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Effects of Organic Zinc, Probiotic and their Combination on Some Production Traits, Immune response, Intestinal Histology and Edible Organs of Broilers

A thesis

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

BY

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بسم الله الرَّحْمٰنِ الرَّحِيمِ

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Summary:

Zinc (Zn) is essential nutritional element that is more addition in poultry diet. Probiotics are used to replenish the gastrointestinal flora and assist maintain a healthy digestive tract. This study aimed to enhances the gastro intestinal tract in broiler chickens to enhance performance and immunity state by increase intestinal villi area in the intestine and measuring minerals content in the pectoral muscle.

The experimental period was five weeks started from 23/1/2023 to 27/2/2023 that carried out in private field. A total of 200 straight run one-day old broiler chicks Ross 308 were divided randomly to four equal groups of 50 chicks each group divided to two replicate contain 25 chicks. The control group (T1) fed basal diet without any additives, the second group (T2) fed on basal diet contain 1.5 g/kg diet Zn, the third group (T3) fed on basal diet contain 1g/kg probiotic diet, the fourth group (T4) fed on basal diet contain combination of Zn 1.5g/kg + probiotic 1g/kg diet. Intestinal samples were collected at 35th 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 status was showed in the combination group, significantly ($P \leq 0.05$) compare with the control group. The result revealed that there was significant increased ($P \leq 0.05$) in the mean body weight values, body weight gain, feed intake , feed conversion ratio and enhance in the carcass traits in the combination group as compare to the other groups. Likewise, the combination group showed significantly increased of antibody titers against Newcastle and infections Bursal (Gumboro disease) vaccines, also the combination group showed increase in intestinal villi area , villi crypt and minerals contain in the pectoral muscle of broilers.

In summary, our work recommends that combination of Zn 1.5g/kg + probiotic 1g/kg could be used in broiler chickens diet lead to enhance gut healthy by enhancing villi and crypt besides enhance performance and immunity.

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List of abbreviations

Abbreviations	Meaning/ Full form
BW	Body weight
DFM	direct feed microbial
FCR	feed conversion ratio
FI	feed intake
GI	gastrointestinal
IBD	Infectious bursal disease
MCP	mono calcium phosphate
ND	Newcastle disease
NRC	National Research Council
ROS	reactive oxygen species
SCFAs	short chain fatty acids
WG	Weight gain
Zn	zinc

Chapter One
Introduction

Introduction

Gastrointestinal system has an essential role for the efficient conversion of feed into its basic components for optimal nutrient absorption. If gut health is enhance, digestion and nutrient absorption will be affected on bird performance and welfare will be compromised maintaining gut health in poultry that is essential to raising top performing birds. When properly managed, good gut health in poultry empowers birds to fight off disease (Ravindran and Abdollahi, 2021).

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 (Nguyen *et al*, 2021). 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) (Mwangi *et al*., 2017).

The bioavailability of organic zinc is higher than that of inorganic zinc. A number of researchers have used organic Zn (Kazemi *et al*., 2020 and Ma *et al*., 2021). Also, its an essential dietary element that has a role in regulating the metabolic processes of amino acids and proteins in the intestinal tract of broiler chickens. The significance of zinc to the health and functioning of the gastrointestinal tract has been the subject of a great number of researches. It has been shown that a zinc deficiency has a deleterious effect on the integrity of gut health by increasing intestinal permeability (Lambert *et al*., 2004; Crane *et al*., 2007; Li *et al*., 2015 and Grande *et al*., 2020).

Zinc increases villi length by product new cells that increase space of the intestine and increase absorption in the intestine (Ohashi and Fukada, 2019). Broiler chickens that are zinc deficiency frequently exhibit evidence of stunted development, frizzled feathers , shortening and thickness of the long

bones with larger hocks. If the deficit has been present for a period of more than two weeks the indications associated with the deficiency may be corrected with timely therapy, however, if treatment is delayed, the chicks will most likely continue to be malformed (Naz *et al*., 2016).

Probiotics are live bacteria, fungus or yeasts that are used to replenish the gastrointestinal flora and assist maintain a healthy digestive tract. As a result, probiotics help chickens develop more effectively and have better overall health. As an alternative to the use of antibiotics and probiotics are increasingly being included into the diets of chickens (Al-Heck *et al*., 2020). The beneficial bacteria are protected from the harmful bacteria while the former are driven out by the probiotic's increased competitiveness, Bacitracin secretion is stimulated as a result.

In addition to improving overall health probiotics boost mucin production by stimulating goblet cells with lactic acid. A glycoprotein called mucin makes up the bulk of the mucous produced by epithelial cells. It has been shown that mucin may improve barrier function and increase competitive exclusion of harmful germs. This exclusion occurs by competition one of active species bacteria on receptor sites and available nutrients to reduce the growth of another species (Rolfe, 1991 and La *et al*., 2018).

The fermentation of indigestible carbohydrates by probiotics has the potential to bring the pH of the digestive tract down. Additionally, these bacteria boost the creation of short-chain fatty acids, which in turn function to improve the competitive exclusion of pathogens and to increase the production of immunoglobulins (Javanshir *et al*., 2021).

Aims of the study:

The current study aimed to study the effect of supplementation of zinc, Probiotics and their combination on productive performance and intestinal morphology of broiler chickens by estimation of:

1. Productive performance (live body weight, weight gain, feed intake, feed conversation ratio).
2. Immune response against ND and IBD disease by using ELISA technique.
3. Carcass characteristics and minerals contents of pectoral muscle (Fe, Mg, Cu, Zn).
4. Intestine histology (villi height, crypt depth, villi Weight, villi area) of small intestine of broiler chickens.
5. Histological study of mucin activity by using special stain (Alcian Blue).

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Chapter Two

Literatures Review

Literatures review

2.1. 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 (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 (Skrajnowska and Bobrowska-Korczak, 2019). Moreover, zinc plays an important role in wound healing and in restoring the integrity of damaged tissues (Jahanian and Rasouli, 2015). Its also has antioxidant effects (Gammoh and Rink, 2017). Zinc ensures normal growth, health and fertility, development of bones, feathers and regulates appetite in broiler chickens (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. 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).

2.1.1 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 (Mahmood *et al*., 2023).

Apparently, due to the high ambient temperature and relative humidity occurring in the regions, it has 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 (Kuter *et al*., 2023).

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 has caused growth and bone mineralization abnormalities. During the short life of broiler chickens their skeletal system undergoes intensive growth. 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 (Yusof *et al*., 2023).

In broiler chickens, Zn deficiency results in insufficient bone mineralization, skeletal malformation and reduction of weight gain (Mohd *et al*., 2023). In addition, Zn-deficient diets reduce egg production and hatchability in layers and breeders (Muttathettu and Anitha, 2023). Zinc is als 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 (Meghrazi *et al*., 2017).

2.1.2 Effect of zinc on immunity:

The immune response of broiler chickens may be modified by the level of zinc in the diet. Organic zinc supplementation has a positive effect on the immunological capacity of broilers by improving the levels of immunoglobulins IgA, IgM and IgG and may also improve the cellular response (Qu *et al*., 2023), however, for this type of response higher levels of supplementation may be

required from organic [120 mg/kg (0 to 3 wk) and or inorganic (80 ppm to 5 wk)] (Gajula *et al*., 2011).

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 , 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 chickens (Mohammadi *et al*., 2015). In broiler chickens, zinc-deficient birds have characteristic microscopic lesions in their lymphoid organs (Hidayat *et al*., 2020).

Lymphoid organs are part of the structure and function of the immune system in broilers that can protect the body from attack by microorganisms. 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 (Mitra *et al*., 2022).

2.1.3 Effect of zinc on gut health status:

Zinc administrating to broiler chicken showed an increasing villus height, crypt depth and villus height to crypt depth ratio (Qu *et al*., 2023).

Zinc is essential for cell proliferation and differentiation, particularly the regulation of DNA synthesis and mitosis division (Rizvi, 2022). Zinc deficiency is also associated with a decrease in the villus height (Kadhim, 2022). On the other hand, 42-day-old broiler chickens treated with(Zinc Glycogen) 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 (Levkut *et al*., 2017).

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(Bourgin *et al*., 2021).

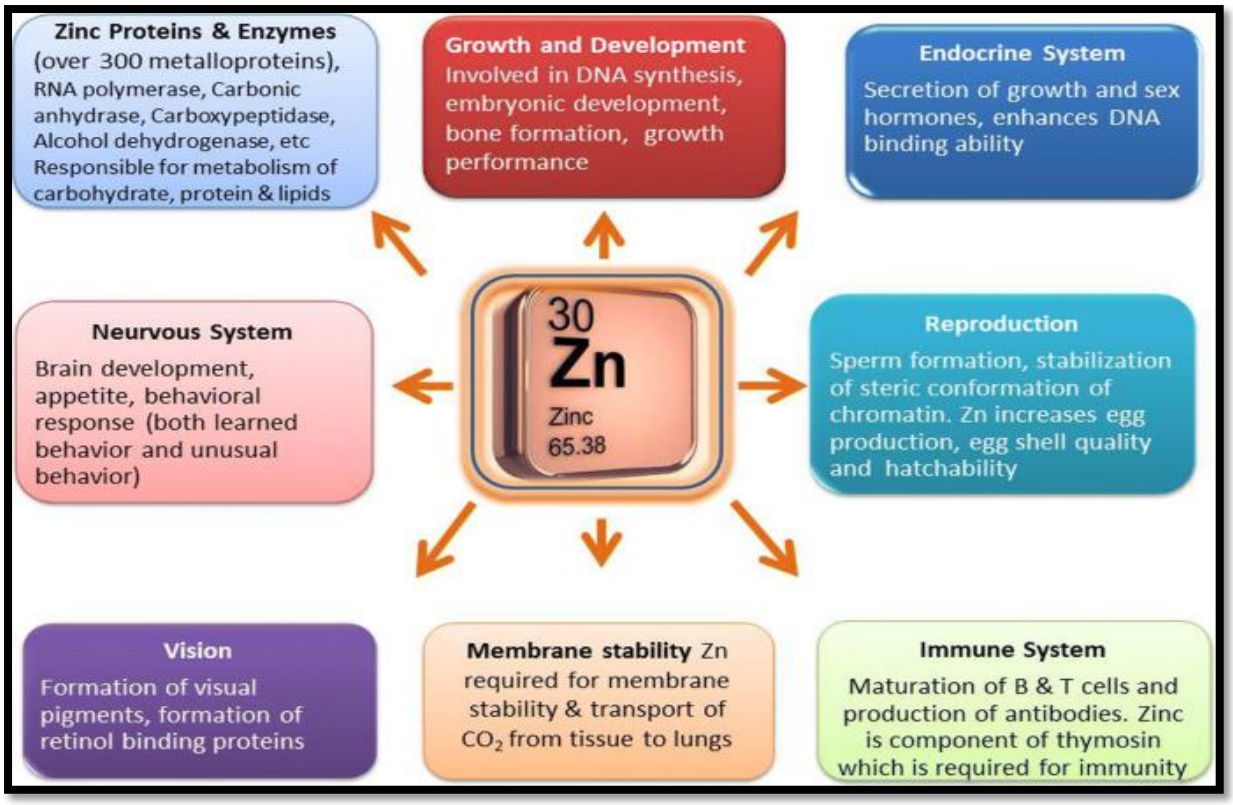

Figure (2-1): Effect of zinc on poultry body (Hassan *et al*., 2020).

2.2. Probiotic:

Probiotic is a living bacteria that when consumed by the host in sufficient quantities, is beneficial to the host's health. Lactic acid bacteria and bifid bacteria are two of the most common probiotic strains (Kimoto-Nira, 2018). Its metabolism is slowed down so that it may survive in harsh conditions like high or low pH and very high or very low temperatures. The effects of these microorganisms on the host include a decrease in intestinal pH due to acid

production, an increase in intestinal antioxidant capacity, stimulation of the gut-associated immune system and stimulation of intestinal intraepithelial lymphocytes (Huang *et al*., 2017).

At low levels of impact in diets antibiotic have been proven in the farm animals for enhancement growth performance due to the emergence of microbes used to treat infections in animals and humans that is resistant to antibiotics (Santovito *et al*., 2018).

There are a number of different possible mechanisms which probiotics work on that include maintaining protective gut mucin, selecting beneficial intestinal organisms or its prevent growth of pathogenic bacteria, improving nutrient uptake, modifying the pH of the gut, improving acid formation and increasing the humeral immune response (Alam *et al*., 2022). Probiotics may modify the immune system and the microbiota of the digestive tract by reducing pathogen colonization, Research on people and on animals have been carried out with the purpose of determining the ability of probiotics to alter the amount and kind of micro flora that are found in the digestive system (Jiang *et al*., 2022 and Nezamdoost-Sani *et al*., 2023).

Studies that have shown a beneficial response in broiler chickens when given dietary supplements including probiotics and organic acids showed there was a discernible increase in both body weight growth and feed consumption in broiler chicks that were given probiotics on a regular basis diet (Angelakis, 2017). Different mechanisms concerning to antagonistic effect on various microorganism include strengthening of the gut epithelial, barrier and modulation of the immune system, competitive adherence to the mucosa and epithelium and secretion of antimicrobial substances.

It is likely that the mechanisms implied the beneficial effect of probiotics is to be multifactorial (Bahaddad *et al*., 2023). Probiotics has been shown to alleviate lactose intolerance (Oak and Jha., 2019). As well as having anti-colorectal

cancer activities (An *et al*., 2019), preventing inflammatory bowel disease (Mishra *et al*., 2022), suppressing diarrhea (Ferguson and Taylor, 2022), and reducing irritable bowel symptomatology (Xu *et al*., 2022). Because the influence of the probiotic effect tends to be strain specific, broad statements about the possible health advantages of probiotics shouldn't be made.

Therefore, the health advantages that are associated with one strain may not necessarily apply to another strain, even if they are associated with the same species.

2.2.1 Type of probiotic:

It is possible to categorize probiotics based on their availability, since they are multiple commercial varieties, modes of action and various metabolic activities. They come in many different forms depending to their aptitude for colonization the gut (McClements and McClements., 2019). *Bacillus* and *saccharomyces cerevisiae* are two examples of free, non-colonizing species that may be used as probiotics. Other examples of probiotics include colonizing species such as *lactobacillus*, *enterococcus*, and *streptococcus* (Goodman-Davis *et al*., 2021) .

The microorganisms should be resisted to the bile , pancreatic juices and hydrochloric acid, stimulate immune system that have anti-carcinogenic activities able to survive both alkaline condition in duodenum and acidic condition of the stomach (Sangamesh *et al*., 2022).

There are two distinct populations of microorganisms that may be discovered living inside the gastrointestinal tract of chickens. The first type of bacteria is called autochthonous bacteria, and they colonize the gut naturally from the environment as a result of feeding behavior or other activities. The second type of bacteria is exogenous in nature, and they are introduced as a dietary supplement into the gastrointestinal tract as direct feed microbial (DFM) or

probiotics. All octhonous bacteria are the name given to these specific types of bacteria (Aruwa *et al*., 2021).

Lactobacillus species can develop in an acidic intestinal environment. Thus, the putrefactive and possibly pathogenic organism that was living in the intestine will be stunted in the intestine due to the fact that it was compose in an acidic environment in the intestine (Bhadra and Banerjee, 2020).

In addition *Bacillus* species are able to secrete enzymes that have been linked to colonization, antibiotic action, and immunological activation. These enzymes include amylase, protease, and lipase. As well as this *saccharomycetes* play a vital role in the creation of amino acids and vitamins (Neveling and Dicks, 2021). Probiotics: an antibiotic replacement strategy for healthy broilers and productive rearing. As a kind of probiotic, bifido bacteria are beneficial for preventing infections and inflammatory bowel disease, in addition to exerting anti-allergenic characteristics on the immune system (García-Burgos *et al*., 2020).

The beneficial properties of a good probiotic include being non-toxic and non-pathogenic, as well as being better capable of fighting off harmful elements of the gastrointestinal tract's environment. In addition to this, they need to be able to cling to the intestinal epithelium while still being compatible with other feed additives (Ashraf *et al*., 2022).

2.2.2 Mode of action of probiotic:

The probiotic efficacy has been increased through the use of a number of different methods and mechanisms. The probiotic efficacy effect is primarily determined by the interactions between the microorganisms of the host and the probiotics mechanism with immune competent cell to the intestinal mucosa (Mazziotta *et al*., 2023).

The traditional mode of action of using bacteria as probiotics is described as the following: competition at the connected site, also known as "competitive exclusion," in which the probiotic bacteria are connected to the bound site,

thereby creating a physical barrier and obstructing the bound in the intestinal mucosa. Additionally, it will produce antibacterial substances. Compounds that are produced by probiotic bacteria and have an antimicrobial effect on pathogenic bacteria are known as bacteriocins and hydrogen peroxide, (Freitas *et al*., 2023).

Some effect result from prevention pathogen establishment and modulation of resident intestinal flora. While other effect result in direct interactions with immune system and host epithelial. In addition stimulation microorganism to modify the gastrointestinal environment to prefer health status and feed efficiency improving (Palkovicsné Pézsa, 2023).

Probiotic actions may stimulate immune system because of ability of creation a natural barrier thought adhesion to the intestinal mucosa and prevent growth of pathogenic bacteria and this enhancing immunity (Rawal and Ali., 2023). Another mode of action may be production of antimicrobial compounds like organic acid and bacteriocins to nutrition competition with pathogens (Chouraddi *et al*., 2023).

Another mechanism that improves efficiency of feed conversion involve enhancement of anaerobic nonpathogenic facultative growth and alteration in intestinal flora, enhancement of digestion and nutrient utilization with growth depression of intestinal pathogen resulting from lactic acid and hydrogen peroxide activity which form by gram positive bacteria (Ngunyangi , 2019).

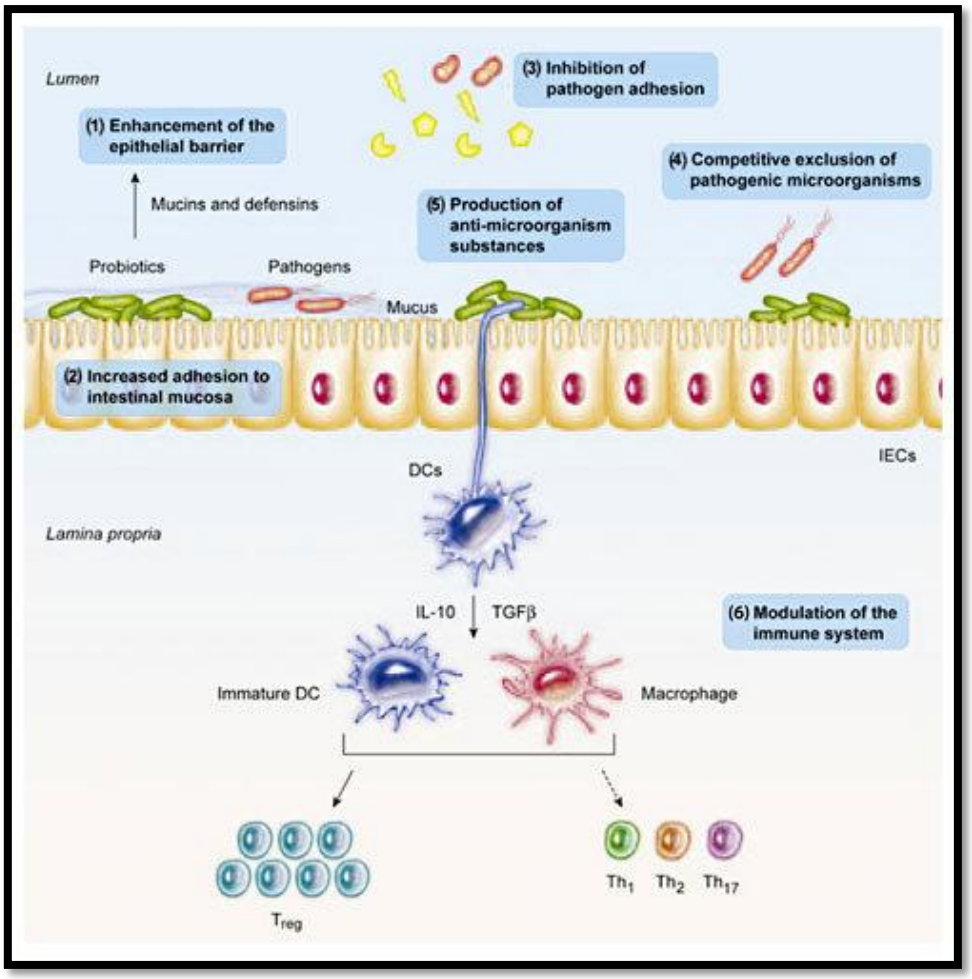

Figure (2-2): Major mechanisms of action of probiotics (Bermudez-Brito *et al*., 2012).

2.2.3 Importance of probiotic:

Animal nutrition researchers found a special attention by using traditional growth promoters (antibiotics), therefore the supplement probiotics strains using diets fed is discovered more as an alternative antibiotic that have been showed the positive effect in animal production and many scientific works in chicken, fish, turkey and other animals (Alagawany *et al*., 2018).

Probiotics bacteria have been used as growth promoters in poultry and livestock feed for increasing feed conversation efficiency improve immune responses and improve growth performance. Probiotics have ability to fight against infectious agents and other stress factors by stimulating the immune system in birds and animals (Patel and Katole, 2023).

Probiotics bacteria have offering digestible proteins, enzyme, vitamins and co-factors as live enzyme factory (Protease, amylase, lipase) which help improving digestion, metabolism and utilization of nutrients for enhancing absorption and digestion of carbohydrates, protein and fats lead to increase feed conversion efficiency. Another hand, helps in synthesis of vitamins (Biotin, B1, B2, and K) and mineral metabolism which responsible for growth properties and metabolism (Li *et al*., 2023).

Probiotic bacteria have been reported to protect the host from any pathogen and stimulation of intestinal immune responses when added in feed to produce of antimicrobial compound (organic acid and bacteriocins) (Tseng, 2023). The advantages of probiotics which increase growth rate and improve productivity have been reported enhance digestibility and utilization of nutrients, reduce stress after transportation, stimulation of immune responses, inhibition of organism and antibiotics therapy of vaccination (Jha *et al*., 2020).

Improvement in feed conversation ratio in animals receiving probiotics up to twenty-one days old and irrespective compared in composition with the group without any addition have been establishment by (Mansilla *et al*., 2022).

Probiotics bacteria compete for attachment a proliferation the microorganism inside the gut mucosa. Thus, beneficial bacteria will prevent the pathogens acquiring foundation in the gut mucosa by rapid colonization . The competition for nutrients resulting in release of antibacterial substances (primary and secondary metabolites) is driving for inhibiting and exclusion of the pathogens (Li *et al*., 2019).

Useful effects of probiotics will rely on various factors including level of consumption, physiological condition of the individual, strains chosen and duration and frequency of exposure. The efficiency or inefficiency of probiotics products may be associated to its composition of microbial species and their livability, bird age, feed composition, method and frequency of application, hygiene facilities and environment stress factors (Tabashsum, 2023).

Some studies observed that probiotics improved the growth performance as efficient as antibiotic growth supporter compared with non-supplement diets. Adding probiotics obtained results are incompatible and spotlight on the importance of its evaluating administration level have been investigated for maximizing efficiency of broiler diet (Ortega *et al*., 2022).

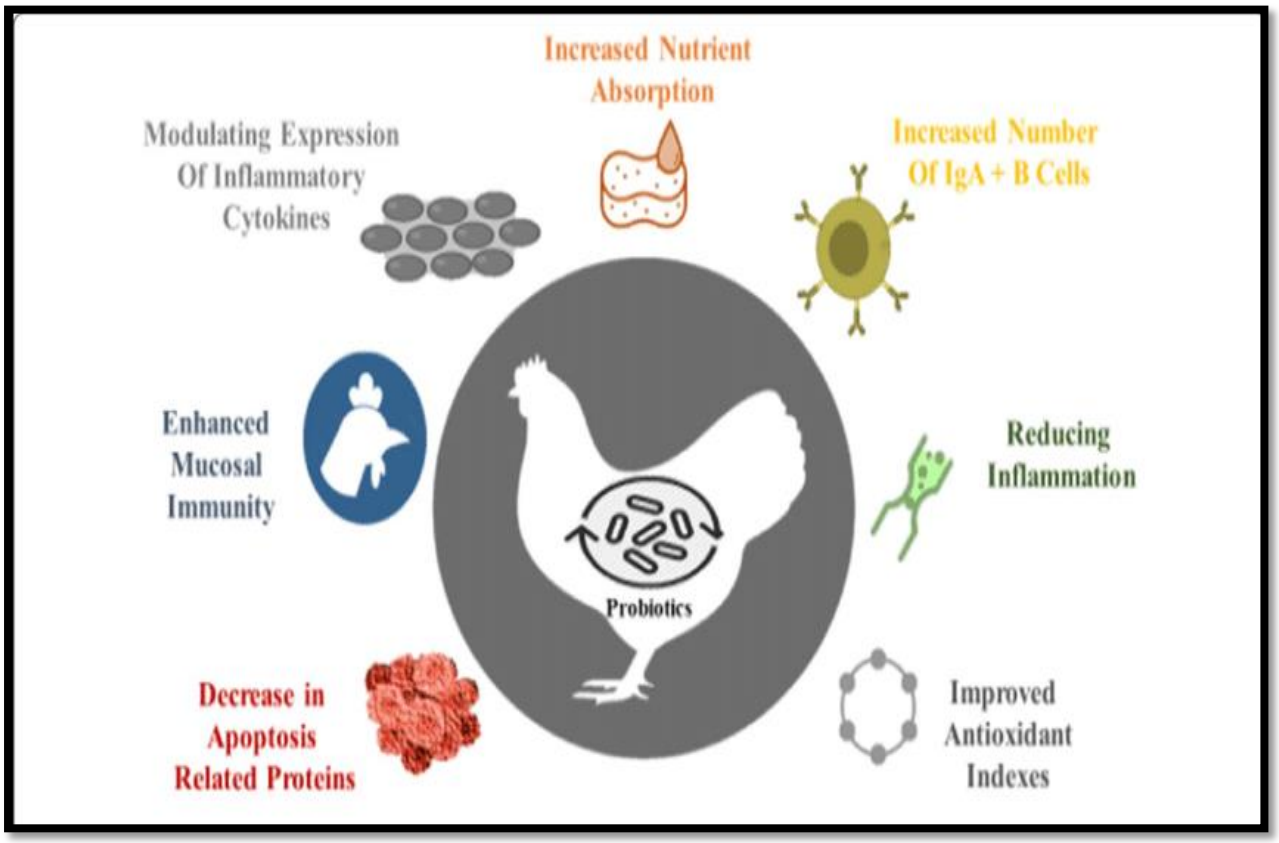

Figure (2-3): effect of probiotic on the poultry intestine (Khalique *et al*.,2020)

2.2.4 Effect of probiotic on the chicken body:

2.2.4.1 Effect of probiotic on growth performance:

Production attribute are beneficial for economic performance monitoring and for evaluating of animal health. The effect of probiotics as alternatives substances when it was added to broiler feed may depend on the breeding system to generate differences in the hygienic conditions for growth promoter. Broiler supplement with probiotics were significantly higher in live body weight gain and carcass yield (Roy and Ray, 2023). other study ,found that laying hen

which were probiotics supplement lowered feed cost and improve egg size(Xu *et al*., 2023).

Adding probiotics have been discovered to enhance feed conversion, improve the immune system and growth performance of birds by effects on intestinal function and morphology and resistance to enteric pathogens in animal's diets (Bajagai *et al*., 2023).

The probiotics have positive effect for improving digestion, absorption, increase digestive and increase an intestinal activities result from availability of nutrients. The measurements of weight gain have shown variable results. Also, renowned a dose-dependent response in poultry receive probiotics (Lokapirnasari *et al*., 2019). In addition, Improvement in digestibility and availability of many nutrients such as fats, carbohydrate, some mineral and protein lead to increase body weight gain have been reported in chicks fed probiotics. Its enhance the effect of endogenous enzyme that one of many of the beneficial bacteria naturally produce within gastrointestinal tract (Onrust *et al*., 2015).

Effect probiotics to stimulate the growth of useful micro flora more desirable balance of bacterium population in small and large intestine. Because of that probiotics naturally rich sources of minerals, vitamin B- complex and protein is ascribed to the growth improvement especially *saccharomyces cerevisiae* (Piccioni *et al*., 2023). Due to improving efficiency of protein ration and/or nitrogen utilization effect of probiotic bacteria and improve growth performance in broiler lead to greater feed conversion efficiency and better physiological activities in animals (Suthama *et al*.,2023).

In addition, there are many advantages of probiotics bacteria such as broke down the hydrocarbon which is the most basic elements that are separated and allowed total absorption in digestive system. Also, growth promotion of animals, as well as majority increase inclusion nutrition and enhance rapid cellular growth and development have been discovered by (Plazy, 2022).

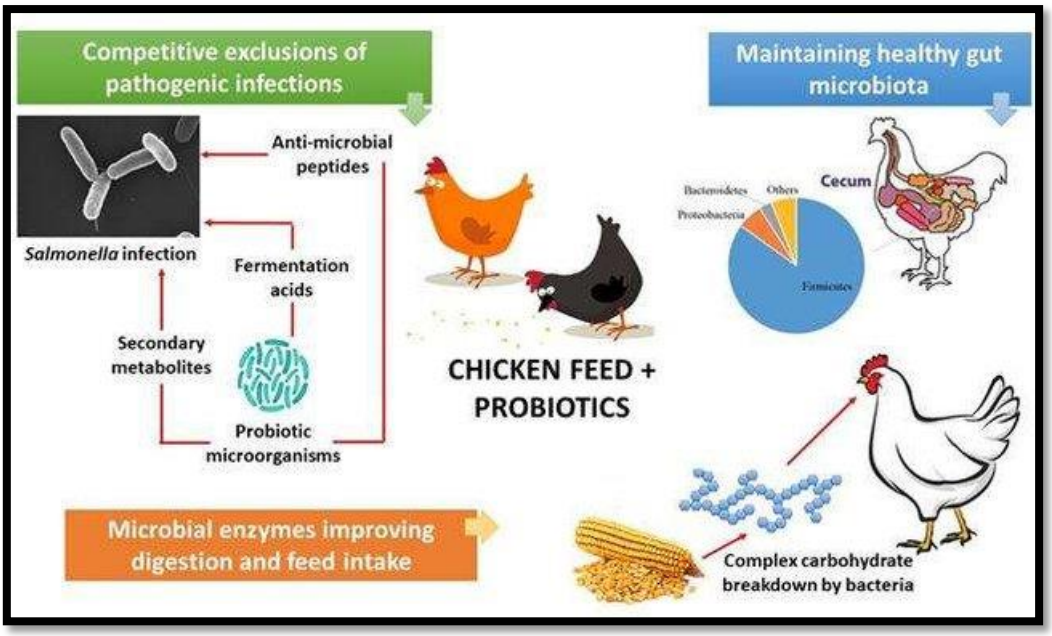

Figure (2-4): The mode of probiotic actions in poultry (Suresh *et al*. 2020)

2.2.4.2 Effect of probiotics on immunity:

Probiotics are mainly used in competitive exclusion of pathogenic bacteria as *Escherichia coli*, *Salmonella* and *Clostridium per fringes* to prevent digestive disorders in diet of birds. Bacteriocins is an antimicrobial secretion that stimulate of immune response and provide maintenance of intestine (Chowdhury *et al*., 2023). Probiotics immune modulatory activities able to fortify intestinal flora micro and improve digestion and nutrient absorption efficiency resulting in growth promotion and feed conversion ratio in the broiler chicken (Ismail *et al*., 2023).

The probiotics have been suggested to stimulate of immune system resulting in increase of Y-interferon production, higher production of immunoglobulin and stimulation macrophage and lymphocyte activity (Zhang *et al*., 2023). Its effect strives many cell types including the adaptive and innate response such as dendritic cells, B- cell, epithelial cells, monocytes/macrophage, NK cells T-cell, involving T-cells with regulatory properties (Ryan *et al*., 2023).

The advantages of probiotics are based on two principle functions inhibition the growth of pathogenic bacteria and stimulation the growth of

beneficial flora micro. Using of probiotics exerts in potential health benefits result in stimulation of gastrointestinal immunity, increased natural resistance to enteric disease and improved digestion (Piccioni *et al*., 2023).

Also probiotics can help in feeding digestion by releasing some enzymes which can completely eliminate the pathogenic bacteria and build up beneficial bacteria flora in intestine.

Probiotic has been suggested positive effect on immune system by enhance production immunoglobulin, natural interferon and stimulation of cell mediated immunity. It plays important role for enhancing the three primary defense system in body defenses against pathogens (the intestinal microbiota) as regeneration immune function and epithelial cells (Al-Mahmud *et al*., 2023). The inhibitory action of probiotics applies on production of primary and secondary metabolite (antibacterial substances), competition of nutrients and stimulation of immunity (Butt, 2023).

It has been demonstrated that there are three different pathways of immune system for enhancing macrophage activity that probiotic increases the ability of intestinal microbiota, increased resistance to infection and increased production of antibodies as immunoglobulin G and M. Some probiotics can enhance resistance to microbial pathogens by stimulate a protective immune response (Obianwuna *et al*., 2023).

Intestinal bacteria are a major source of antigenic material that stimulate of gut development and have intense effect on the immune development of gastrointestinal which associated with production of immunoglobulin A, lymphoid tissue and payer's patches, and production of antimicrobial peptide . Lactic acid producing bacteria has been indicated enhanced macrophage activity (Cheng *et al*., 2023).

Probiotics may have indirect effect to change the microbial population of the gastrointestinal tract lumen, moreover, it stimulate immune cells to secrete

cytokine. *Lactobacillus* and *Bifid bacterium* gram positive bacteria as probiotics that will improve immune response (Alchalaby and AL-Abed, 2023).

Probiotics are enhancing mucosal barrier via stimulating innate immune activity associated with secretion of immunoglobulin A, phagocytosis and increase natural killer cell activity, as well as it has been indicated stimulating the anti-inflammatory effect (Rawal and Ali, 2023). Probiotics fed supplement have been observed higher antibody titer against Newcastle and Infectious Bursal disease during the period three to thirty-five days of age in broiler chicken (Abed et al., 2018).

Probiotic could help relieved the immune response in birds against secondary infection that observed during immune suppressive condition or viral disease (Adhikari *et al*., 2020). Probiotics have been considered to lower pathogenic microbes to help preventing various infectious agent involve bacterial, protozoan, viral, fungal, also enhance the resistance of birds and protect against the negative growth effect (Yadav *et al*., 2016).

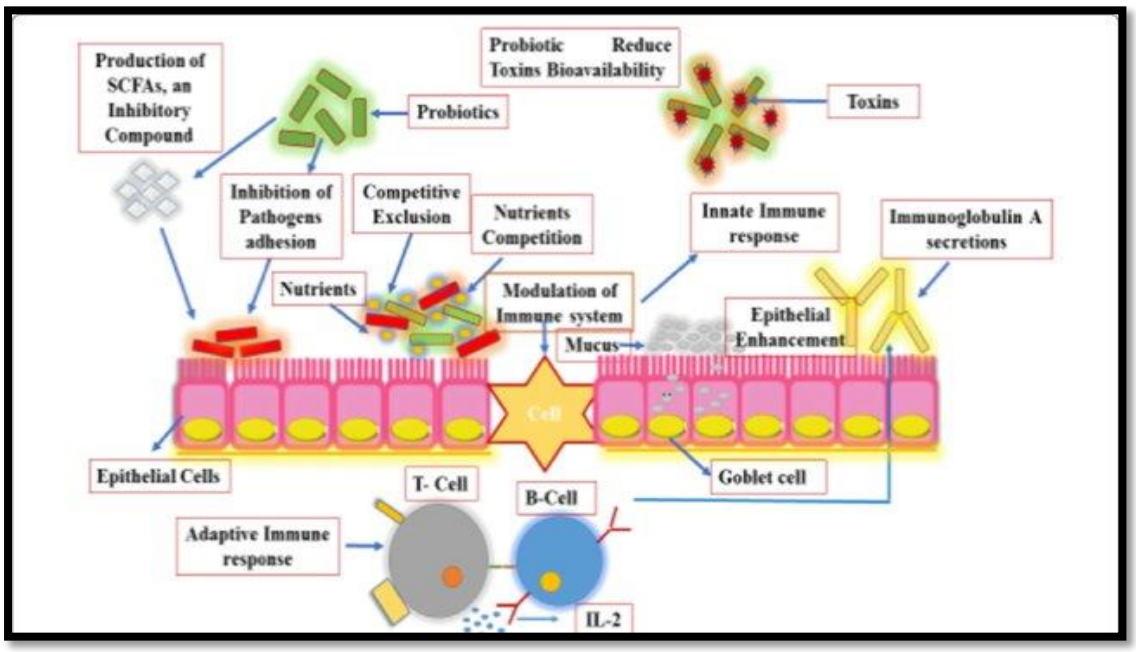

Figure (2-5): Probiotics reduce colonization of pathogens through competitive exclusion (CE) and enhance immune response (Rajput *et al*., 2020).

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Chapter Three

Materials and Methods

3.1 Experiment design:

This study was carried out in a private hall. Chicks were obtained from commercial hatchery . A total of 200 straight run one-day broiler chicks Ross 308 were divided randomly to four groups (50 chicks) per group each group sup divided to two replicate each replicate contain (25)chicks . The control group (CON)(T1) fed basal diet without any additives. The second group (T2) fed on basal diet contain 1.5 g/kg Zn. The third group (T3) fed on basal diet contain 1g/kg probiotic. The fourth group (T4) fed on basal diet contain combination of Zn 1.5g/kg + probiotic 1g/kg. Zn was used from the company ZINPRO.

This experimental period was five weeks started from 23/1/2023 to 27/2/2023 carried out in Albudayr private hall. The weight of chicken and consumed fed were acquired at the end of every week. After the end of the experiment the chicken were sacrifice and sample of blood serum, pectoral muscle and intestine sample were acquired and carcass characteristic.

3.2 Preparation of poultry farm:

After cleaning the walls, ceiling and floor by clean water and disinfectant (sodium hypochlorite). All windows were opened and all ventilation was switched for ensuring removal of toxic gases completely before chick's admittance, all waterers and feeders were cleaned and disinfectant and then distributed to the groups. All the groups were provided with suitable litter (wood sawdust), lighting and ventilation were controlled according to recommendation. All chicks were reared according to Aviagen guide (Aviagen, 2014).

3.3 Vaccination programs:

There is no drug therapy used on the day of hatch. All birds were vaccinated with commercial Newcastle Disease (ND) and Infectious Bronchitis Disease (IB) live vaccine Volvac® LaSota stain 10^{55} EIDS + Massachusetts serotype 10^{55} DIO 30 from Boehringer IngeIheim company at 7 days of age by drinking water. Then, all birds were vaccinated with live Izovac GUMBORO 2 from IZO S.r.I by drinking water at 14 days of age. At day 23 of age Chickens were vaccinated with Izovac CLONE dried live attenuated ND vaccine 10^{6} EID_{50} in drinking water.

Chickens were thirsty by preventing water supply from the chickens for 2 hours depending on the ambient temperature. The vaccine was dissolved in the water according to manufacturer instructions .

Table (3-1) shows the program of vaccination.

Age of chicks (days)	Disease	Type of vaccine	Administration rout
7	ND+IB	Volvac® LaSota stain 10^{55} EIDS + Massachusetts serotype 10^{55} DIO 30/USA	drinking water
14	Gumboro	Izovac GUMBORO2 from IZO S.r.I/USA	drinking water
23	ND	Izovac CLONE 30/Holanda	drinking water

3.4 Nutrients used in the experiment:

All chicks fed *ad libitum* and the diet was formulated according Avian Company requirements. The diet used in the experiment is shown in the table **(3-2)**.zinc was used from Zinpro /USA, probiotic used from Biofine Company / Austria

Table (3-2): Composition of basil diet of broiler chickens (kg for ton)

Content	Starter	Grower	Finisher
Yellow Corn	521	556	596
Soy been meal	411	370	325
Oil	24	35	43
Premix	25 /1000	25	25
Limestone	10	9	6
MCP(mono calcium phosphate)	9	5	5
Chemical composition			
Energy Kcal/Kg	3000	3000	3200
Crud protein CP%	23	21.5	20
Calcium%	0.98	0.87	0.77
Av. Phosphorus%	0.50	0.40	0.40
Av. Lysine%	1.23	1.14	1.04
Av. Methionine%	0.51	0.49	0.47
Av. TSAA %	0.80	0.77	0.73
Av. Threonine %	0.75	0.70	0.63
Calcium%	0.98	0.87	0.77
phosphate%	0.50	0.40	0.40

Effective material in 1 kg of premix: vitamin E 50 000 mg; vitamin D 3 800 000 IU; niacin 12 000 mg; pantothenic acids 3 000 mg; riboflavin 1 800 mg; thiamn 600 mg; menadion 800 mg; ascorbic acid 50 000 mg; folic acid 400 mg; vitamin A 2 500 000 IU; biothin 40 mg; pyridoxin 1 200 mg; vitamin B12 10 mg; cholín 100 000 mg; betain 50 000 mg; Mn 20 000 mg; Zn 16 000 mg; Fe 14 000 mg; Cu 2 400 mg; Co 80 mg; I 200 mg; Se 50 mg.

3.5 Blood sampling:

All blood samples were collected at days 35 of age randomly from each group which were obtained from the wing vein in 6 bird a test tube without anticoagulant. Tubes without anticoagulant were allowed to clot and centrifuged for 10 minute/ 3000 rmp to collect serum. Serum was collected and stored in the freezer until analysis. Blood serum was used to determine ELISA antibody titer against ND and IBD diseases vaccine.

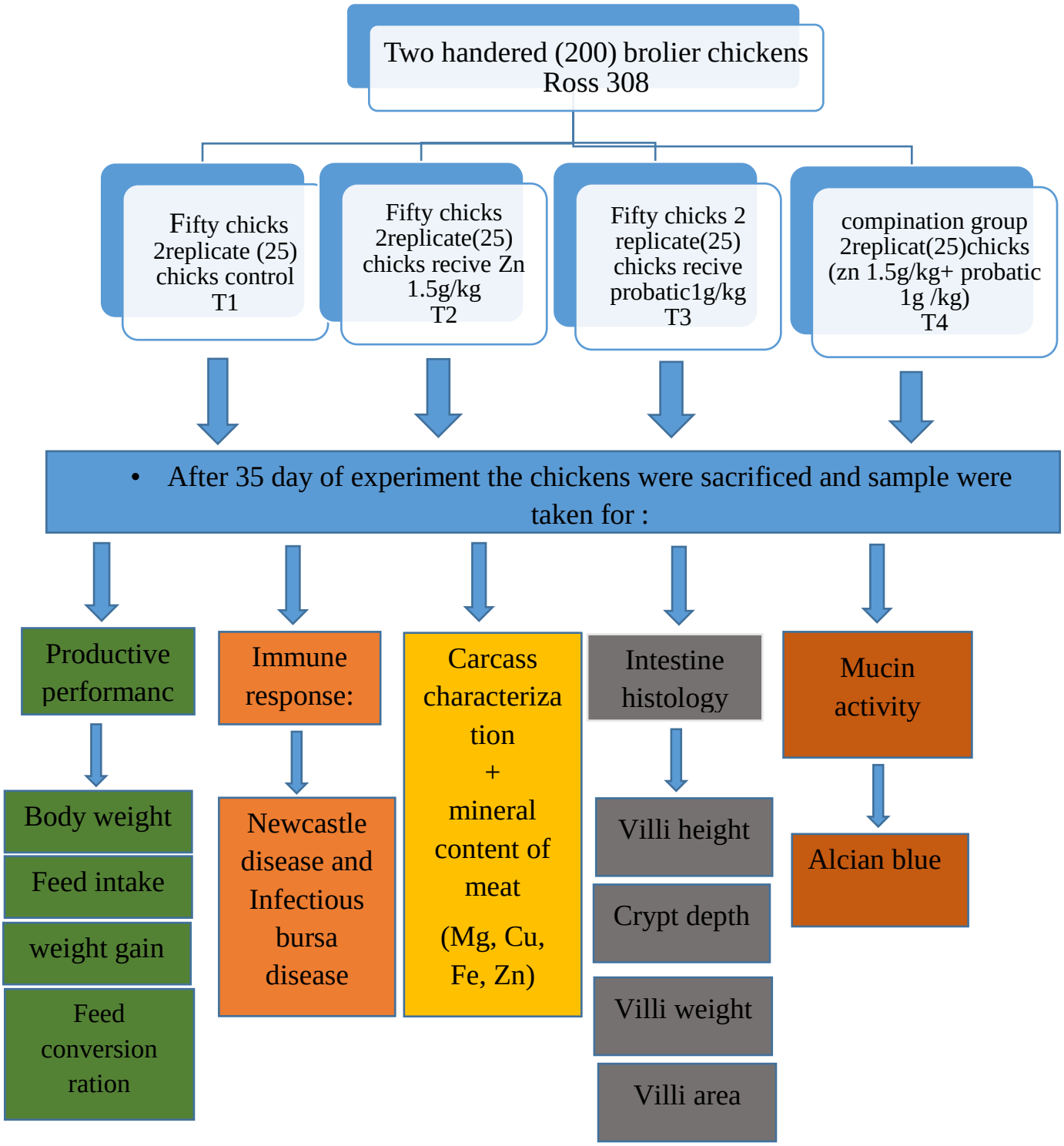


Figure (3-1) experimental design

3.6 Instruments، equipment and kits:

The apparatuses and tools that used in this study are summarized in the table (3-3) with their origin or provider.

Table (3-3) instruments and equipment with their origin.

instruments and equipment	origin
Centrifuge	Japan
Colorimetric determination of Fe	Ltaonline/ Italy
Colorimetric determination of Mg	Ltaonline/ Italy
Colorimetric determination of zinc CU	Ltaonline/ Italy
Colorimetric determination of zinc kit	ltaonline/Italy
Cooler box	China
Deposable syringe (1, 2, 3) cc	China
Disposable gloves	China
Eosin Stain	Himedia Lab India
Eppendorf tubes and micropipettes tips	China
Formalin	Chemanol SA
Graduated glass pipettes size (2, 5, 10) cc	Silber®brand/Germany
Hematoxylin Stain	Himedia Lab India
Infectious Bursal Disease (IBD) Antibody Elisa Test Kit	green spring/USA
Medical cotton	Turkey
Multi-channel pipette type-12	Transferpette ® -BRAND /Germany
Newcastle(ND) Antibody Elisa Test Kit	green spring /USA
Refrigerator	Beko ® Turkey
Sensitive electrical balance	Mettler, Switzerland
Single channel pipette (micropipette 1-50) microliter)	Transferpette ® -BRAND/ Germany
Sterile glass tube without anticoagulant	Venoject ® Terumo / Belgium
Test tube rack (stainless steel)	Germany

3.7 Parameters of the study:

3.7.1 Broiler performance:

3.7.1.1 Mean weekly body weight (BW) (gm/birds):

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

3.7.1.2 Weekly feed intake (F.I.) (gm):

The feed intake has been calculated 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 day. According to this equation which was mentioned by **(AL-Fayadh and Naji, 1989),** for calculated the food intake of chicks.

Weekly 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.

3.7.1.3 Weekly mean weight gains (WG) (gm/birds):

The mean body weight gain was calculated 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).**

3.7.1.4 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)** reported the equation for measurement of FCR.

$$\mathbf{FCR} = \frac{\textit{mean weekly feed intake (gm)}}{\textbf{mean weekly body weight gain (gm)}}$$

3.7.2 Immunological tests:

Serum Immunological tests for ND and IBD were done by using ELISA kit as shown in appendix (I and II).

3.7.3 Mineral content of meat:

3.7.3.1 Content of Mg in meat:

Mg was determined by use special Colorimetric method kit as shown in appendix III

37.3.2 Content of Cu in meat:

Cu was determined by use special Colorimetric method kit as shown in appendix IV

37.3.3 Content of Zn in meat:

Zn was determined by use special Colorimetric method kit as shown in appendix V

3.7.3.4 Content of Fe in meat:

Fe was determined by use special Colorimetric method kit as shown in appendix VI

3.8.3 Histological examination:

Tissue samples (duodenum, jejunum and ilium) were taken after the sacrifice and have been placed in 10% formalin and the histological slide stain with (HandE and alcian blue) is shown in appendix (VII and VIII).

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

3.9 measuring of edible organs weight:

The weight of the organs was measured after the slaughter process and their removal from the body using an electronic scale and compared with the body weight

3.10 Statistical analysis:

Data ware analyzed as one-way ANOVA using the general linear model (GLM) procedure to SPSS 22.0 software **(Corp, 2011).** Four treatment means were separated using a "protected" Duncan`s analysis in level (0.05).

Chapter four

Results and Discussion

4. Results and Discussion

4.1. Production performance

4.1.1. Effect of zinc, probiotic and their combination on live body weight, weight gain, feed intake and feed conversation ratio of broilers:

The results of current study, in the tables, showed a significant ($p \leq 0.05$) increase in the live body weight and weight gain of combination group as compared to the other groups, while zinc and probiotic groups show a significant increase when compared to control group, as shown in table (4.1 and 4.2), while in the age 35th days, we found a significant increase of live body weight as mean ± SE (2516 gm±16) in the (Zn+ Prob) group rather than control group as mean ± SE (2077 gm ±10.84).

The results in (table 4.3) indicated that the (Zn+ Prob) 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 ,the results were recorded as mean ±SE (1146.65 gm ±28) rather than (1123.59 gm ±1) in the control group. It is 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 period from 1to 35 days of age with supplementing broiler diets with zinc is a common industry practice (Sunder *et al*., 2012).

As a result it positively affects intestinal activity and increases digestive enzymes to improve digestion and intestinal absorption. Probiotic led to improving BW, WG, FI and FCR that due to enhancing body health and gut health by promoting mucin secretion which acts to improve barrier function and competitive exclusion of pathogenic bacteria. This result of the current study shows a significant increase in the feed intake and decrease in feed conversation ratio of combination group as compared to the other groups

while zinc and probiotic groups show a significant increase when compared to control group as shown in table (4-3and4-5) and this result is in agreements with (Ogbuewu *et al*., 2023) and (Derakhshan *et al*., 2023). Probiotic enhance antimicrobial substances production such as lactic acid and acetic acid which lower of pH intracellular of bacterial cell(Russell and Diez., 1998); Mack *et al*., 2003) and Gonzalez *et al*., 2012) who reported that probiotic supplement led to enhance growth performance and health body in broiler chicken.

Table (4.1): Effect of Zinc, probiotic and their combination on broilers live body weight (gm).

Groups / Weeks	T1	T2	T3	T4
Day1	42.64±.168A	42.76±.168A	42.54±.183A	42.28±.151A
week1	183.6±1.1C	199.2±2.0B	196.7±1.4B	209.4±1.1A
week2	430.88±4.8C	463.38±14.5B	463.16±2.5B	528.16±6.7A
week3	908.3±8C	1047±10B	1042 ±173B	1108±20A
week4	1446 ±16C	1634.40±11B	1621 ± 16B	1724.00±16A
week5	2077 ± 10.84C	2334 ± 5.5B	2318 ±24 B	2516±16A

Different letters among groups showed a significant difference at ($p \leq 0.05$).
The control group (T1) fed basal diet without any additives, (T2) contain 1.5 g/kg Zn, (T3) contain 1g/kg probiotic, (T4) contain combination of Zn 1.5g/kg + probiotic 1g/kg.

Table (4.2): Effect of Zinc, probiotic and their combination on broilers weight gain (gm) (Mean ± SE).

Groups / Weeks	T1	T2	T3	T4
week 1	140.96±1.1C	165.48±1.9B	154.20±1.3B	167.16±1.0A
week 2	247.28±5.2B	268.8±12.5B	266.42±3.7B	318.72±5.7A
week 3	477.46±10.8B	584.5±21.2A	579.4±18.5A	579.94±15.1A
week 4	538.16±12.7B	586.5±19 AB	579.3±17 AB	615.90±24.7A
week 5	613.22±16.4C	699.62±11B	666.2±21 BC	792.14±25.8A

Different letters among groups showed a significant difference at ($p \leq 0.05$) .
The control group (T1) fed basal diet without any additives, (T2) contain 1.5 g/kg Zn, (T3) contain 1g/kg probiotic, (T4) contain combination of Zn 1.5g/kg + probiotic 1g/kg.

Table (4.3): Effect of Zinc, probiotic and their combination on broilers Feed intake (gm) (Mean ±SE):

Groups / Weeks	T1	T2	T3	T4
week 1	203.27±5.68C	225.76±.36B	220.05±5.8B	239.51±4.26A
week 2	389.71±11.8C	404.48±1.1B	380.1±2.4BC	447.62±3.61A
week 3	784.44±15.3C	824.5±11.3B	865.68±9.6A	846.75±6.5AB
week 4	872.68±.77AB	888.55±1.8A	848.85±4.9B	865.99±16AB
week 5	1132.59±1AB	1057.74±18B	1063.53±28B	1146.65±28A

Different letters among groups showed a significant difference at($p \leq 0.05$).
The control group (T1) fed basal diet without any additives, (T2) contain 1.5 g/kg Zn, (T3) contain 1g/kg probiotic, (T4) contain combination of Zn 1.5g/kg + probiotic 1g/kg.

Table (4.4): Effect of Zinc, probiotic and their combination on Feed conversion ratio in broiler chickens (Mean ±SE).

Groups / Weeks	T1	T2	T3	T4
week 1	1.48±.01A	1.44±.01A	1.43±.0.3A	1.41±.02A
week 2	1.60±.04A	1.51±.07AB	1.42±.01B	1.40±.03B
week 3	1.66±.04A	1.42±.04B	1.50±.05B	1.46±.03B
week 4	1.62±.03A	1.52±.04AB	1.47±.05AB	1.41±.05B
week 5	1.80±.04A	1.49±.01BC	1.58±.03B	1.45±.04C
mean	1.67±.00A	1.47±.00BC	1.51±.01B	1.43±.02C

Different letters among groups showed a significant difference at ($p \leq 0.05$).
The control group (T1) fed basal diet without any additives, (T2) contain 1.5 g/kg Zn, (T3) contain 1g/kg probiotic, (T4) contain combination of Zn 1.5g/kg + probiotic 1g/kg.

4.2. Effect of zinc, probiotic and their combination on immune response against Newcastle disease and Infectious bursa disease in broiler chickens:

The current study showed a significant ($p \leq 0.05$) increase in Immunological parameters against ND and IBD viruses in the combination group as compared with the other groups while there is significant ($p \leq 0.05$) increase in probiotic group as compared with zinc and control groups. On the other hand, zinc group show significant ($p \leq 0.05$) increase as compared with control group as shown in table (4-5). The immune response of broiler chickens may be modified by the level of zinc in the diet; it has an important role in many physiological functions in the body.

The role of zinc as an enhancer of broiler immunity is necessary because of the number of restrictions on the use of antibiotics in the diet imposed by many countries. The immune status of the broilers improves, with an improvement in the function of immune organs such as Lymphoid organs that are part of the structure and function of the immune system in broilers that can protect the body from attack by microorganisms. Also 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 (Hidayat *et al*., 2020).

Zinc has been shown to be necessary for DNA synthesis, which is an essential step in cell division. Specifically, zinc is required for the activity of many enzymes involved in DNA replication, repair, and transcription. Zinc may also play a role in regulating the cell cycle, which is the sequence of events that leads to cell division and increase the immune cells production (Zhang *et al*., 2023).

Probiotic bacteria are capable of enhancing both specific and nonspecific immune responses by activating macrophages, increasing cytokine production by intra epithelial lymphocytes (IEL),and increasing the levels of the body immunoglobulins especially IgA (Roselli *et al*., 2022). Probiotics bacteria have been a cause to protect the body from any infections and stimulation of intestinal immune responses when added in diet to produce antimicrobial compound (organic acid and bacteriocins) (Ellin, 2001).

Table (4.5): Effect of Zinc, probiotic and their combination on immune response / mean ±SE

Groups / Parameters	T1	T2	T3	T4
ND	2878.0±49.89D	3190.0±93.45C	3428.0±80.14B	3778.0±79.72A
IBD	169.40±5.5 C	245.00±6.1 B	261.00±3.9 B	343.80±15.0 A

Different letters among groups showed a significant difference at ($p \leq 0.05$).
The control group (T1) fed basal diet without any additives, (T2) contain 1.5 g/kg Zn, (T3) contain 1g/kg probiotic, (T4) contain combination of Zn 1.5g/kg + probiotic 1g/kg.

4.3 Effect of zinc, probiotic and their combination on carcass characterization in broiler chickens:

The results of this study show a significant ($p \leq 0.05$) increase in the live body weight and carcass without feather, this examination did at age of broiler chickens 35 days. We notice an increase in the size of the heart, liver and gizzard; this is due to the good use of organic zinc, as it is included in the synthesis of protein and helps in building the muscles of the bird's body. This increase in the size of the heart is appropriate for the weight increase of the bird's body in the combination croup (T4) when compere with other group and the differences are similar between (T2 and T3), it is considered less significantly the control group (T1).

The growth performance of animals is affected by the muscle protein, which is the net balance of protein synthesis and degradation. Thus, any factor that increases the synthesis and/or reduces breakdown will lead to higher muscle mass, while reducing the synthesis and/or increasing in breakdown will result in muscular atrophy (Tesseraud *et al*., 2011). Amino acids are the building block of protein that can also regulate signal transduction pathways involved in cell function and metabolism, and modulate protein metabolism in the body (Dickinson *et al*., 2011).

Zinc plays a role in collagen synthesis and deficiencies of this nutrient result in reduced production of skin collagen (Leeson and summers, 2005).

Zinc which is the second most abundant trace element in the body plays an essential role in protein and DNA synthesis, immune cell function, regulation of cell growth, and enzyme’s co-factor. Inorganic Zn has been used as an additive in the chicken diet. However, inorganic Zn is easily dissociated in the upper GIT and interacts with other minerals, thereby its bioavailability is reduced in the intestine, and excretion increases in the environment (Yenice *et al*., 2015).

Additionally dietary probiotic has been used to modulate the immune response in poultry. Their effect may include immuno-stimulation, anti-inflammatory reactions, exclusion and killing of pathogens in the intestinal tract and reduction of bacterial contamination on processed broiler carcasses. Also the balanced interaction between the intestinal microbiota, epithelium and immune system provides resistance to enteric pathogens (Patterson and Burkholder, 2003).

The study showed an increase in the size of the heart and this is due to the good use of organic zinc, as it is included in the synthesis of protein and helps in building the muscles of the bird's body. This increase in the size of the heart is appropriate for the weight increase of the bird's body. When body weight increases, the size of the heart may naturally increase to meet the needs of the larger body. An increase in body weight is usually associated with an increase in volume, muscle mass and adipose tissue in the body, which require the heart to increase in strength and efficiency to pump blood to these additional areas (Hajiazizi *et al*., 2023).

As broiler chickens consume a high-energy diet to promote rapid growth, the gizzard adapts to the increased workload by growing in size and developing stronger muscular walls. This adaptation allows for better grinding and digestion of the feed, aiding in nutrient absorption (Ravindran *et al*., 2021).

Table (4-6): Effect zinc and probiotic on carcass characteristic/ mean ±SE

Groups / Parameter gm	T1	T2	T3	T4
Live body Wight	2077.72±10.84D	2334.02±5.56B	2251.04±2.50C	2516.14±16.41A
Carcass Without feather	1923.88±10.07D	2161.30±5.15B	2084.46±2.32C	2329.94±15.19A
Carcass Without visra	1525.04±7.96D	1713.17±4.08B	1652.26±1.84C	1846.84±12.04A
Carcass with edible	1615.24±11.01D	1826.97±10.09B	1765.46±4.30C	2001.64±8.79A
Gizzard	42.40±3.24C	59.80±3.08B	59.00±4.22B	75.40±3.32A
Liver	36.60±1.87B	39.40±3.72B	41.20±1.40B	60.20±2.42A
Heart	11.20±.47B	14.60±1.81B	13.00±.25B	19.20±1.98A

Different letters among groups showed a significant difference at ($p \leq 0.05$).
The control group (T1) fed basal diet without any additives, (T2) contain 1.5 g/kg Zn, (T3) contain 1g-/kg probiotic, (T4) contain combination of Zn 1.5g/kg + probiotic 1g/kg.

4.4 Result of mineralogy metric examination (Mg, Cu, Zn, and Fe) in pectoral muscle of broiler chickens.

The current study showed a significant ($p \leq 0.05$) increase in Mg in zinc group as compared to the other groups, while there is no significant difference when compared to the combination group as shown in table (4.6). The Cu shows no significant deference between the experiment groups.as shown in table (4.6). Zinc show a significant ($p \leq 0.05$) increase in the combination group as compared to the other groups while there is a no significant deference between the zinc group and the probiotic group while the control group shows a significant ($p \leq 0.05$) decrease when compared to the other groups.

Iron shows a significant ($p \leq 0.05$) increase in the combination group as compered with the zinc and probiotic groups, the control group showed a significant decrease as compered with the other groups.

Table (4.7): Effect of Zinc, probiotic and their combination on mineral contained in muscle µg/kg/mean ±SE

Groups / Minerals	T1	T2	T3	T4
Mg	7.07±.05C	7.55±.07A	7.23±.01BC	7.54±.12AB
Cu	50.88±1.7A	51.85±1.7A	50.20±1.1A	52.80±1.6A
Zn	57.94±7.4C	162.67±21.0A	96.41±14.9BC	121.36±11.2AB
Fe	200.58±7.2A	215.20±5.7A	219.10±9.4A	218.80±6.7A

Different letters among groups showed a significant difference at ($p \leq 0.05$).
The control group (T1) fed basal diet without any additives, (T2) contain 1.5 g/kg Zn, (T3) contain 1g/kg probiotic, (T4) contain combination of Zn 1.5g/kg + probiotic 1g/kg.

Zinc facilitates the absorption of iron in the intestine through various mechanisms. Zinc aids in the synthesis and secretion of a protein called hepcidin, which regulates iron metabolism. Zinc deficiency can lead to decreased hepcidin levels, resulting in impaired iron absorption. Also, zinc promotes the activity of an protein called ferroportin, which transports iron across the intestinal cells and into the bloodstream. By enhancing ferroportin function, zinc facilitate the movement of iron from the intestinal lumen into the body (Zhang *et al*., 2018).

Furthermore, zinc plays a role in the structure and function of the intestinal lining. It helps maintain the integrity of the intestinal cells and supports the development of microvilli, which are finger-like projections on the surface of the intestines that increase the absorptive area. This enhanced absorptive surface allows for improved uptake of iron and other nutrients (Yang and Liao, 2019).

Probiotics can increase the expression and activity of proteins involved in iron uptake, such as divalent metal transporter 1 (DMT1). This regulation of iron transporters facilitates the absorption of dietary iron in the intestines (Garcés *et al*., 2018). Also, Probiotics have anti-inflammatory properties and can help alleviate gut inflammation. Chronic inflammation can impair iron absorption, so reducing inflammation promotes better iron uptake (Deriu *et al*., 2013). Probiotics can compete with other intestinal bacteria for zinc-binding

sites in the gut. This competition reduces the binding of zinc to less beneficial bacteria, allowing more zinc to be available for absorption by the body (Scarpellini *et al*., 2021).

4.5. Effect of zinc, probiotic and their combination on villi length:

The current study showed a significant increase ($p \leq 0.05$) intestinal villi area in the combination group comparison with other groups. Zinc increase villi length by product new cells that increase space of the intestine and increase absorption in the intestine (Ohashi and Fukada, 2019).The role of gut health is pivotal in broiler performance from hatch to the point of marketing (Shannon and Hill, 2019).

Table (4-8): Duodenum villi measurements

Groups	Villi length(mm) Mean ±**S. E**	Villi width(mm) Mean ±**S.E**	Villi area(mm)^2 Mean ±**S.E**
T1	1.48 ± 0.10 C	0.12 ± 0.02 C	0.18 ± 0.04 C
T2	1.61 ± 0.15 B	0.11 ± 0.02 C	0.17 ± 0.03 C
T3	1.71 ± 0.13 A	0.15 ± 0.02 B	0.26 ± 0.03 B
T4	1.71 ± 0.13 A	0.18 ± 0.02 A	0.31 ± 0.03 A

Different letters among groups showed a significant difference at ($p \leq 0.05$). The control group (T1) fed basal diet without any additives, (T2) contain 1.5 g/kg Zn, (T3) contain 1g/kg probiotic, (T4) contain combination of Zn 1.5g/kg + probiotic 1g/kg.

Table (4-9): Jejunum villi measurements**.**

Group	Villi length(mm) Mean ±**S.E**	Villi width (mm)Mean ±**S.E**	Villi area (mm2) Mean ±**S.E**
T1	1.00 ±0.13 C	0.11 ± 0.03 C	0.12 ± 0.04 C
T2	1.06 ± 0.07 C	0.12 ± 0.03 C	0.13 ± 0.04 C
T3	1.29 ± 0.06 B	0.15 ± 0.04 B	0.20 ± 0.05 B
T4	1.45 ± 0.06 A	0.22 ± 0.03 A	0.31 ± 0.04 A

Different letters among groups showed a significant difference at ($p \leq 0.05$).
The control group (T1) fed basal diet without any additives, (T2) contain 1.5 g/kg Zn, (T3) contain 1g/kg probiotic, (T4) contain combination of Zn 1.5g/kg + probiotic 1g/kg.

Table (4-10): Ileum villi measurements

Group	Villi length(mm) Mean ±**S.E**	Villi width(mm)Mean ±**S.E**	Villi area (mm2) Mean ±**S.E**
T1	0.95 ± 0.10 B	0.09 ± 0.01 C	0.09 ± 0.01 D
T2	0.79 ± 0.16 C	0.16 ± 0.02 B	0.12 ± 0.03 C
T3	0.94 ± 0.13 A	0.20 ± 0.04 A	0.19 ± 0.03 B
T4	1.10 ± 0.10 A	0.23 ± 0.03 A	0.25 ± 0.03 A

Different letters among groups showed a significant difference at ($p \leq 0.05$).
The control group (T1) fed basal diet without any additives, (T2) contain 1.5 g/kg Zn, (T3) contain 1g/kg probiotic, (T4) contain combination of Zn 1.5g/kg + probiotic 1g/kg.

Table (4-11): Crypt depth measurements(mm)

Group	Duodenum Mean **±S.E**	jejunum Mean **±S.E**	Ilium Mean **±S.E**
T1	0.18 ± 0.03 C	0.17 ± 0.02 C	0.19 ± 0.03 C
T2	0.28 ± 0.08 B	0.28 ± 0.06 B	0.27 ± 0.04 B
T3	0.23 ± 0.02 B	0.24 ± 0.03 B	0.29 ± 0.05 B
T4	0.37 ± 0.05 A	0.39 ± 0.03 A	0.37 ± 0.03 A

Different letters among groups showed a significant difference at ($p \leq 0.05$).
The control group (T1) fed basal diet without any additives, (T2) contain 1.5 g/kg Zn, (T3) contain 1g/kg probiotic, (T4) contain combination of Zn 1.5g/kg + probiotic 1g/kg.

The results of the current study showed the crypt depth and villus length in intestine were increased significantly ($p \leq 0.05$) in the groups respectively compare with the control (T1). The results indicated that the combination group recorded the highest mean the crypt depth and villus among treatments we found a significant increase of the crypt width and villus height as (mean ± SE) according to duodenum (0.71±0.13), (0.18±0.02) and jejunum (1.45 ±0.06), (0.22±0.4) and ileum (1.10±0.23), (0.39±0.3) respectively.

The Combartion with control group was recorded duodenum (1.48±0.10), (0.12±0.2) and jejunum (1.00±0.13), (0.11±0.3) and ileum (0.95±0.10), (0.9±0.01) respectively. 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). 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).

It is believed that organic forms of zinc are more easily absorbed in the small intestine, leading to higher zinc bioavailability and subsequently influencing cellular processes involved in villus development and growth (Long *et al*., 2022). Zinc deficiency can disrupt cellular processes involved in villus development, including cell proliferation, differentiation, and migration. Zinc is also involved in the synthesis and stabilization of structural proteins in the intestinal epithelium, which are crucial for maintaining the integrity of the villi (Wan and Zhang, 2022).

Zinc is involved in protein synthesis, which is crucial for growth and development. Zinc deficiency can hinder protein synthesis, resulting in reduced growth rates and overall performance in broiler chickens (Hidayat *et al*., 2020).

Enzymatic production by different strains of bacteria has caused rapid growth and advancement in the field of probiotics. *Bacillus licheniformis* strains have been heavily used in the industry because of its ability to produce amylase, alkaline, protease, keratinase and B-mannanase (Hmidet *et al*., 2009; Zhang *et al*., 2002).

Addition of indigenous lactic acid bacteria probiotics to the diets increased villus height, jejunum and ileum, and villus width of duodenum, and improved the expression of mucin mRNA in the ileal part, These results conclude that lactic acid bacteria may stimulate proliferation of intestinal epithelium and regulate mucosal barrier formed by mucin in the small intestine of birds (Ariyadi and Harimurti, 2015).

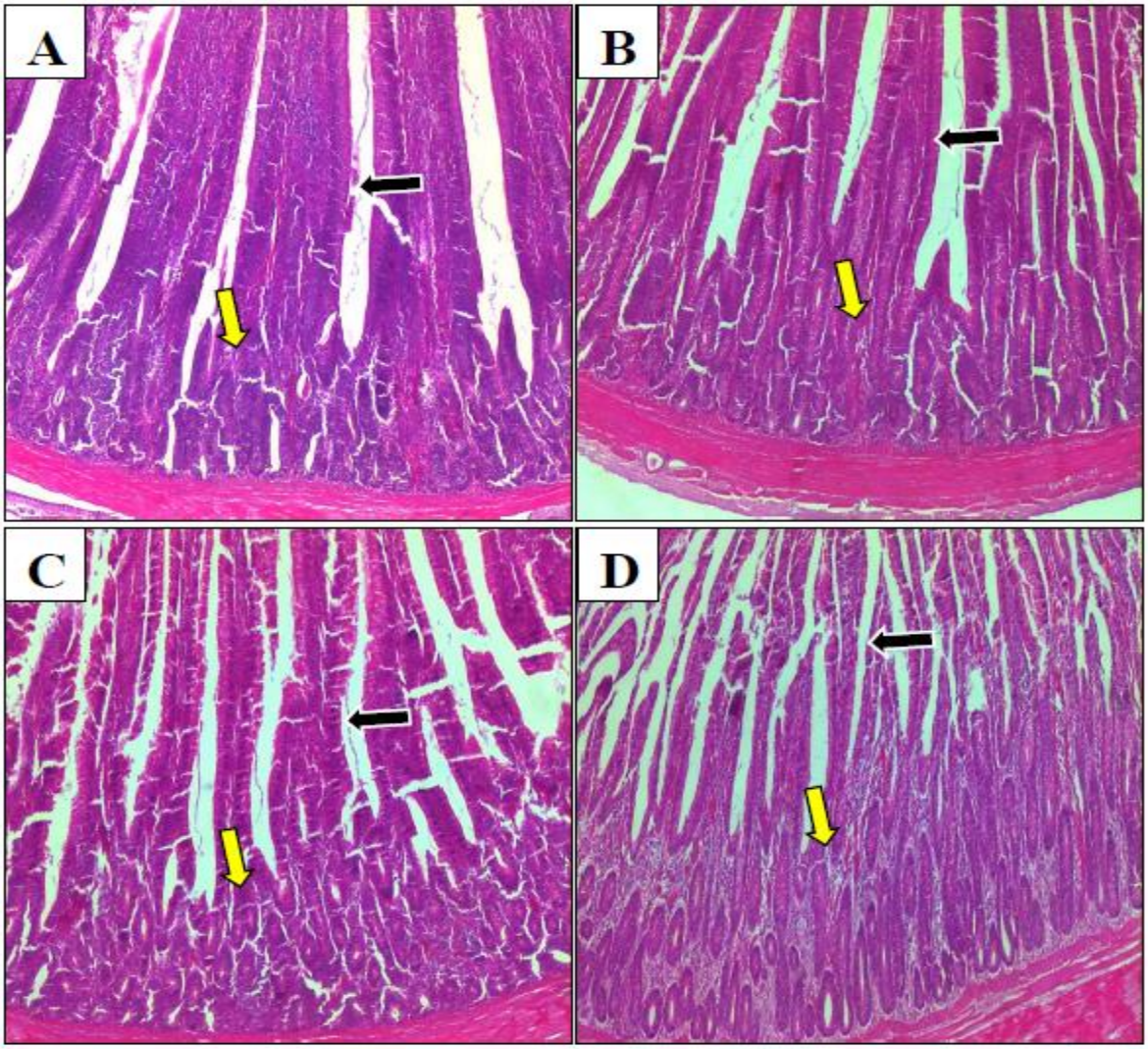
4.6 Effect of zinc, probiotic and their combination on intestinal histology:

Organic forms of zinc are thought to be more easily absorbed in the small intestine, resulting in higher zinc bioavailability and, as a result, influencing cellular processes involved in villus development and growth (Long et al., 2022). Zinc deficiency can wreak havoc on cellular processes involved in villus development, such as cell proliferation, differentiation, and migration. Zinc is also involved in the synthesis and stabilization of structural proteins in the intestinal epithelium, which are necessary for villi integrity (Wan and Zhang, 2022).

Zinc aids in protein synthesis, which is necessary for growth and development. Zinc deficiency can impair protein synthesis in broiler chicks, resulting in lower growth rates and overall performance (Hidayat et al., 2020).

The area of probiotics has experienced fast expansion and improvement due to the synthesis of enzymes by various bacterial strains. Because it can generate amylase, alkaline, protease, keratinase, and B-mannanase, Bacillus licheniformis strains have been widely employed in the industry (Hmidet et al., 2009; Zhang et al., 2002).

The jejunum, ileum, and duodenal villus width increased with the addition of indigenous lactic acid bacteria probiotics to the diets, and the expression of mucin mRNA in the ileal region improved. According to these findings, lactic acid bacteria may promote the growth of intestinal epithelium and control the mucous membrane barrier that mucin creates in the small intestine of birds (Ariyadi and Harimurti, 2015).



Figur (4-1): Photomicrograph of duodenum of control and treated groups.

A / Control group(T1). Normal histological architectures of villi (black arrow) and crypt of Lieberkuhn (yellow arrow). Where shows long villi relative to the short crypts**. B/ Group (T2).** Normal histological architectures of villi (black arrow) and crypt of Lieberkuhn (yellow arrow). However, villi showed higher numbers compared with control group**. C/ Group (T3).** Normal histological architectures of villi (black arrow) and crypt of Lieberkuhn (yellow arrow). However, villi showed higher numbers compared with control group, also the crypt of Lieberkuhn was thicker in comparison with control group. **D/ Group (T4).** Normal histological architectures of villi (black arrow) and crypt of Lieberkuhn (yellow arrow). However, villi showed higher numbers compared with control group, also the crypt of Lieberkuhn was thicker relative to villi length in comparison with other groups. **HandE A, B, C and D: 40x.**

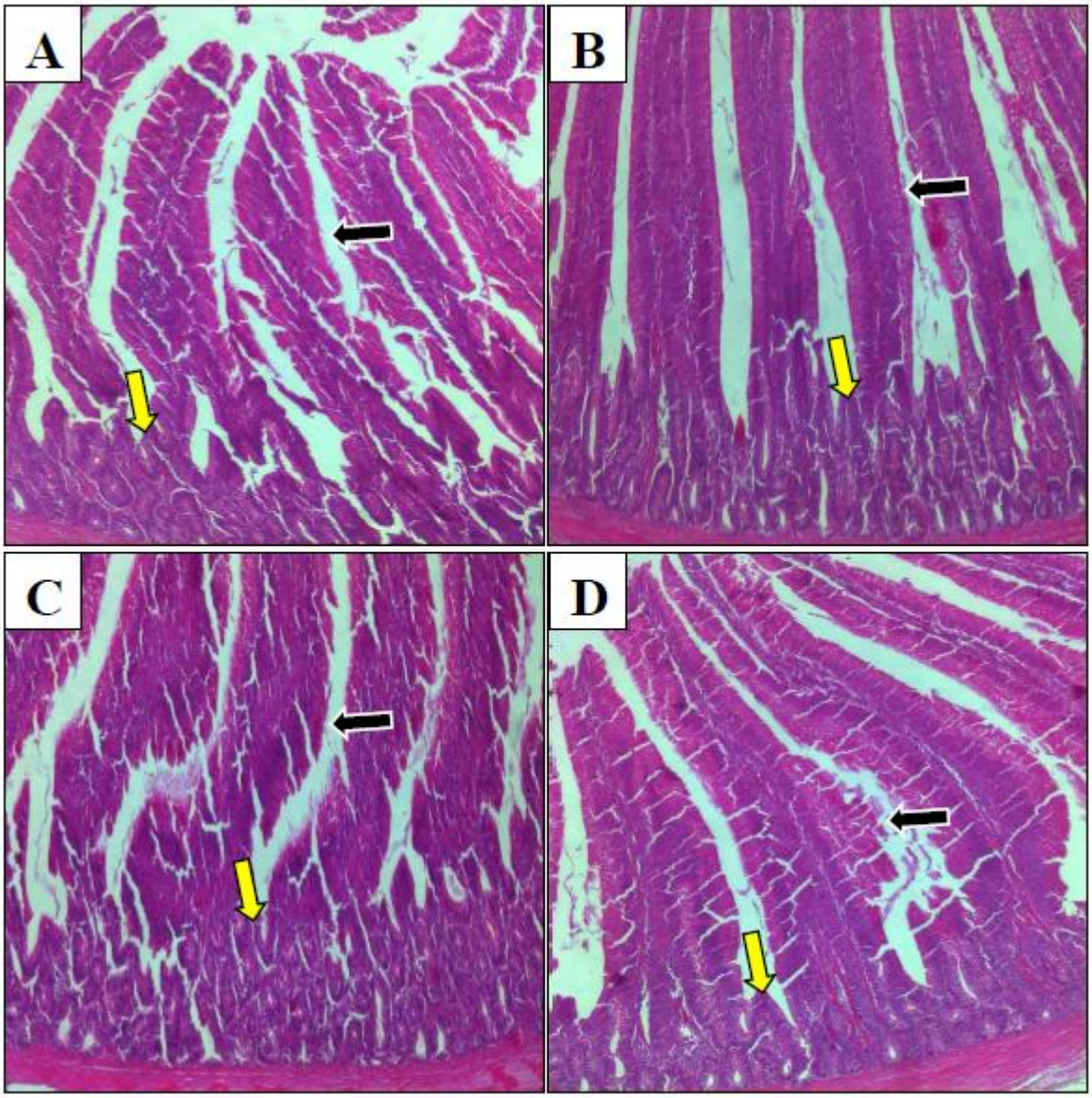


Figure (4-2) : Photomicrograph of jejunum of control and treated groups.

A / Control group(T1). Normal histological architectures of villi (black arrow) and crypt of Lieberkuhn (yellow arrow) of jejunum. **B/ Group (T2).** Normal histological architectures of villi (black arrow) and crypt of Lieberkuhn (yellow arrow). **C/ Group (T3).** Normal histological architectures of villi (black arrow) and crypt of Lieberkuhn (yellow arrow). However, villi showed larger area compared with control group, also the crypt of Lieberkuhn was thicker in comparison with control group. **D/ Group (T4).** Normal histological architectures of villi (black arrow) and crypt of Lieberkuhn (yellow arrow). However, villi showed larger area compared with other groups. **H and E A, B, C and D: 40x.**

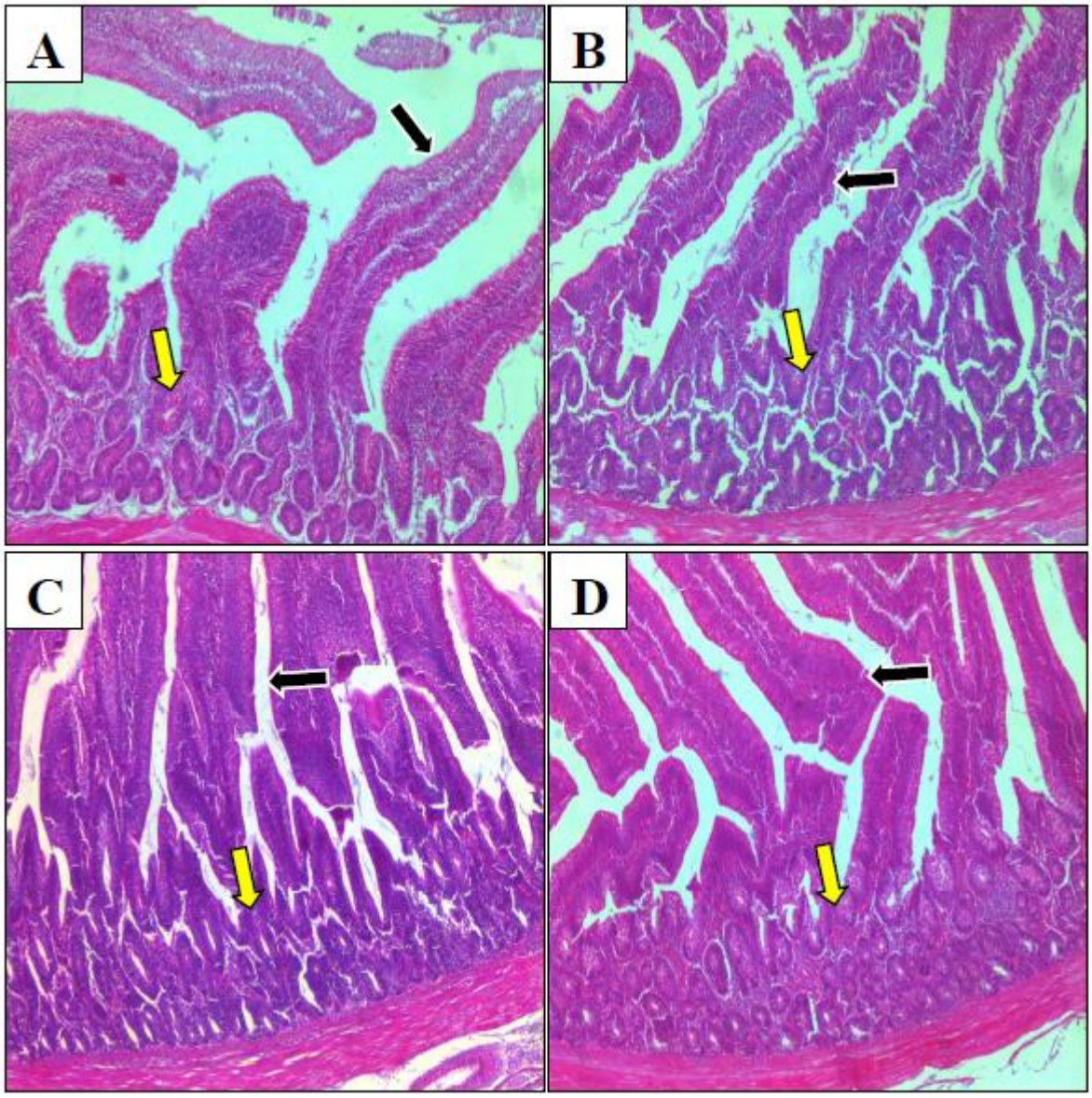


Figure (4-3) : Photomicrograph of ileum of control and treated groups.

A / Control group(T1). Normal histological architectures of villi (black arrow) and crypt of Lieberkuhn (yellow arrow) of ileum. **B/ Group (T2).** Normal histological architectures of villi (black arrow) and crypt of Lieberkuhn (yellow arrow). **C/ Group (T3).** Normal histological architectures of villi (black arrow) and crypt of Lieberkuhn (yellow arrow). However, villi showed higher numbers and larger area compared with control group. **D/ Group (T4).** Normal histological architectures of villi (black arrow) and crypt of Lieberkuhn (yellow arrow). However, villi showed higher numbers and larger area compared with control group. Also, the crypt of Lieberkuhn was thicker in comparison with control group. **H and E A, B, C and D: 40x.**

4.7 Effect of zinc, probiotic and their combination on intestinal mucin activity:

The study showed a significant increase in the intestine mucin in the duodenum, jejunum and ilium in the zinc ,probiotic and combination group as compared to the control group as shown in figure (4-4 , 4-5 and 4-6).

Zinc is involved in the production of mucus proteins, which are essential for forming the mucus layer that lines various surfaces in the body, including the respiratory and gastrointestinal tracts (Abd El-Hack *et al*., 2020).

One of the key mechanisms by which zinc influences mucin production in the intestine is through its involvement in protein synthesis. Zinc acts as a critical cofactor for numerous enzymes that are responsible for protein synthesis and modification. Among these enzymes are those involved in the synthesis of mucin (Zhang *et al*., 2012).

Zinc deficiency has lead to alterations in the expression and secretion of mucin in the gastrointestinal tract, potentially compromising the mucosal barrier. This can result in increased susceptibility to infections, inflammation, and other gastrointestinal disorders (Wan and Zhang, 2022).

Probiotics influence mucin production indirectly by interacting with the gut epithelial cells and immune cells. Probiotic microorganisms can stimulate the production of certain molecules, such as short-chain fatty acids, that are beneficial for gut health. These molecules can enhance the expression and secretion of mucin, promoting the formation of the mucus layer in the intestine One of the main Short Chain Fatty Acid SCFAs produced in the gut is butyrate. Butyrate, along with other SCFAs like acetate and propionate, serves as an energy source for the cells lining the intestinal wall, Intestinal epithelial cells preferentially use butyrate as a fuel for their energy needs, enhancing their function and promoting overall gut barrier integrity. Also, the butyrate can increase the absorption of minerals, including zinc, from the intestinal lumen into the bloodstream (Salvi and Cowles 2021).

SCFAs also promote gut motility, which can indirectly influence zinc absorption. Adequate gut motility ensures that food and nutrients spend enough time in the small intestine, allowing for sufficient nutrient absorption. Improved

gut motility helps prevent conditions like small intestinal bacterial overgrowth (SIBO) that may interfere with zinc absorption (Hughes et al., 2021).

SCFAs have been shown to exert anti-inflammatory effects in the gut. They can modulate immune responses and reduce the production of pro-inflammatory cytokines. By promoting a balanced inflammatory environment, SCFAs help to maintain the health and function of the intestinal mucosa (Ney et al., 2023).

Also, probiotics have been shown to modulate the immune system in the gut. They can influence the activity of immune cells and the production of immune-regulating molecules, which can impact mucin synthesis and secretion. A well-regulated immune response is crucial for maintaining the integrity of the mucosal barrier and ensuring proper mucus production in the intestine (Javanshir *et al*., 2021).

Furthermore certain probiotics can produce bioactive compounds, such as exopolysaccharides, that may directly or indirectly influence mucin production. These bioactive compounds can contribute to the development of a favorable gut environment, promoting the growth and activity of beneficial microorganisms that support mucosal health (Ferguson and Taylor, 2022).

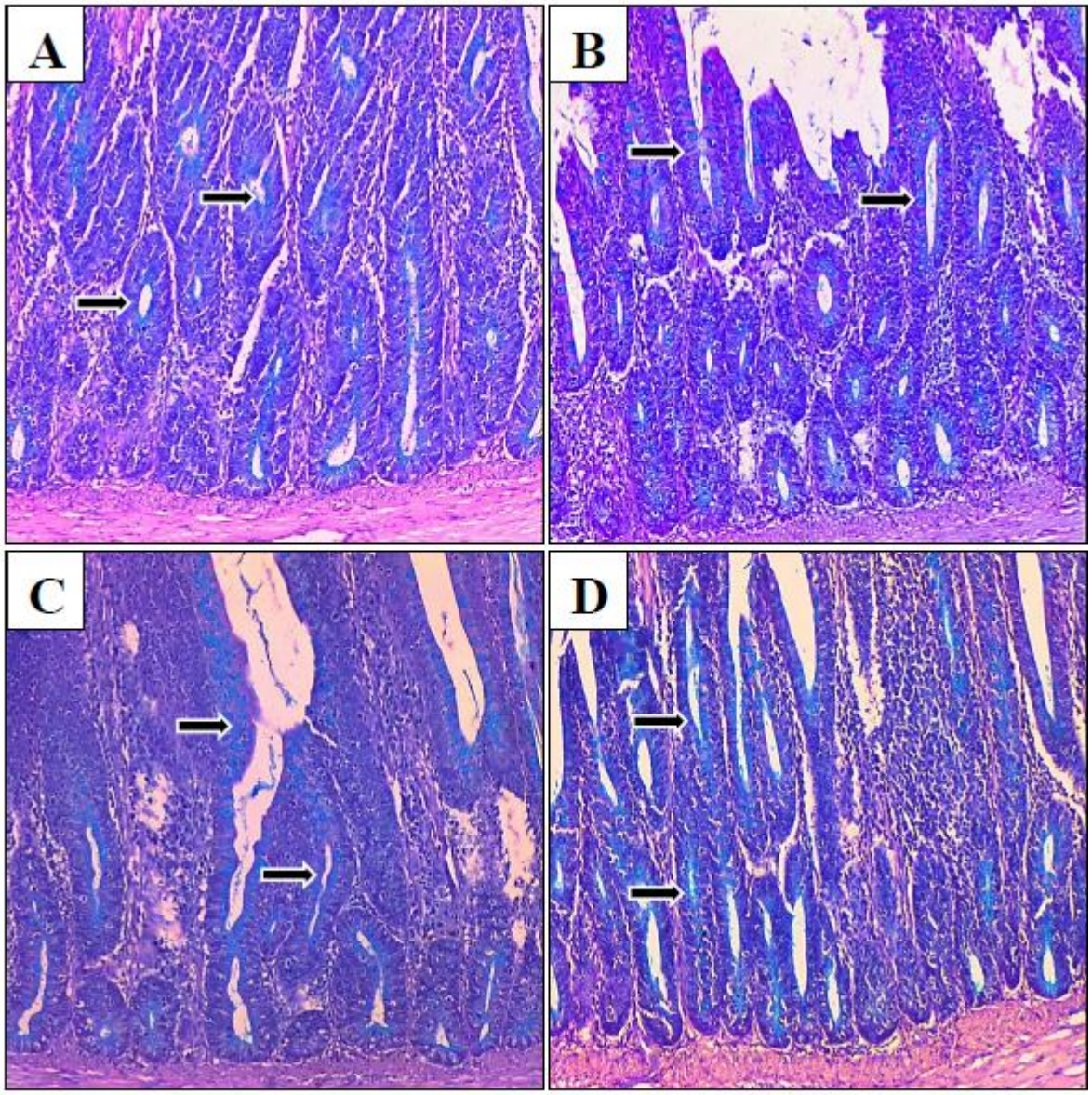


Figure (4-4): Photomicrograph of duodenum of control and treated groups.

A/ Control group(T1). The light blue color that expressed the presence of mucin (black arrow) in cytoplasm of secretary epithelial cells of crypt of Lieberkuhn and villus epithelial cells. **B/ Group (T2).** The light blue color that expressed the presence of mucin (black arrow) in cytoplasm of secretary epithelial cells of crypt of Lieberkuhn and villus epithelial cells. **C/ Group (T3).** Note the secretary epithelial cells showed mucin density in its cytoplasm compared with control group. **D/ Group (T4).** Note the secretary epithelial cells showed higher mucin density in its cytoplasm compared with control group. **Alcian-PAS. A, B, C and D: 100x.**

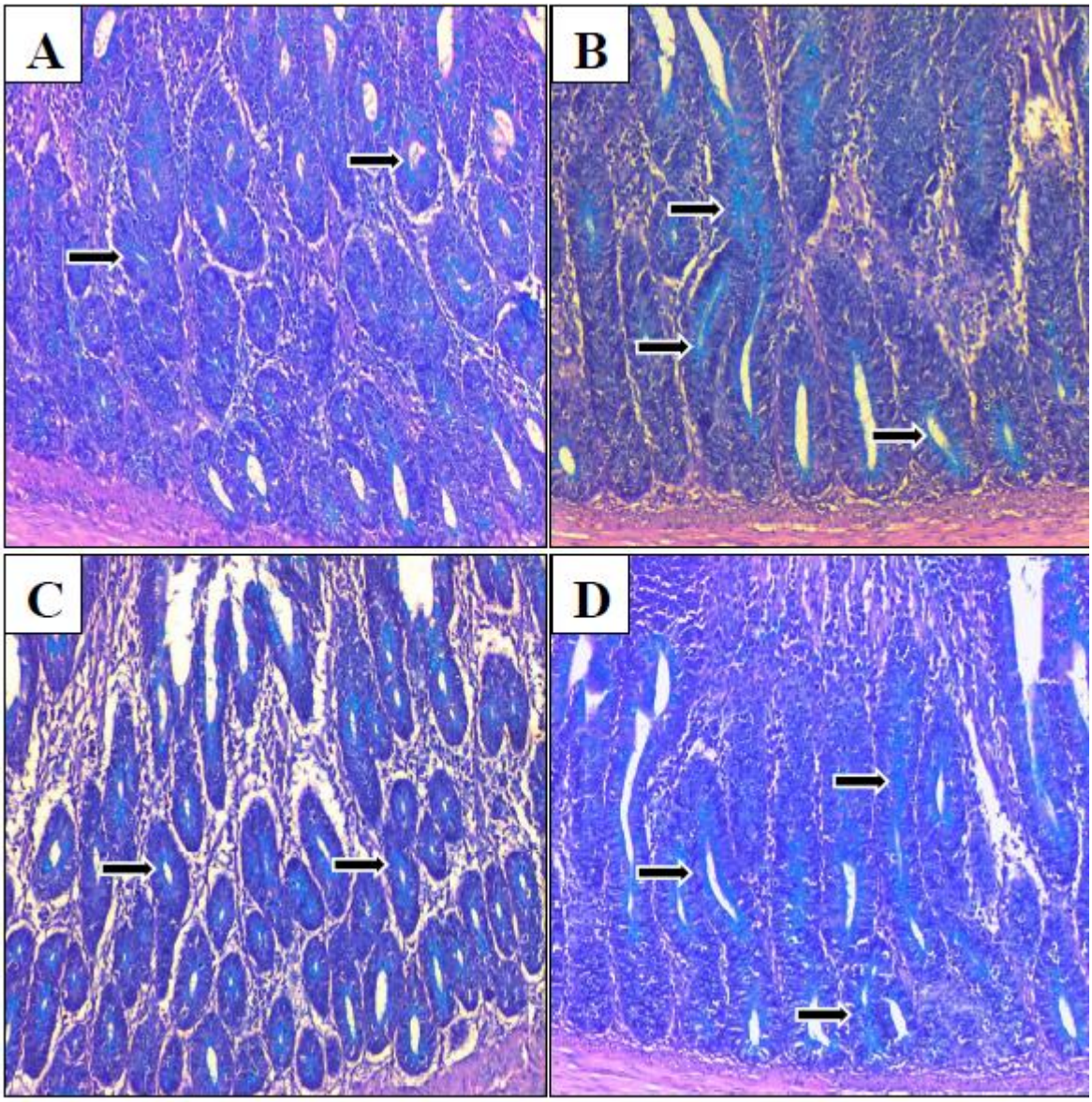


Figure (4: 5)Photomicrograph of jejunum of control and treated groups.

A/ Control group(T1). The light blue color that expressed the presence of mucin (black arrow) in cytoplasm of secretary epithelial cells of crypt of Lieberkuhn and villus epithelial cells. **B/ Group (T2).** The mucin (black arrow) in cytoplasm of secretary epithelial cells of crypt of Lieberkuhn and villus epithelial cells showed higher density compared with control group. **C/ Group (T3).** Note the secretary epithelial cells showed mucin density in its cytoplasm compared with control group. **D/ Group (T4).** Note the secretary epithelial cells showed higher mucin density in its cytoplasm compared with other groups. **Alcian-PAS. A, B, C and D: 100x.**

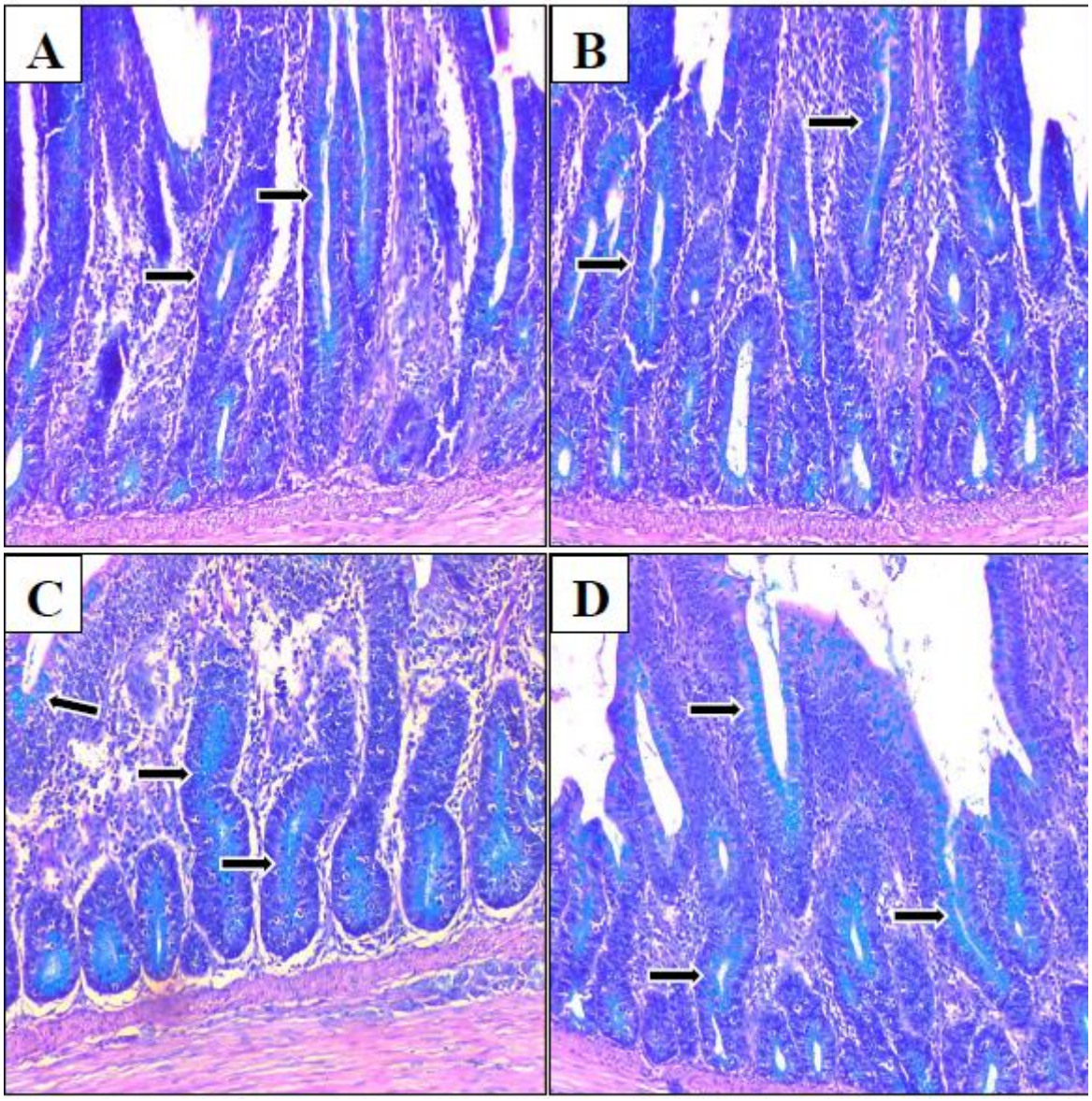


Figure (4-6): Photomicrograph of ileum of control and treated groups.

A/ Control group(T1). The light blue color that expressed the presence of mucin (black arrow) in cytoplasm of secretary epithelial cells of crypt of Lieberkuhn and villus epithelial cells. **B/ Group (T2).** The mucin (black arrow) in cytoplasm of secretary epithelial cells of crypt of Lieberkuhn and villus epithelial cells showed higher density compared with control group. **C/ Group (T3).** the secretary epithelial cells showed more mucin density in its cytoplasm compared with control and A groups. **D/ Group (T4).** Note the secretary epithelial cells showed higher mucin density in its cytoplasm compared with and A groups. **Alcian-PAS. A, B, C and D: 100x.**

Chapter five

Conclusions and Recommendations

5.1. Conclusions:

Depending on the study findings. The conclusions are as the following:

1. The use of combination (zinc 1.5g/kg and probiotic 1g/kg) led to enhance growth performance of broiler chickens.
2. There is an improvement in the immune response in broiler chickens in the group that feed a combination of (zinc 1.5 g/kg and probiotic 1g/kg) improve the immune response broiler chickens.
3. Feeding on combination of (zinc 1.5g/kg and probiotic 1g/kg) improve the carcass characteristics of the broiler chickens.
4. Dietary combination may enhance the intestinal health of broiler chickens through increasing the efficacy villus length, crypt depth and villus area.

5.2 .Recommendations:

1. Using a combination (Zn + Probiotic) in laying hens diet and studying their egg production..
2. Studying the effect of addition of combination of zinc and probiotic on broiler chicken under heat stress.
3. Study the apoptotic factor before and after supplementation of Zinc and probiotic.
4. Studying the effect of addition of combination of zinc and probiotic on antioxidant activity.
5. study the effect of Zinc and probiotic on laying hens diet on mucin secretion and gen expression.

Chapter six

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Appendices

Appendix I

Newcastle Disease Virus Antibody titer:

Preparation

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

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

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

Test procedure

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

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

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

4. Add 100μL enzyme conjugate into each well.

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

6. Repeat step 3.

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

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

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

Appendix II

Infectus Bursa Disease Virus Antibody titer

reparation

- Bring ELISA reagents to the room temperature (20-25 ℃) for 30 min to get best results.
- Sample dilution: use the sample diluent to dilute the sample at 40 times, mix the diluted sample evenly can get better result.
- Washing solution preparation: Dilute the 20×concentrated washing buffer with deionized water at 20 times.

Test procedure

1. Adding sample: Take out the required coated plates according to sample quantity (Can be detached) and record the sample position on a worksheet. Set 2 wells for negative control serum and 2 wells for positive control serum, add undiluted negative and positive control serum to its well accordingly, 100 μL/well. Others are sample wells, add diluted sample, 100μl/well (both single-well and double-well test is OK).
2. Incubation: cover with Adhesive Foil after adding sample,incubate at 37℃for 30 min.

3. Remove adhesive foil. Pour the liquid out of the wells, add the diluted Washing solution into each well fully, be static for 10s, pour out directly. Repeat 3 times, at last time pat to dry on absorbent paper.
4. Add 100 μL enzyme conjugate into each well.
5. Cover with adhesive foil andincubate at 37℃for 30 min.
6. Repeat step 3.
7. Add 100 μL substrate into each well, mix properly,Color for 10 min at 37℃in the dark.
8. Add 50uL stop solution into each well, shake for 10s, and determine the result.
9. Read OD value of each well with ELISA Reader at double-wave length: 450/630nm.

Appendix III

Content of Mg in meat

PRINCIPLE

Magnesium forms a purple chelate with Xylidyl Blue-I at an alkaline pH. The intensity of this colored complex is proportional to the magnesium concentration in the sample and is measured at 660 nm.

SAMPLE PREPARATION

1. Serum or plasma

Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma samples cannot be used as EDTA interferes with this assay.

2. Tissue extracts, cell lysates, and other samples such as urine or other biological fluids:

If the sample is turbid, centrifuge at 6,000 rpm for 15 min. Collect the

supernatant and use for the assay

If necessary, add small amounts of 6M HCl to the sample and adjust pH to 2.0 - 3.0. For example, add ~5-10 µL of 6M HCl per 1 mL of sample.

3. Tissue samples

Add 5% TCA solution, vortex 1 min. and incubate at 4 - 8°C for 30 min. Centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use for the assay.

ASSAY PROTOCOL (Microplate and Microplate Reader)

(Total reaction volume = 253 µL)

1. Add 3 µL of Blank (purified water), Magnesium Calibrator, or Sample to each

well.

2. Add 250 µL of Chromogen to each well, mix, and incubate at room temperature

for 5 minutes. Mix carefully using a pipette to avoid foaming. If a plate mixer is used for mixing, there is a risk of obtaining poor reproducibility.

3. Read the OD absorbance at 660 nm.

Assay Protocol				
Step	(µL)	Blank	Calibrator	Sample
1	Purified water	3	-	-
	Magnesium Calibrator	-	3	-
	Sample	-	-	3
2	Chromogen	250	250	250
	Mix and incubate for 5 minutes at room temp.			
3	Read the OD absorbance at 660 nm.			

CALCULATION OF SAMPLE CONCENTRATION

$$\frac{\text{OD sample - OD blank}}{\text{OD calibrator - OD blank}} \times 2 = \text{Magnesium (mg/dL)}$$

Appendix IV

Content of Cu in meat

PRINCIPLE OF TEST

The chromogen 3,5-Di-Br-PAESA react with cupric ions and forming a blue-violet compound, which intensity is proportional to the copper concentration in the sample. The method does not require de-proteinization of the serum nor the blank sample.

REAGENTS PREPARATION

Prepare the Work Reagent mixing in equal quantity the Reagent A with Reagent B. Reagents are stored at 2-8°C and are stable until expiration date on label. Work Reagent is stable 20 days at room temperature.

REAGENTS	BLANK	STANDARD	SAMPLE
Work Reagent	1 ml	1 ml	1 ml
Distilled water	66 µl	--	--
Standard	--	66 µl	--
Sample	--	--	66 µl
Mix and wait for 10 minutes then read the absorbances against the blank at 580 nm. The colour is stable for 30 minutes.			

CALCULATION:

$$\text{Copper } \mu g/dl = \frac{A \text{ (sample)}}{A \text{ (standard)}} \times 200$$

Appendix IV

Content of Zn in meat

PRINCIPLE OF TEST

Zinc reacts with the chromogen present in the reagent forming a coloured compound which colour intensity is proportional to the zinc concentration present in the sample.

SAMPLE PREPARATION

1. Serum or plasma

Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma samples cannot be used as EDTA interferes with this assay.

2. Tissue extracts, cell lysates, and other samples such as urine or other biological fluids:

If the sample is turbid, centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use for the assay

If necessary, add small amounts of 6M HCl to the sample and adjust pH to 2.0 - 3.0. For example, add ~5-10 µL of 6M HCl per 1 mL of sample.

3. Tissue samples

Add 5% TCA solution, vortex 1 min. and incubate at 4 - 8°C for 30 min. Centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use for the assay.

REAGENTS PREPARATION

Prepare the Work Reagent mixing in equal quantity the Reagent A with Reagent B. Reagents are stored at 2-8°C and are stable until expiration date on label. Work Reagent is stable 20 days at room temperature.

REAGENTS	BLANK	STANDARD	SAMPLE
Work Reagent Distilled Water Standard Sample	1 ml 50 µl -- --	1 ml -- 50 µl --	1 ml -- -- 50 µl
Mix and read the absorbance against blank at 578 nm. Colour is stable for 30 minutes.			

CALCULATION:

Zn mg/dl = [A(sample) / A(standard)] x 200

Appendix IV

Content of Fe in meat

PRINCIPLE

The method is based on the properties of Chromazurol S (CAS), a chromogenic iron-binding dye, that under acidic conditions in presence of cetrimide (CTAB) forms an intense purple complex proportional to the concentration of iron present in the sample

$$Fe^{+3} + 3\ CAS + CTAB \xrightarrow{pH\ 4.7} Fe\ (CAS)_3\ complex$$

PROCEDURE

1. Bring reagents and samples to room temperature.
2. Pipette into labelled test tubes:

TUBES	Blank	Sample	CAL. Standard
R1.Reagent	1.0 mL	1.0 mL	1.0 mL
Sample	–	50 µL	–
CAL. Standard	–	–	50 µL

3-Mix and let the tubes stand 10 minutes at 37ºC.

4- Read the absorbance (A) of the samples and the standard at 635 nm against the reagent blank.

Appendix IIV

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 to24 h at 4 oC, then rinsed and stored in 70% ethanol at 4 oC 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 .

الخلاصة:

الزنك وهو العنصر الغذائي الاساسي لجميع أشكال الحياه، وهو الاكثر اضافة في اعلاف الدواجن. تستخدم المعزز الحيوي للمساعدة في الحفاظ على صحه الجهاز الهضمي. هدفت هذه الدراسة الى تعزيز صحة الجهاز الهضمي في دجاج اللحم لتحسين الاداء الانتاجي والحالة المناعية للطير بزياده مساحه الزغبات في الامعاء زياده البروتين في عضلات الصدر .

كانت فتره التجربة خمسه اسابيع تبدأ من (2023/1/23 الى 27-2-2023) و نفذت التجربة في قاعه خاصه تم تقسيم اجمالي 200 فرخ على التوالي عمر يوم واحد نوع (Ross 308) بشكل عشوائي الى 4 مجاميع متساوية تتكون كل مجموعه من 50 فرخا كل مجموعه مقسمه الى مجموعتين تحتوي على 25 فرخا مجموعه السيطرة غذيت على عليقه اساسيه بدون اضافات(T1)، المجموعة الثانية(T2) تتغذى على العليقة الأساسية مضاف لها الزنك 1,5غم/كغم من العلف، المجموعة الثالثة(T3) تتغذى على المعزز الحيوي 1غم/كغم من العلف، بينما مجموعه الخليط(T4) تمت تغذيتها على العليقة الأساسية مضاف لها خليط من الزنك 1,5 غم /كغم و 1 غم/ كغم من المعزز الحيوي.

تم جمع عينات من الامعاء في اليوم الخامس والثلاثين من الدراسة عن طريق اخذ عينه من نسيج الاثني عشر والصائم واللفائف لملاحظه وفحص التغيرات الشكلية والنسيجية في الامعاء الدقيقة ،اظهرت التغيرات النسيجية تحسنا في عرض الخبايا ، كما اظهرت تحسن مساحة الزغابات وتحسن الحاله الصحية للامعاء في مجموعة الخليط بصورة معنويه ($P\leq0.05$) مقارنه مع مجموعه السيطرة بالإضافة الى زياده معنويه ($P\leq0.05$) في متوسط وزن الجسم الحي وكميه العلف المستهلك ومعامل التحويل الغذائي بالمجموعة الخليط مقارنه بالمجموعات الاخرى . بلاضافة الى تحسن في وزن الاعضاء الداخلية الصالحة للاكل بصورة جيدة مقارنة مع وزن الجسم للطير و زيادة في كمية الطبقة المخاطية للامعاء بلمقارنة مع المجاميع الاخرى .

وبالمثل اظهرت هذه المجموعة زياده معنويه في معيار الاجسام المضادة ضد مرض النيوكاسل ومرض الكومبورو وتم فحص وقياس نسبه امتصاص العضلات للمعادن المفيدة للجسم خاصه مجموعة الخليط وتبين زياده تصنيع البروتين بالعضلات عن طريق زياده في وزن الذبيحة والاحشاء العضلية خاصه القلب.

باختصار يوصي عملنا باستخدام مزيج الزنك 1,5 غم لكل كغم من العلف مع المعزز الحيوي 1غم/ كغم من العلف في عليقه الدجاج اللاحم .

جمهوريه العراق
وزارة التعليم العالي والبحث العلمي
جامعه كربلاء
كليه الطب البيطري
فرع الصحه العامه

تأثير الزنك العضوي، المعزز الحيوي وخليطهما على بعض الصفات الإنتاجيه، الاستجابة المناعية، الأنسجة المعوية والاعضاء المأكولة لفروج اللحم.

الرسالة

مقدمة الى مجلس كلية الطب البيطري في جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في علوم الطب البيطري/ الصحة العامة

من قبل

سارة سعيد هيلان

بأشراف

أ.د. ياسر جمال جميل

أ.د. لطيف إبراهيم كاظم

2023 م

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