blood sampling:

All blood samples were collected at day 21th 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 antibody titer against (ND) and (IB) vaccine at the end of the study.

3.7 Equipments and instruments:

The apparatuses and tools were used in this study are summarized in the Table (3.3) with their origin or provider.

Table (3.3) shows equipment's and instruments with their sources.

Equipment's and Instruments	**Company *(Origin)***
Disposable syringes (1, 3, 5) cc	China
Disposable gloves	Malaysia
Medical cotton	Turkey
Cooler box	China
Eppendorf tubes and tips	China
Test tube rack (stainless steel)	Germany
Centrifuge rotofix 32	Hettich® / Germany
Graduated glass pipettes size(2,5,10)cc	Silber® -Brand / Germany.
Multi channel pipettes type -12	Tansferpette® -BRAND /Germany
Electrical incubator	Incucell® / Germany.
Autoclave (121°-131°) C	SELECTA /Spain.
Refrigerator	Beko® /Turkey.
Deep freeze refrigerator (-20°C)	GFL Burgwedel / Germany.
Sterile glass tube without anticoagulant	venoject ® terumo /Belgium
Incubator	Binder® /Germany
Centrifuge	China

3.8 Parameters studied :-

3.8.1 Production performance :

3.8.1.1 Weekly mean body weight (B.W.) (gm/bird):

The weight of broiler body was calculated every week by weighing chicks individually at one day old and at end of each week by sensitive balance. Mean body weight was measured from the total weight of all chicks divided on the number of chicks (Al-fayadh and Naji, 1989).

3.8.1.2 Weekly mean weight gain (W.G.) (gm/bird):

The mean body weight gain was measured weekly for each group by recording the weight gain at the beginning of the week and at the end depending on the following equation.

Mean weekly weight gain=body weight at the end of the week - body weight at the beginning of the week (Al-fayadh and Naji, 1989).

3.8.1.3 Weekly mean feed intake (F.I.) (gm/bird):

The feed intake has been measured each week depending on weighting the remaining feed at each end of the week and substrate from the feed that offered at the beginning of the same week, taking with concern the number of the dead chicks and number of feeding days. According to this equation which was mentioned by (AL-fayadh and Naji, 1989). For calculated the food intake of chickens.

Weekly mean feed intake (gm/chick) = $\frac{W}{L+D}$

W= Quality of feed intake through the week (gm).

L= number of live chicks fed through the week.

D= number of dead chicks × number of their feeding days.

3.8.1.4 Weekly mean feed conversion ratio (F.C.R)%:

Feed Conversion Ratio was calculated weekly for each group up to the end of experiment. (AL-fayadh and Naji, 1989) was reported the equation for measurement of F.C.R.

$$\text{F.C.R} = \frac{\text{mean weekly feed intake(gm)}}{\text{mean weekly body weight gain(gm)}}$$

3.8.2 Histological examination

Histological sampling were collected from each replicate at 35 days. Section from the middle of duodenum, jejunum and ileum (about 0.6 cm in length) were excised longitudinally at the antimesenteric attachment and gently flushed with NaCl (9 g·L-1). These samples were 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 (Aqua mount improved gun, VWR, West Chester, PA). Ten villi and 10 crypts of Lieberkühn from each segment of each bird were measured using an optical microscope. The sample of duodenum, jejunum 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 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)**.

3.8.3 Estimation of Immune Response:

3.8.3. Enzyme Linked Immunosorbent Assay (ELISA)

Antibody titers against Newcastle Disease and Infectious bronchitis Virus in broiler chicks, Serum samples were measured at the 21-day old of study by using Enzyme Linked Immunosorbent Assay (Spalatin *et al.*, 1973).

3.9 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 ($P \leq 0.05$).

Chapter four

Results and Analysis

4.1 Production traits:

4.1.1 Results of live body weight, feed intake, weight gain, and feed conversion ratio of broiler chickens as show in:

Tables (4.1,4.2,4.3,4.4) The results showed a significant increase ($P \leq 0.05$) compared with (the control group) the BW, FI, WG and FCR for the (Thr) and (Zn) groups and combination groups (Thr+Zn).

Table (4.1) Effect of supplementation of threonine, zinc and their combination on weekly live body weight (gm/birds) of broiler chickens (Mean±SE).

Groups age	1st.group (Con)	2nd.group (Thr)	3rd.group (Zn)	4th.group (Thr+Zn)
1st day	44.42±0.09	44.50±0.08	44.42±0.09	44.52±0.09
1st wk.	180.67±2.5 C	200.22±0.8 B	198.66±1.27 B	209.84±0.37 A
2rd wk.	417.14±4.08 D	491.10±3.32 B	462.78±2.25 C	536.32±1.94 A
3th wk.	907.58±8.29 D	1054.86±5.77 B	1009.59±6.06 C	1147.74±3.73 A
4th wk.	1472.74±10.23 D	1663.60±3.86 B	1604.34±4.10 C	1817.78±6.66 A
5th wk.	2081.50 ±11.69 D	2333.72±4.34 B	2249.60±6.37 C	2540.70±4.50 A

-Different letters in the same row showed a significant difference among groups at ($P \leq 0.05$)

Table (4.2) Effect of supplementation of threonine, zinc and their combination on weekly feed intake (gm/birds) of broiler chickens (Mean±SE).

Groups Age	1st.group (Con)	2nd.group (Thr)	3rd .group (Zn)	4th.group (Thr+Zn)
1 st wk.	175.708±0.622 D	226.34±0.303 B	210.618±0.397 C	253.76±0.363 A
2 nd wk.	354.14±0.665 D	407.45±0.482 B	380.18±0.450 C	444.02±0.549 A
3 rd wk.	740.41±0.766 D	907.38±0.812 B	862.57±0.818 C	945.018±0.682 A
4 th wk.	971.62±0.596 D	1086.74±1.609 B	1046.59±0.670 C	1141.57±0.643 A
5 th wk.	1124.83±1.448 D	1225.98±1.454 B	1186.94±1.267 C	1304.05±1.280 A
Cumulative feed intake	3366.71±3.044 D	3853.91±2.128 B	3686.91±1.129 C	4088.42±1.991 A

-Different letters in the same row showed a significant difference among groups at (P≤0.05).

Table (4.3) Effect of supplementation of threonine, zinc and their combination on weekly weight gain (gm/birds) in broiler chickens (Mean±SE).

Groups Age	1st.group (Con)	2nd.group (Thr)	3rd.group (Zn)	4th.group (Thr+Zn)
1 st wk.	136.34±2.63 C	155.72 ±0.78 B	154.24 ±1.29 B	165.32±0.39 A
2 nd wk.	236.38±4.74 D	290.88±3.58 B	264.12±2.66 C	326.48±2.04 A
3 rd wk.	490.44±9.65 C	563.76 ±5.60 B	546.72 ±6.66 B	611.42 ±3.88 A
4 th wk.	565.16 ±7.06 C	608.74 ± 4.86 B	594.84 ±3.09 B	670.04±4.20 A
5 th wk.	609.76±3.25 D	670.12 ±0.83 B	645.26 ±4.85 C	722.92 ±8.34 A
Cumulative weight gain	2038.08±11.77 D	2289.22±4.30 B	2205.18±6.38 C	2496.18±4.50 A

-Different letters in the same row showed a significant difference among groups at ($P \leq 0.05$).

Table (4.4) Effect of supplementation of threonine, zinc and their combination on weekly feed conversion ratio of broiler chickens (Mean±SE).

Groups Age	1st.group (Con)	2nd.group (Thr)	3rd.group (Zn)	4th.group (Thr+Zn)
1 st wk.	1.29±0.027 D	1.453±0.008 B	1.365±0.011 C	1.53±0.003 A
2 nd wk.	1.50±0.028 A	1.401±0.018 AB	1.44±0.013 B	1.36±0.008 C
3 rd wk.	1.610±0.029 A	1.512 ±0.016 C	1.578±0.018 AB	1.545±0.010 BC
4 th wk.	1.785±0.021 A	1.72±0.013 BC	1.759±0.008 AB	1.704±0.01 C
5 th wk.	1.844±0.01 A	1.8295±0.002 A	1.819± 0.015 A	1.804±0.021 A
Cumulative F.C.R	1.683±0.010 A	1.6522±0.002 B	1.672±0.004 A	1.637±0.003 B

-Different letters in the same row showed a significant difference among groups at (P≤0.05).

4.2 Effect of supplementation of threonine, zinc and their combination on crypt width and villus height of broiler chickens:

Tables (4.5) showed crypt width and villus height of different sites of gut separately. The results showed significant differences (P≤0.05) compared with (the control group) Crypt width and villus height between (Thr) and (Zn) groups and combination groups (Thr+Zn) figure (4.5).

Table (4.5): Effect of threonine, zinc and their combination on crypt width and villus height of broiler chickens (Mean±SE).

Group Intestine(μm)	1st.group (Con)	2nd.group (Thr)	3rd.group (Zn)	4th.group (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 among group at (P≤0.05)

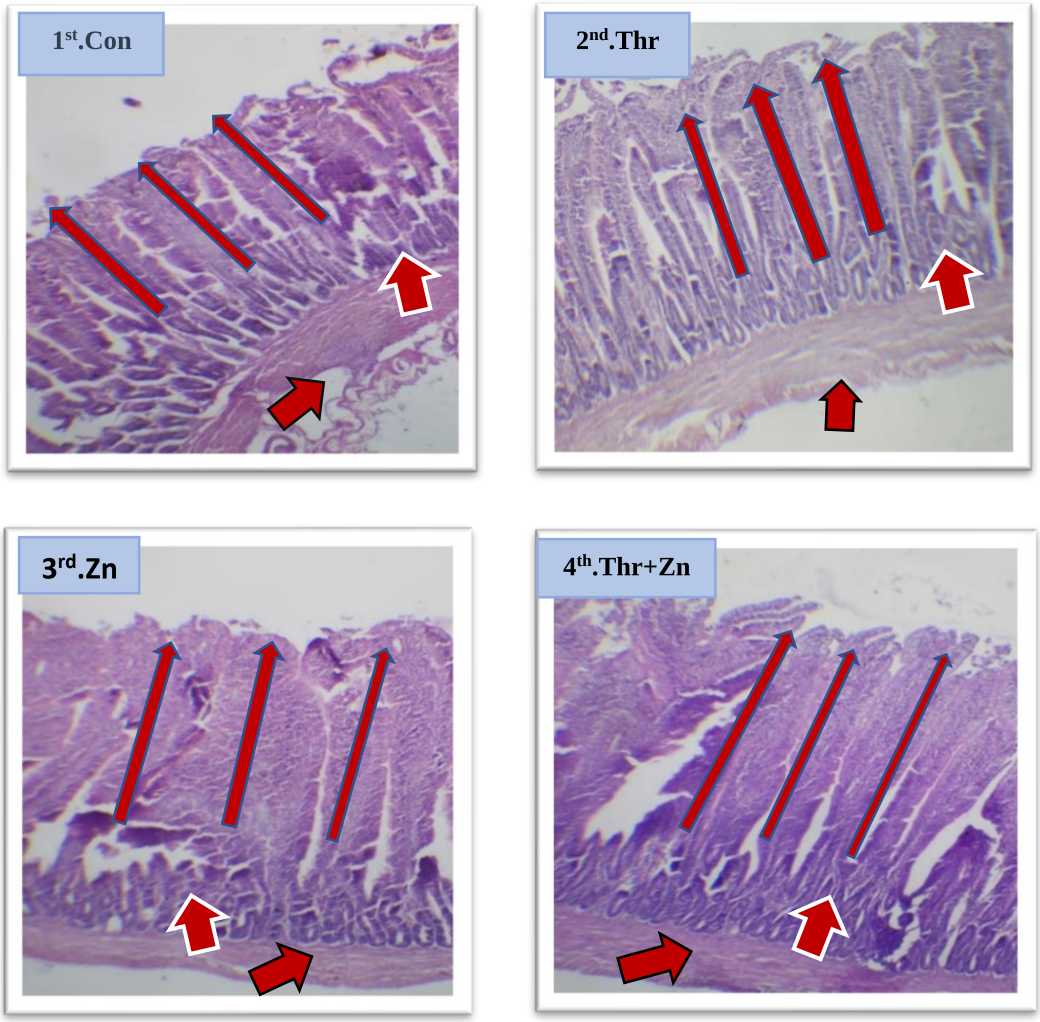

Figure 4.1: Photomicrograph of duodenum in intestine chicken Con. It look like normal slightly length of villi. (Thr) and (Zn) revealed moderate length of villi while high length which include in (Thr+Zn) groups.

The arrow ( )showed villus hight , the arrow ( ) showed crypt width and ( ) showed intestinal wall with magnification power 40X (E and H staining).

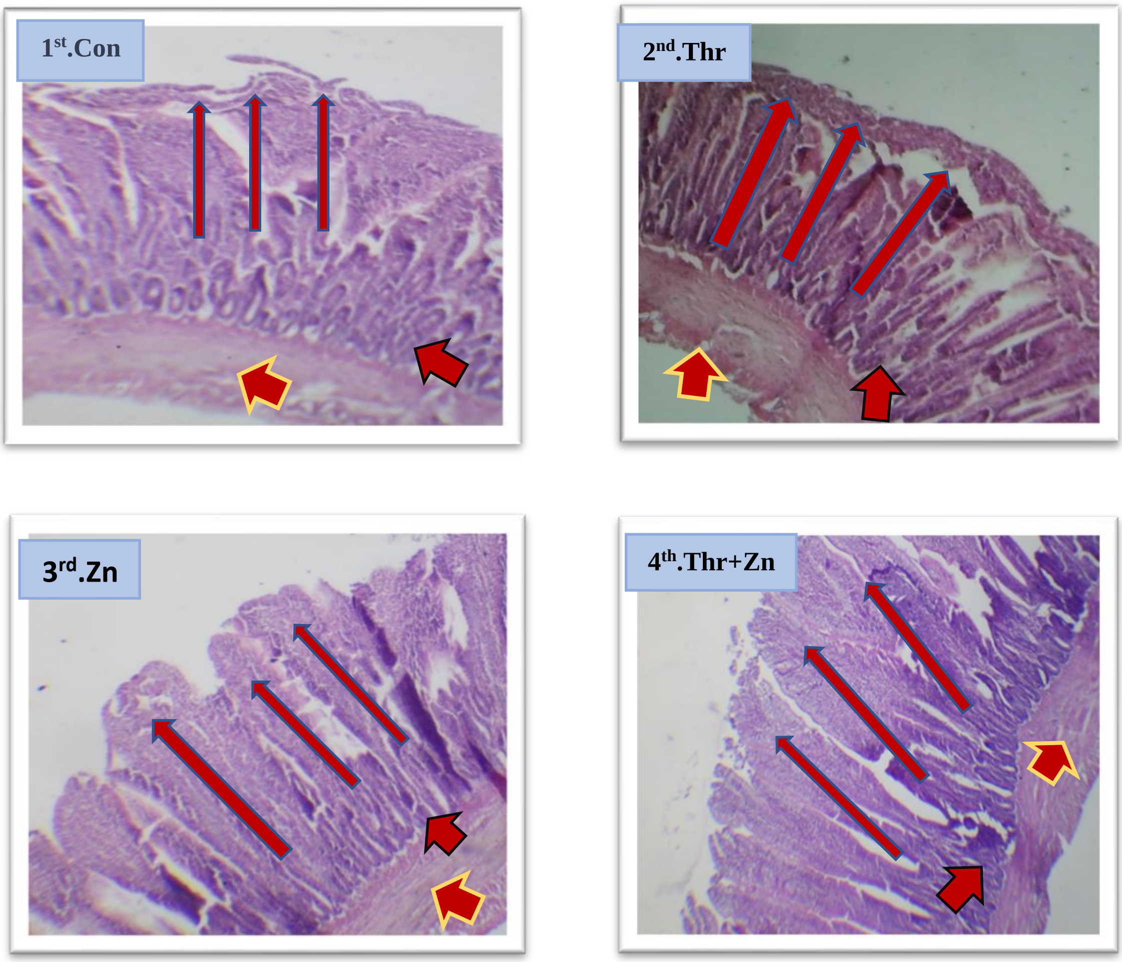

Figure 4.2: Photomicrograph of Jejunum in intestine chicken Con. It look like normal slightly length of villi. (Thr) and (Zn) revealed moderate length of villi while high length which include in (Thr+Zn) groups.

The arrow ( )showed villus hight , the arrow ( ) showed crypt width and ( ) showed intestinal wall with magnification power 40X (E and H staining).

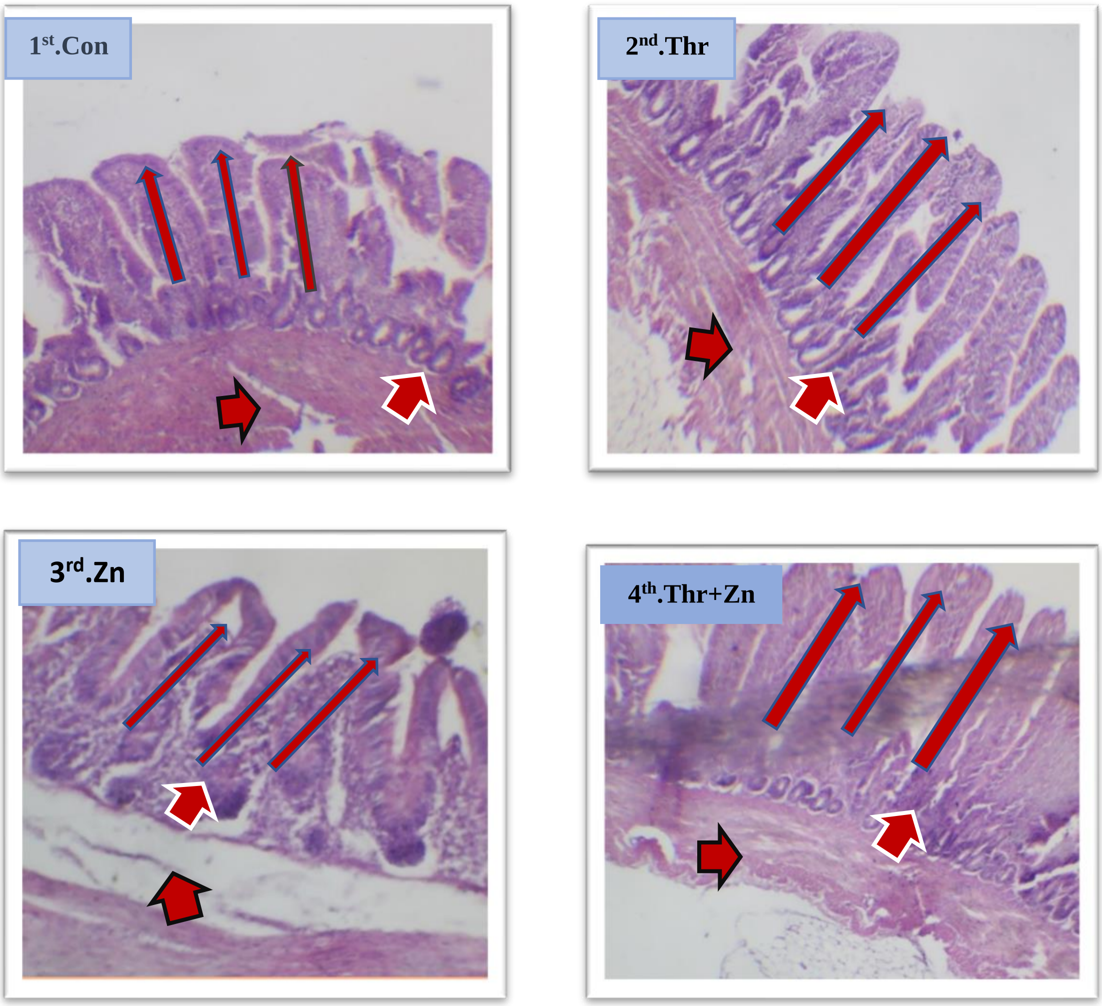

Figure 4.3: Photomicrograph of ileum in intestine chicken Con. It look like normal slightly length of villi. (Thr) and (Zn) revealed moderate length of villi while high length which include in (Thr+Zn) groups**.**

The arrow ( )showed villus hight , the arrow ( ) showed crypt width and ( ) showed intestinal wall with magnification power 40X (E and H staining).

4.3 Immune response against Newcastle disease and Infectious bronchitis in broiler chickens:

The results table (4.6) of the immunity response that showed significant differences among exp.groups ($p \leq 0.05$). The improvement of immunity response Ab titer against Newcastle Disease and Infectious bronchitis were improved significantly ($P \leq 0.05$) in the Thr+Zn, Thr, and Zinc groups respectively compared with the Con in the twenty-one days old.

Table (4.6) Effect of supplementation of threonine, zinc and their combination on immune response in broiler chickens at 21 days of the study (Mean± SE).

Group / Vaccine	1st.group (Con)	2nd.group (Thr)	3rd.group (Zn)	4th.group (Thr+Zn)
ND	7800 ±334.664 C	8926 ±161.70 B	9842 ±323.32 B	12332±402.64 A
IB	2878 ±61.106 C	3190±114.455 B	3428 ±98.152 B	3778 ±97.642 A

-Different letters represent a significant difference among groups at ($P \leq 0.05$)

Chapter five

Discussion

5.1 Production traits:

5.1 Effect of supplementation of threonine, zinc and their combination on weekly live body weight, feed intake, weight gain, and feed conversion ratio of broiler chickens:

The results of the current study showed the live body weight, feed intake, weight gain, and feed conversion ratio in tables (4.1,4.2,4.3,4.4) were increased significantly ($P \leq 0.05$) in the (Thr+Zn), (Thr), and (Zn) groups respectively compared with the control. The results in (table 4.1) indicated that the (Thr+Zn) group recorded the highest mean body weight among treatments, while in the age 35th days, we found a significant increase of live body weight as mean ± SE (2540.70 ± 4.50) in the (Thr+Zn) group rather than control group as mean ± SE (2081.50 ±11.69). The results in (table 4.2) indicated that the (Thr+Zn) group recorded a significant ($P \leq 0.05$) increased in feed intake and improved in the feed conversion ratio as compare with the control group and the results were recorded as mean±SE (1304.05±1.280) greater than (1124.83±1.448) in the control group.

Threonine led to improving BW, WG, FI and FCR that due to enhancing body health and Gut health by promoting mucin secretion which act to improve barrier function and competitive exclusion of pathogenic bacteria. A ratio Thr 0.9% showed a significant increase of daily BW, WG, FI and FCR of broilers for the overall period of 1-35 days of age, reported that the broiler fed diets containing 0.9% Thr led to improve of BW, WG, FI and FCR compared with those broiler chickens fed control diets.

The positive effects of Thr on growth performance in broiler chickens may be due to the involvement of Thr in the development of intestinal mucosa as well as in digestive enzymes function (Dozier *et al*., 2001). A healthy gut plays a key role in an ideal growth performance of broilers because it supports a better digestion and absorption of nutrients. Threonine is one of the main constitutes of mucin structure, providing 11% of its amino acid content as encoded by mucin (Gum *et al.* 1992). Improved growth performance of broiler chickens was observed in response to dietary of threonine higher than recommended demand by the other researchers (Rasheed *et al*., 2018).

Also, Zinc organic led to improve of growth performance (BW, WG, FI and FCR) may be because of enhance body health and Gut health with improve barrier function and competitive exclusion of pathogenic bacteria. The results showed that Zn organic to improve growth performance body weight at level (0.1%) had the best result. Therefore, it positively affects intestinal activity and increases digestive enzymes to enhance digestion and intestinal absorption. The positive effects of organic Zn (Burrell *et al*., 2004), in broiler diets and found an increase in body weight and weight gain. Our result showed a significant increase in the feed intake with organic Zn inclusion, while the overall feed conversion improved with organic Zn diet. (Liu *et al*., 2013).

Stated that Zn had a significant beneficial effect on weight gain in broilers. A ratio Zn showed a significant increase of daily weight gain and feed efficiency of broilers for the overall period of 1-35 days of age with supplementing broiler diets with zinc is a common industry practice (Sunder *et al*., 2008), moreover, it plays an important role in wound healing and in restoring the integrity of damaged tissues (Batal *et al.,* 2001; Jahanian and Rasouli, 2015), zn increases the synthesis of growth factors, such as insulin-like growth factor 1 (IGF-1), and influences the activity of calcium-regulating hormones (Lowe *et al.,* 2002), thus, Zn appears to have multiple important functions in bone development, formation, and metabolism (Seo *et al.*, 2010), bones contain about 30% of total body Zn (Molokwu and Li, 2006), in poultry, Zn deficiency results in insufficient bone mineralization, skeletal malformation, and reduction of weight gain (Sahraei *et al*., 2012).

In plants, zinc is mostly bound to phytate, forming an insoluble complex that hampers absorption. This can be solved by adding phytases to broiler chickens' diets (Lönnerdal, 2000; Tamim and Angel, 2003) observed a higher digestibility coefficient for ZnAA complexes compared to ZnS, confirming the higher bioavailability reported by Star *et al*., (2012), the bioavailability of zinc supplements is affected by competition with other minerals or inhibition by antagonists present in the diet (Lönnerdal, 2000; Sauer *et al*., 2017), therefore, It has been shown that ZnAA complexes are taken up by amino acid transporters as opposed to zinc salts which are taken up by zinc transporters (Gao *et al.,* 2014; Sauer *et al.,* 2017), the latter can be inhibited by zinc uptake antagonists (Lönnerdal, 2000), This alternative supply route of zinc might explain the higher bioavailability.

Thus, Zinc amino acid chelates (such as zinc threoninate) can improve availability and absorption by reducing antagonism, compared to the inorganic form, which may react with no absorbable compounds in the gastrointestinal tract (Hu *et al.,* 2010; Zhao *et al*., 2010), the combined effects from (Thr+Zn) to enhance the digestive and absorptive capacity and antioxidant status in the intestine, Hong *et al.,* (2015) are led to improve growth performance. Combined effects of Thr and Zn are suggested on the intestinal absorption and digestion, according to Rodríguez-Yoldi *et al*., (1993).

5.2 Effect of supplementation of threonine, zinc and their combination on villus height and crypt width of gut:

The results of the current study were showed the crypt width and villus height in intestine in the table (4.5) were increased significantly (P ≤ 0.05) in the (Thr+Zn), (Thr) and (Zn) groups respectively compare with the control. The results indicated that the (Thr+Zn) group recorded the highest mean the crypt width and villus among treatments we found a significant increase of the crypt width and villus height 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.

The threonine can improve intestinal morphology and barrier function, increase intestinal goblet cell (Faure *et al*., 2006), increasing villus height, crypt width and villus height to crypt width ratio (De Grande *et al.*, 2020), in broiler chickens, Chee *et al.* (2010b) showed that an excess intake of Thr can increase the amount of mucin in intestine. Additionally, Mucin protects intestinal epithelium from acids, digestive enzymes, and works as a filtering fence against outside pathogens (Kim and Ho, 2010), mucin is made up of Thr, serine and proline, with Thr being the major (28 to 40%) component of mucin (Carlstedt *et al.*, 1993). Eftekhari *et al.,* (2015), that are the main component of mucous layers that cover intestinal epithelium and mucin is secreted by goblet cells, because of increase intestinal goblet cell density (Hamard *et al.*, 2010;

Wang *et al.*, 2010; Azzam *et al.*, 2011, 2012), the main protect the intestinal cells from digestive enzymes and acids (Kim & Ho 2010). Thus, Mucins act as a physical barrier against any pathogens and allows nutrients passing through a gut Zaghari *et al.*, (2011), moreover, Rezaeipour *et al.*, (2012), Observed that the villus length and crypt width were increased in duodenum, jejunum and ileum segments as a result of the use dietary Thr and the crypt width gives increase of the number of cells in intestinal villi. This can be explained on the basis that Thr fortification maintained the gut health integrity and immunity Chen *et al*., (2017).

Organic Zinc at level showed the best result on increasing villus height, crypt width and villus height to crypt width ratio (De Grande *et al.,* 2020). Zn increased villus length and villus length to crypt width ratio. Movement of cells from crypts to the villus tip is the cause of renewing, which makes them ready for absorption, Length in crescent of villus is associated with enzyme increscent suitable for digestion and absorption, organic Zn (De Grande *et al.* 2020). Our result with agreement (Faure *et al*., 2006; De Grande *et al.,* 2020; Kim and Ho 2010), who reported that the villus length and crypt depth enhance by adding (Thr+Zn) by increasing mucin. 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, threonine and zinc increasing villus height, crypt width and villus height to crypt width 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 in broiler chickens.

5.3 Effect of supplementation of threonine, zinc and their combination on immune response against Newcastle disease and Infectious bronchitis viruses in broiler chickens:

The results of the current study were showed the improvement of immune response Ab titer against Newcastle Disease (ND) and Infectious bronchitis (IB) virus in the table (4.6) were increased significantly ($P \leq 0.05$) in the (Thr+Zn), (Thr), and (Zn) groups respectively compare with the control. The results indicated that the (Thr+Zn) group recorded in the table(4.6) we found a significant increase of the highest mean the of the titer against Newcastle Disease and Infectious bronchitis virus among treatments, as (mean ± SE) that recording (12332) for Newcastle disease vaccination and (3778) for Infectious bronchitis viruses, Threonine, and (Zinc) groups respectively compare with the Con with mean (7800) for ND and (2878) for IB virus, at 21days old.

The improvement of immune response was showed that Ab titer against Newcastle Disease (ND) Infectious bronchitis (IB) virus were improve significantly (P≤0.05) in the (Thr+Zn), (Thr), and (Zn) groups respectively compare with the control. Thr as an essential amino acid is involved in the barrier integrity and in the synthesis of mucin and immunoglobulins (Wang *et al*., 2010; Ren *et al.*, 2014), In the current study, extra Thr improved the intestinal morphology of broilers as evidenced by increased intestinal villus height and the ratio of villus height to crypt width. This improvement may be due to threonine act to enhance the immunity by promoting mucin secretion which improve barrier function and competitive exclusion of pathogenic bacteria by competition on receptor sites and availability nutrient. Threonine on have been reported to exert an immunity effect by ability to interact with epithelial. Which was threonine possibly associated with enhanced mucin synthesis and immunoglobulin secretion resulting from reduced pathogens in the intestine (Ren *et al*., 2014; Trevisi *et al*., 2015), however, all the broiler chickens of this study recorded significantly (P ≤ 0.05) increment in antibody titers against Newcastle Disease and infectious bronchitis vaccine, This increment in Ab appear obviously in the (Thr) as compare with the control at (21) days of age, Increasing (Thr) concentration in diet might enhance immune response, stimulate the synthesis of antibodies and relieve immune stress caused by Newcastle disease and infectious bronchitis vaccine (Azzam *et al.,* 2012; Trevisi *et al*., 2015), 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).

Zinc plays an important role in the antioxidant defense system (Prasad and Kucuk 2002), It also is effective in the immune system (Huang *et al.*, 2007), In addition, Zn plays a role in the weight of broiler chickens' immune system, (Sahraei *et al*., 2012). Rasooli *et al*., (2018) found the increased levels of Zn in the diet caused an increase in the body weight and immune response. Zinc improves the immunity of broilers, and several studies have reported that zinc can improve immunity. First, zinc functions as a cofactor of thymulin and induces proliferation and modulates cytokine release (Maggini *et al.*, 2007), Second, (Sajadifar *et al*., 2013) reported that zinc has a role as a nonpharmacologic booster of immunity in broiler chickens. Third, zinc acts as an immunostimulator that is able to enhance the immune systems (Underwood, 2001), the role of zinc as an enhancer of broiler chickens' immunity is necessary However, the doses of zinc needed to improve the broiler immune response differ across reports, with some studies reporting that the required dose is greater than the NRC recommendation and that zinc has the ability to enhance antibody production. Accordingly, the aim of the current study was to determine the effect of zinc addition to broiler chickens' diets on the immune response and production performance.

Vaccine efficacies have been investigated in large number of studies of chicken additive fed based on immunological focusing mainly on response 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 compared to the control group in broiler chickens diet attributed to Thr+Zn have immune-stimulatory and immune response 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), it was reported that broiler chickens fed diet containing Thr+Zn increase in ND and IB antibody titer compare with those fed control diet.

Chapter six

Conclusions and Recommendations

6.1 Conclusions:

Based on the current study findings, the conclusions are as follows:

1. Adding a mixture (0.9%+0.1%) of threonine and zinc to diet of broiler lead to enhancing growth performance of broiler chickens.

2. Dietary threonine with zinc may improve the intestinal health of chicken broiler through increasing the efficacy of crypt width and villus height.

3. Adding a mixture (0.9%+0.1%) of threonine and zinc to their diet can improve the immune response broiler chickens.

6.2 Recommendations:

1.We prefer to use threonine more than methionine, because zinc is loaded with threonine instead of methionine because its price is lower and its need is more.

2. Use of different levels more 1% of threonine and levels more 1% of zinc and their combinations in broiler chickens' diet.

3. Using a mixture (0.9%+0.1%) of supplementation (Thr+Zn) in laying hens.

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الخلاصة

قد يتأثر أداء الدجاج اللاحم والاستجابة المناعية بصحة الجسم، لذلك هدفت هذه الدراسة إلى تقييم تأثير الثريونين، الزنك على الاستجابة الإنتاجية والصورة النسيجية والمناعية لدجاج اللاحم. كانت فترة التجربة خمسة أسابيع بدأت من 2021/12/7 حتى 2022/1/11 نفذت في حقل خاص. استخدمت 200 فروج لحم غير متجانسة بعمريوم واحد وزعت بشكل عشوائيا إلى أربع مجاميع تغذوية (50 فرخا / مجموعة) صممت كل مجموعة بواقع مكررين (25 فرخا/مكرر). المجموعة الاولى (Con):غذيت على عليقة أساسية بدون أي إضافة. المجموعة الثانية مجموعة الثريونين (Thr):غذيت على عليقة أساسية واضيفت لها ثريونين (0.9%), المجموعة الثالثة مجموعة الزنك(Zn): غذيت على عليقة أساسية واضيفت لها زنك (0.1%), بينما كانت المجموعة الرابعة هي مجموعة الخليط (Thr+Zn): غذيت على عليقة أساسية مضافا لها ثريونين (0.9%) والزنك (0.1%), تم توفير الأعلاف والمياه بصورة حرة حتى نهاية الدراسة, بينما جمعت عينات الدم في يوم 21 من الدراسة للتغيرات المناعية. أظهرت النتائج تحسن في الاستجابة المناعية ضد مرض نيوكاسل (ND) والتهاب الشعب الهوائية المعدي (IB) وبشكل ملحوظ ($P\leq0.05$) في مجموعات Thr+Zn وThr و Zn مقارنة مع Con, وتحسن أداء نمو فروج اللحم بشكل كبير ($P\leq0.05$) فيThr+Zn و Thrو Zn في استهلاك العلف (FI) ووزن الجسم(BW) ونسبة تحويل العلف (F.C.R) والزيادة الوزنية (WG) مقارنة بـمجموعة (Con). من ناحٌية أخرى ، تم جمع العينات المعوية بعمر 35 يوم من الدراسة عن طريق أخذ عينة من نسيج الاثني عشر؛ الصائم واللفائفي لملاحظة التغيرات المظهرية في الأمعاء الدقيقة، أظهرت النتائج إن التغيرات النسيجية لعمق خبايا الأمعاء وارتفاع الزغابات اختلافًا كبيرًا ومعنوياً ($P\leq0.05$) في مجموعات Thr+Zn وThr و Zn مقارنة بمجموعة Con. في الختام ، فإن إضافة الثريونين والزنك وخليطهما إلى النظام الغذائي الأساسي يمكن أن يحسن من الاستجابة المناعية، صحة الأمعاء بالإضافة الأداء الانتاجي لدجاج اللحم.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الطب البيطري

فرع الصحة العامة

تأثير مكملات الثريونين، الزنك وخليطهما في بعض الصفات الإنتاجية والصورة النسيجية لامعاء فروج اللحم

رسالة

مقدمة إلى مجلس كلية الطب البيطري في جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في فرع الصحة العامة

من قبل

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