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Effect of Enzyme Supplementation on Broiler Chicken: A Literature Review

Az enzim kiegészítés hatása brojler csirkében: Irodalmi áttekintés

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Abstract:

While broiler chicken breeding is becoming more expensive and the market demand for chicken meat is constantly increasing, farmers are in need of a supplement that could boost their production while maintaining the animal's welfare. When antibiotics usage as growth promoters got banned in many countries due to negative impacts on the consumer's health, enzyme supplementation started increasing worldwide for different reasons. Several studies have been conducted on the effects of exogenous enzyme supplementation on the broiler chicken's body, showing their impacts on different traits such as immunity, nutrition, intestinal microflora, and growth rate. This thesis demonstrates how different exogenous enzymes can have a positive impact on broiler chickens by boosting their immunological system or by reducing the pathological lesions associated with different viral or bacterial infections. It also shows how enzyme supplementation can positively affect the gastrointestinal tract of chickens by promoting the development of specific intestinal structures and promoting the development of a complete and healthy intestinal microflora.

Absztrakt:

Miközben a brojlercsirke tenyésztése egyre drágább, és a csirkehús iránti piaci kereslet folyamatosan növekszik, a gazdáknak olyan kiegészítőre van szükségük, amely az állatok jólétének megőrzése mellett növelheti termelésüket. Amikor számos országban betiltották az antibiotikumok növekedésserkentőként való használatát a fogyasztók egészségére gyakorolt negatív hatások következtében, az enzim kiegészítés világszerte növekedni kezdett különböző okok miatt. Számos tanulmányt végeztek az exogén enzim kiegészítés brojlercsirkékre gyakorolt hatásairól, amelyek kimutatták, hogy ezek milyen hatással vannak a különböző tulajdonságokra, például az immunitásra, a táplálkozásra, a bél mikroflórájára és a növekedési rátára. Ez a dolgozat bemutatja, hogy a különböző exogén enzimek milyen pozitív hatással lehetnek a brojlercsirkékre azáltal, hogy erősítik immunrendszerüket, vagy csökkentik a különböző vírusos vagy bakteriális fertőzésekkel összefüggő kóros elváltozásokat. Továbbá azt is bemutatja, hogy az enzim kiegészítés miként képes pozitívan befolyásolni a csirkék gyomor-bél traktusát azáltal, hogy elősegíti a specifikus bélstruktúrák kialakulását és elősegíti a teljes és egészséges bélmikroflóra kialakulását.

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1. Introduction

Having very high nutritional values and excellent taste as well as reasonable prices in most countries, chicken meat is present in almost every kitchen in the world and is considered one of the most consumed types of meat worldwide. Behind this product of high protein level, low fat, and a relatable amount of polyunsaturated fatty acids, is a huge industry spread all around the world trying to optimize the growth and the production of broiler chickens [1]. While all other meat products consumption has been decreasing constantly worldwide, chicken is the only type of meat whose consumption was expected to reach 98 million metric tons by the end of 2023, double the amount eaten in 1999. As for the future, an increase in production up to 16% is expected by 2025 [2]. By that, in less than a couple of years, humans for the first time will be consuming far more chicken than any other type of protein, which pushed the farmers and the producers to find new techniques in order to keep up with the market demand. It all started with the genetic improvement of the broiler chicken breeds, which have undergone an amazing and huge genetical improvement in terms of feed conversion ratio as well as lean muscle size and mass. In 1985, under good husbandry and a high-energy diet, at 35 days of age a 1.40 kilograms broiler required 3.22 kilograms of feed, while in 2010, under the same conditions, a 2.44-kilogram broiler was produced on 3.66 kg of feed. In parallel, from 1957 to 2005, broiler growth increased by over 400%, with a concurrent 50% reduction in the feed conversion ratio [3]. While the breeds predisposed in the market are almost the same worldwide, producers were also trying to find more efficient ways to increase the growth of chickens and to boost their production as well, from where came the idea of supplementations. For as long as 50 years, the use of antibiotics, like tetracyclines most commonly, has been spread to promote growth and improve production. But since the 1950s, scientists have raised concerns about the development of resistant bacteria which resulted in a decrease in the usage of antibiotics for these purposes [4]. After this period, safer ways to promote the growth of broilers were researched deeply, till enzyme supplementation came into light after noticing a lack and insufficiency of certain enzymes like lipase, amylase, or protease. Many research confirmed a positive correlation between the supplementation of exogenous enzymes and growth rate in broiler chicken, till most farmers and producers worldwide adopted this technique that became a key to the industry, confirming that exogenous enzyme supplementation with certain specific feeds helped in better digestion, absorption, and utilization of dietary nutrients which led to a better growth rate in broilers, and therefore better production costs and better supply that can meet the needs of the market [5]. But here comes an important question about this adopted habit that may not be officially and scientifically confirmed yet. In this thesis, we will address the hypothesis that enzyme supplementation has a positive effect on the growing and production of broiler chicken.

2. Literature review

2.1. Broiler chicken breeding

Domestic chickens are members of the genus *Gallus*, which contains four morphologically different species: the Red junglefowl *Gallus gallus*, the Grey junglefowl *Gallus sonneratii*, the Ceylon or Sri Lanka junglefowl *Gallus lafayettii* and the Green junglefowl *Gallus varius* which all origin from different regions across Asia [6].

The evolution of modern chicken breeds has taken place over billions of years through natural selection, whilst artificial selection has been used for the benefit of business. But in the second half of the 20th century, since the introduction of industrial scale, the most significant improvements have been seen so far in chicken genetics since their domestication [7].

Broiler breeding techniques can be summarized as follows: the breeding companies own and control the pure breeding lines to the highest degree. These lines are exposed to large-scale selection programs; all of a company's broiler products are descended from these lines. Great-grandparent stocks derived from pure-bred lines are exposed to mass selection for specific traits. Since the 1950s, the primary selection trait has been growth rate, with a more recent focus on breast meat yield, livability, and feed use efficiency. Crossbreeding of specific grandparent lines produces parent stock, which is then distributed to particular farmers and integrated producers. The last stage in intensive artificial selection is crossbreeding between these hybrids (parent stock) to yield production broilers for slaughter by production companies [7].

2.2. Nutrition of broiler chicken

Poultry are known for their swift and efficient conversion of feed into food products, causing less environmental impact compared to other livestock. Due to their high productivity, they have increased nutrient demands. Poultry necessitates a well-balanced diet containing at least 38 essential nutrients in suitable concentrations and proportions [8].

2.2.1. Drinking water requirements

Broiler chickens drink about 1.6 to 2.0 times the weight of their feed in water. Water plays a crucial role in the metabolic and nutritional processes of birds. Physiologically, the water ingested by the birds serves multiple purposes, including transporting nutrients, facilitating enzymatic and chemical reactions within the body, regulating body temperature, and lubricating joints and organs [9].

2.2.2. Energy and amino acids requirements

Designing broiler diets based on an ideal protein concept addresses challenges related to protein and amino acid metabolism-related energy use and enhances the efficiency of protein utilization. An ideal protein and amino acid profile in the diet implies that the digestible essential and non-essential amino acid levels precisely fulfill the demands for optimal growth and performance, leaving no excess amino acid nitrogen for elimination. The practical application of an ideal protein and amino acid approach involves selecting easily digestible protein sources that complement each other and formulating a diet based on digestible amino acids. By formulating diets according to digestible amino acid content, the aim is to achieve the ideal protein and amino acid levels essential for broiler growth without relying on large safety margins for poorly digestible protein sources [10]. An important note to remember is that poultry are unable to synthesize 9 amino acids, including tryptophan, methionine, cysteine, lysine, threonine, and arginine, due to their deficiency of specific digesting enzymes. Although birds can produce histidine, glycine, and proline, their synthesis rates are often inadequate to fulfill the metabolic demands of highly productive poultry. To achieve optimal performance, a dietary supply is necessary. Cysteine and tyrosine can be derived from methionine and phenylalanine, respectively, yet their presence in the diet is crucial if methionine or phenylalanine levels are insufficient. Histidine, glycine, proline, cysteine, and tyrosine are termed conditionally essential amino acids [8].

2.2.3. Vitamins requirements

The source of vitamins in poultry diets can be traced to two origins: they naturally occur within the ingredients used to formulate the diet, or they can be introduced separately as concentrated supplements. Vitamin supplementation is vastly related to the energy and amino acid requirements of broiler chicken, by creating a bond between these two that is crucial for optimal growth and health of broiler chicken. In fact, vitamins play a catalytic role, helping in the synthesis of nutrients. For example, glycine may be synthesized from keratin and serine only if vitamin B12 is available in the body [11]. The absence or inadequate absorption and utilization of vitamins in the diet leads to specific diseases or deficiency syndromes in poultry. Insufficient vitamin levels can also result in various health issues. For instance, a lack of vitamin A can result in stunted growth, ataxia, weakness, and loss of vision, while a deficiency in vitamin E which is a biological antioxidant that enhances the growth performance and physiological as well as immunological well-being of broiler chickens by reducing lipid peroxidation and counteracts free radicals present in both skeletal muscle and plasma, can cause exudative diathesis and encephalomalacia. In parallel, a lack of vitamin B complex can result in perosis, curled toe paralysis, polyneuritis, and impairment of feed utilization [12]. The B vitamins play significant roles in avian metabolism, primarily acting as coenzymes. These coenzymes combine with larger enzyme molecules, enhancing a range of metabolic activities. Vitamins B1, B2, B6, niacin, pantothenic acid, and biotin contribute to energy metabolism, while vitamin B12 and folic acid influence growth and cell upkeep. It's crucial to note that specific B vitamins engaged in distinct metabolic functions have interactions among themselves, complicating the determination of individual requirements within the B vitamin group [13].

2.2.4. Minerals requirements

Minerals are important feed components and supplements required for a healthy broiler with normal and optimal physiological development. Minerals play a vital role by being considered activators for hormones and enzymes, which will lead to the development of the bones of the broiler and eggshells and help regulate the body's pH equilibrium. Calcium and phosphorus are the predominant mineral components within the body and fall under the category of macro-minerals, which also include sodium, potassium, chloride, sulfur, and magnesium. Typically, about 60 to 80 percent of the overall phosphorus found in plant-based ingredients exists as phytate-phosphorus. In regular dietary circumstances, poultry struggle to effectively utilize phytate phosphorus due to the absence of natural phytase in their digestive enzymes [14].

Enzymes are not only essential in broiler chicken nutritional requirements for the processing and the catabolism of digestion reactions which will help achieve optimal growth and body score conditioning, but also are beneficial for the intestinal flora in broiler chicken.

2.3. Intestinal tract flora of broiler chicken

Right after hatching, the poultry's digestive system encounters external microorganisms, and subsequently, it becomes a perfect environment for a complex microbiome dominated by anaerobic bacteria including facultative anaerobic cocci, Gram-negative cocci, streptococci, *Clostridium*, *Propionibacterium*, *Eubacterium*, *Peptostreptococcus*, and *Bacteroides* [15]. As the host matures, this microbiome diversifies significantly, eventually settling into a constant but very active state. Significant interactions take place between the broiler and its intestinal flora. These interactions will be noticed on many levels, especially on the nutritional and immunological levels.

2.3.1. Nutritional importance of the microbiota

The bacteria in broiler chickens' and other animals' intestines serve a crucial role in nutrient digestion, which is one of the initial stages of healthy animal growth. Each bacteria constituting the intestinal microflora of broiler chickens plays an important function in feed digestion.

The digestion process starts with starch hydrolysis in the crop, which is mostly dominated by Gram-positive facultative anaerobic bacteria such as *Lactobacillus* that attach to the epithelium and have 10^8 to 10^9 colony-forming units per gram. The crushing of the feedstuff continues in the proventriculus and gizzard of the broiler, which have an acidic and low pH of 2.6, which not only helps with digestion but also reduces as much as possible the number of pathogens and microbes entering the intestines [16].

After being crushed by entering through the stomach, digestion continues in the small intestines, where the total estimated number of microflora count is expected to be lower at around 10^5 colony-forming units per gram of digestible matter because of the amount of bile and pancreatic secretions [17]. The bacteria found in the small intestine, where carbohydrates are broken down, include streptococci, clostridia, lactobacilli, and enterobacteria types [18].

The caecum, which is characterized by more diversified bacterial activity and nature, is where bacteria feed on indigestible and residual carbohydrates that are digestible entering from the small intestines. The caecum consists of a densely populated microbiota, estimated to have 10^{10}

colony-forming units per gram of digesta, which is classified into two prominent groups of bacteria, Gram-positive Firmicutes and Gram-negative Bacteroidetes [19].

By colonizing the entire gastrointestinal tract of broiler chicken, the microbiota is able not only to achieve nutritional functions but also immunological too.

2.3.2. Immunological importance of the microbiota

The colonization of microorganisms in the avian gut starts shortly after hatching, and a sequence of microbial changes takes place until a complex and ever-changing microbiome is established. The gastrointestinal tract serves as the principal reservoir for these bacteria, enabling significant interaction between those foreign cells and the immune system of the host. A gel-like mucus layer produced by goblet cells and containing mucin glycoprotein covers the lining of the avian stomach. This mucin layer comprises an external, porous section where microorganisms can establish themselves, along with an internal, dense layer that effectively blocks the attachment of most bacteria [20]. The mucus layer, which is a component of the natural immune defense of the intestinal mucosa, works as a barrier that prevents gut bacteria from entering the intestinal epithelium. This layer acts as the initial defense against infections [21]. The small intestine boasts a highly developed set of innate immune mechanisms. These processes protect against potential invasive pathogens while also preventing germs from getting into contact with the epithelial cells or the underlying lamina propria. Along with factors such as the rapid passage of ingested food, an abundance of bile salts, and digestive enzymes, the natural immune system is critical in preserving the duodenum and jejunum's relatively low microbiota density. These innate immune responses include a protective mucus layer, secretory Immunoglobin A, host-specific defense peptides such as defensins and cathelicidins, and lysozyme, which are all strategically adapted to guard against invading microbes [22]. It has been proven for instance, that broiler's intestinal mucus can lower *Campylobacter jejuni* pathogenicity by decreasing the bacteria's capacity to attach to and penetrate intestinal epithelial cells [23]. MWANGI et al. discovered that the diversity of microorganisms in the poultry intestinal microbiota has an immediate effect on the complexity of the receptor for T cell variety. This effect can be found in addition to the stomach and the spleen, suggesting that gut bacteria have systemic effects on the immune system [24].

In addition, the intestinal microbiota plays a role in regulating B cell response and immunoglobin A generation, which is a key factor in controlling the intestinal microbiota composition by selectively attaching to the bacterial epitopes [25].

Being part of most of the reactions happening in the body, enzymes are also part of different reactions happening in the gastrointestinal tract and will impact all the functions taking place in the gut, including immunity. But this function can be highlighted better by discovering first the pathological lesions assimilated with the intestinal microflora and their connection to the enzyme deficiency.

2.4. Pathological lesions associated with enzyme deficiency

The effective functioning of poultry digestive systems is strongly related to their health and productivity. Enzymes are essential for breaking down complicated nutrients into forms that the body can absorb and use. However, enzyme deficits can cause a chain reaction of physiological disturbances. Pathological lesions form as a result, which might have a severe impact on the overall health of animals. In this section, we seek to investigate the complex interaction between enzyme deficits and pathological lesions in animals, to provide insight into the mechanisms that link these two elements of animal health. Understanding this connection is crucial for improving animal nutrition, welfare, and management practices.

2.4.1. Direct and indirect effects of enzyme deficiency on the gut

As the broiler matures, its intestines become more competent, leading to a higher level of enzymes and better digestion of feed. This, in turn, creates a more stable environment in both intestines, which will help create a balance between the broiler’s ability to digest and its nutritional needs [26]. However, some feed components may reduce the digestion process to a very minimal level by causing irregular activities inside the intestines due to the lack of specific enzymes to digest these feeds. For example, non-starch polysaccharides constitute a significant category of antinutritional compounds found in various feed ingredients, particularly cereals. What characterizes non-starch polysaccharides across different sources is their resistance against the digestive enzymes of animals and their capacity to create a thick, viscous environment within the intestinal lumen. This heightened viscosity within the intestinal contents has been demonstrated to give rise to digestive and health issues. Non-starch polysaccharide plays an important role in reducing the passage rate of digesta, limiting the availability of essential nutrients. Furthermore, the prolonged retention of feed particles favors bacterial colonization that will lead to an abnormal change in the intestinal microbiota and heightened activity within the small intestine. Cereals such as barley, wheat, rye, and oats are particularly rich in non-starch polysaccharides, and their consumption has been linked to elevated digesta viscosity, reduced passage rate of digesta, diminished enzymatic activity in digestion, lower nutrient digestibility, weakened immune system, compromised feed conversion efficiency, and decreased growth rates in poultry because broilers lack the enzyme xylanase and NSPase that is necessary to break down the non-starch polysaccharides found in the cell walls of grains, which will force them to accumulate inside the intestines and cause all of the problems mentioned [27, 28].

Not only does non-starch polysaccharide digestion pose a problem due to their deficiency of digesting enzymes, but so do proteins. In high-performing broiler chickens, there is incomplete utilization of dietary protein in the small intestine. On average, approximately 84 grams of crude protein per kilogram of feed are excreted in the feces when broilers are provided with unrestricted access to feed [29]. This rise in the crude protein levels in diet broilers leads to higher moisture content in the litter. This connection could be attributed to the need for increased water to eliminate excess amino acids through uric acid excretion. It could also stem from an elevated protein influx into the ceca, disrupting the microbiota balance and causing inflammation of the intestinal lining along with increased mucus secretion. Most importantly, it could be due to an imbalance between the amount of crude protein in the diet and the amount of protease available to help with digestion. Ammonia, amines, and other byproducts of protein fermentation might impact the integrity of the intestinal barrier, contributing to the link between dietary protein and issues with wet litter in poultry. Consequently, broilers may experience secondary health problems and complications such as footpad dermatitis, irritation in the breast area, hock burns, and a decline in overall performance [30]. In addition, it has been proven that when there is a reduction in pepsin activity within the proventriculus-gizzard, it not only leads to reduced protein digestibility in the ileum but also substantially boosts protein fermentation and the creation of putrefaction byproducts in the caeca. This illustrates that when the body's proteases do not operate effectively, the result is an accumulation of harmful protein fermentation byproducts in the lower intestine [31].

Another problem that could be derived from maldigestion due to enzyme deficiency is necrotic enteritis. High viscosity in the gut created by the lack of NSPase and the disability to digest non-starch polysaccharides causes a delay in the digestion of proteins. Simultaneously, it promotes an anaerobic environment in the small intestine, which is beneficial for the growth of a large number of *Clostridium perfringens* bacteria, which may enhance their ability to reproduce in such an environment. The combination of prolonged digestion of proteins and the presence of *Clostridium perfringens* can cause digestive issues, decrease gut health, and potentially serious conditions like necrotic enteritis. This issue emphasizes the significance of regulating nutritional factors, such as non-starch polysaccharides that increase the viscosity to maintain a healthy gut environment in poultry production, by providing exogenous supplements of enzymes to achieve an adequate and absolute metabolism of both non-starch polysaccharides and proteins [32].

The negative effects of enzyme deficiencies create a cascade of events that impact each other. So by altering and reducing the digestion of some feed components, the intestinal microbiota of broiler chickens will also be negatively altered.

2.4.2. Enzyme deficiencies having a direct effect on different pathogens

A lack of various enzymes can directly affect the gut microflora, which, as was already established, is essential to the innate immune system of the intestines. This suggests that, depending on the particular circumstance, the absence or deficiency of various enzymes may contribute to the development of diverse bacteria in the intestines.

For example, the jejunum's environment is more suitable for the growth and colonization of *Clostridium perfringens* when alkaline phosphatase is absent. This deficiency results in several significant changes in the gut, including a decline in alkaline phosphatase activity, a decrease in the activity of jejunum lysozyme mRNA, and a weakening of the immune system's defenses against the development, colonization, and invasion of pathogens. This may help to explain why *Clostridium perfringens* can develop quickly and perhaps cause infections or overgrowth in the gut when there is insufficient alkaline phosphatase activity in the intestines [33].

Another great example of the development of pathogens due to a lack of enzymes is the deficiency in lysozymes.

Lysozymes, also called 1,4-*β*-N-acetylmuramidase, are enzymes secreted in the broiler's intestines as part of the innate immunity, that cause the loss of cellular permeability and cellular death by cleaving the glycosidic connection between the N-acetylglucosamine and N-acetylmuramic acid in the bacterial peptidoglycan of the cellular wall [34]. Their mode of action is also connected to immunoglobulins and the monocyte-macrophage system [35]. This explains that lysozyme deficiency impairs the chicken's capacity to regulate the number of dangerous bacteria in its intestines, such as *Clostridium perfringens*, *Escherichia coli*, and *Campylobacter,* which will predispose the chicken to different pathogenic lesions [36].

2.5. Effect of different enzyme supplementation on broiler chicken

After examining how the lack of specific enzymes may have negative effects on different levels concerning the health and well-being of broiler chicken, we will discuss in the section how the supplementation of different enzymes can have a positive impact on the different aspects of the broiler chicken's body.

Different enzymes have been shown to have a great impact on the digestion, nutrient absorption, immunity, and intestinal flora of chicken.

2.5.1. Effect of Non-Starch Polysaccharide Degrading Enzyme (NSPase) supplementation

One of the most commonly supplemented enzymes in poultry is the NSPases. As discussed earlier, non-starch polysaccharides, naturally present in cereals such as wheat, rye, and oats can help in the development of an unwanted thick and viscous intestinal environment. As a consequence, the passage rate of the digesta will be minimal, which will reduce the availability of essential nutrients and favors bacterial overgrowth. By that, the optimal growth and healthy state of broilers will be disturbed and reduced. But this whole unwanted process can be avoided by supplementing the most crucial enzyme for the digestion of non-starch polysaccharides which are the NSPases [27].

Non-starch polysaccharide degrading enzymes, one of the most significant exogenous enzymes, are frequently added to broiler chicken diets and perform a variety of vital functions including breaking down non-starch polysaccharides, releasing nutrients contained within the cellular wall, decreasing the viscosity of the intestines, enhancing the absorption of nutrients, and influencing the structure and metabolic capacity of bacterial communities [37].

The positive effects of NSPases can be seen on many levels. Firstly, on the nutritional level, an addition of NSPases to the diet of broiler chickens can help significantly improve both the body weight and the feed conversion ratio. According to research carried out by KLEIN et al., NSPase supplementation was able to counteract the adverse effects of low-energy diets: broilers in the negative control (NC) group, where energy intake was decreased, showed lower body weight, and increased FCR. NSPase supplementation assisted in reversing these negative effects, proving that NSPase can boost performance even in low-energy diets [38]. The positive effects of NSPase supplementation can also be seen in intestinal absorption. In fact, by adding xylanase to a wheat-based diet, protein's ileal digestibility can be raised from 67% to 73%, which results in the host absorbing 10% more protein. It also causes a noticeable 20% decrease in the amount of protein that leaves the ileum, though. Starch and protein digestion can be significantly delayed when the diet becomes excessively viscous. When combined with an anaerobic environment, this delay can cause the fermentation rate in the ileum to increase by 10 times more than usual. However, enzymatic degradation of the viscous substance done by xylanase and mixtures of NSPases can completely eliminate this problem [39].

Secondly, NSPases can play a significant role in positively altering the intestinal microbiota. NSPases are able to supply the microbiota with fermentable oligosaccharides as a result of their action on polymeric non-starch polysaccharides and can limit the supply of readily fermentable resources such as starch and protein to the microbiota [40]. When an NSPase enzyme is active, it randomly breaks the structure of its target non-starch polysaccharide to reduce it. The bigger polymeric fragments produced initially by this process eventually become smaller as more hydrolysis takes place. The oligosaccharide produced as an end result will be assimilated into the intestinal microbiota according to its length and size. In addition, the supplementation of an NSPase causes increased luminal amounts of oligosaccharides, many of which are prebiotics, in the small intestine and potentially the caecum [41]. It appears that at least a portion of the mechanism of action of this class of enzymes involves the production of oligosaccharides via an NSPase. Such an activity would be particularly beneficial between the ages of 12 and 20 days when the ileum's and the caeca's fermentable substrate limitations become critical and the consequent decrease in the flux of nutrients into the caeca rapidly impacts the structure and number of the microbiota [42]. In addition to that, as previously shown, undigested NSPs, such as arabinoxylans, encourage the growth of pathogenic bacteria in the intestine and act as a foundation for their fermentation processes. The related pathogen-induced inflammatory response can cause morphological changes in the intestine and result in a high turnover of gut epithelial cells which is related to erosion and replacement of cells at the tips of the villus in part. This results in decreased nutrient absorption and a shift in energy from productive growth to maintenance of the gut itself. By making nutrients less accessible to bacteria, NSPases such as xylanase indirectly help preserve the shape of the intestine's mucosa and its capability to absorb nutrients.

Thirdly, NSPases can play a major role indirectly and directly in preserving and boosting the immunity of broiler chickens by promoting a strong and persistent immunity defense in the intestines. On the indirect level, NSPases boost feed passage rates and improve energy use in the foregut, which reduces the availability of nutrients for pathogenic bacteria in the lower gastrointestinal tract and lowers the possibility of multiplication of pathogenic bacteria [43]. On the direct level, according to research conducted by LIU and KIM, the length of villus and villus height to crypt depth ratio of the duodenum, jejunum, and ileum were enhanced by supplementing dietary xylanase levels. Additionally, the supplementation of xylanase to diets enhanced the ileal and cecal *Lactobacillus* counts, while most importantly, xylanase supplementation lowered the ileal and cecal *E. coli* counts. This point was proved by showing that supplementing xylanase to broilers' wheat-based diets encouraged the development of lactic acid bacteria in the ileum, which was put into evidence by greater lactic acid concentrations, while it has been also demonstrated that the presence of xylanase reduced the quantity of ileal *E. coli* and *Salmonella* while increasing the population of *Lactobacillus* in the ileum [44]. According to these findings, xylanase supplementation reduced the growth of potentially harmful bacterial communities in the colon and enhanced intestinal health.

In general, by increasing body weight, feed conversion, and nutrient absorption, NSPases boost broiler chicken performance. By providing fermentable oligosaccharides and reducing pathogenic bacteria, it has a favorable impact on the intestinal flora. NSPases also promote immunity by limiting pathogens' access to nutrients, both directly and indirectly. NSPases enhance the immunity, intestinal health, and growth of broiler chickens overall.

These effects are not only common for non-starch polysaccharide degrading enzymes but also for proteases.

2.5.2. Effect of protease enzyme supplementation

Proteases are a broad and complex category of hydrolytic enzymes that are categorized according to their site of action, the structure of the enzyme active site, and particular reaction processes. Proteases are widely distributed in broiler chickens, where they play a biochemical and physiological role in a variety of cellular and organismal functions, such as growth, adaptability, regulation, and the breakdown of proteins [45].

The beneficial effects of protease enzymes can be seen on different levels. Firstly, on the nutritional level, research conducted that protease supplementation in broiler diets significantly enhanced the body weight gain of broilers over days 1-18 and 19-35 as well as over the broiler chicken's overall growth phase from day 1 to 35 as the dietary protease levels rose from 0% to 0.09%. Furthermore, groups supplemented with protease had a lower and better feed conversion ratio than the control group, which was also marked by a greatly better digestibility of crude protein, dry matter, and energy. In addition, with increasing dietary protease addition in broiler chicken diets, a number of amino acids, including histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, and cysteine, which as discussed earlier cannot be synthesized by broiler chicken, showed a considerable improvement in digested rate [46].

Secondly, protease supplementation has been demonstrated to have a positive effect on the intestinal morphology of broiler chickens. In fact, supplementation with protease is capable of increasing villus height and the villus height to crypt depth ratio in the duodenum, as well as the villus height of the jejunum, and the villus height to crypt depth ratio of the ileum [47].

Thirdly, exogenous protease can play an important role in boosting the immunity of broiler chickens directly and indirectly. On a direct level, protease has been demonstrated to increase the serum IgM levels in broilers [48], while on the indirect level, the inclusion of protease in the meals of broiler chickens can significantly reduce litter moisture and reduce the risks of foot pad dermatitis due to *E.coli*. This is due to the enzyme's ability to increase protein breakdown. An increase in the level of protease will result in an increase in the level of protein digested, which will result in less undigested protein reaching the caecum. As a consequence, microbial fermentation and ammonia production will be lower [49]. In addition to that, protease supplementation can lead to certain changes in the gut flora and has the capacity to increase the number of beneficial bacteria inhabiting the microflora such as Bacteroidetes [50].

2.5.3. Effect of phytase supplementation

Phytases also known as myo-inositol hexaphosphate hydrolases are phosphatases, which are enzymes that may catalyze the hydrolysis of phosphate ester linkages. Phytases are able to hydrolyze one or more phosphate groups from phytic acid [51].

Firstly, phytase supplementation has been demonstrated to have several beneficial effects on the nutrient digestion and availability level of broilers while increasing body weight gain and decreasing feed conversion ratio. In fact, phytase is capable of greatly increasing the ileal digestibility of phosphorus regardless of the dose supplemented, which leads to an increased level of phosphorus in the broiler’s serum [52]. Exogenous phytase supplementation also had a great impact on calcium and phosphorus absorption even on broilers fed insufficient levels of both calcium and phosphorus. Indeed, even when the amount of calcium and phosphorus in the feed is insufficient, phytase has been proven to elevate the calcium and phosphorus availability [53].

Secondly, the positive effects of phytase supplementation are not only limited to digestion but to bone mineralization and litter moisture as well. The addition of phytase to the diet of broiler chickens is capable of increasing the length, ash content, and bone marrow density of the tibia while in parallel increasing the bone marrow density and concentration of the femur. Simultaneously, phytase is capable of reducing excessive litter moisture content which can help reduce the risks of foot pad dermatitis in broilers [52, 54].

Thirdly and most importantly, exogenous phytase supplementation can have a positive impact on intestinal morphology and microbiota. On the morphological aspect, phytase supplementation can increase the villus height and width of the jejunum, as well as improve the crypt depth of the ileum and the villus height to crypt depth ratio of the ileum. The positive modifications made by phytase supplementation on the intestines also reached the microflora that can become more resilient to pathogenic bacteria due to the direct and indirect effects of phytases. In fact, exogenous phytase can create a lower pH in the small intestines which has a bacteriostatic effect on pathogenic bacteria. For instance, phytase has been demonstrated to directly reduce the number of Enterobacteriaceae and Helicobacteriaceae, while increasing the number of beneficial bacteria inhabiting the intestines such as the *Lactobacillus reuteri*. This specific bacterium has been linked with the production of antimicrobial substances such as reuterin, ethanol, and organic acids. In addition to that, the supplementation of phytase in a broiler's diet can improve the intestinal barrier and reduce the number of bacteria that are transferred from the lumen of the gut to the surrounding tissues [55].

In general, we can conclude that the supplementation of phytase to broiler feeds has many benefits. It improves the digestion and absorption of nutrients, especially phosphorus and calcium, which results in better growth. Additionally, phytase lowers the moisture content of the litter and lowers the chance of foot pad dermatitis while improving bone mineralization. In addition, it has a positive effect on intestinal shape and microbiota, maintaining an optimal intestinal environment and lowering the predominance of pathogenic bacteria.

2.5.4. Effect of mannanase supplementation

Mannanases also called β-mannanases are enzymes required for the breakdown of mannan, an undigestible type of hemicellulose present in feedstuff and that represents anti-nutritive compounds that could have a deteriorating effect on different levels of the broiler's health if not digested, such as a decrease in the voluntary feed intake, nutrient absorption, metabolism, and immune system [56].

On the first hand, the positive effects of mannanase supplementation can be seen in the average daily gain and the feed conversion ratio of broilers of all age categories, by increasing the level of average metabolizable energy while increasing the level of different nutrients such as methionine, valine, and leucine [56, 57].

On the other hand, mannanase can play a very crucial role in enhancing the immunity of broiler chickens by different mechanisms. Due to their inflammatory and viscous properties, galactomannans are thought to damage the intestinal epithelial cell lining. By depolymerizing them, mannanases are able to reduce this inflammatory process [58]. In addition, mannanase supplementation has been demonstrated to increase blood serum levels of immunoglobins A, G, and M [59]. Not only do the positive effects of mannanase supplementation have a direct impact on immunity, but indirectly as well. Indeed, adding mannanase to broiler chicken diets leads to better intestinal integrity which results in lower risks of footpad lesions [58].

In general, we can conclude that mannanase has a beneficial effect on broiler chickens by increasing nutrient availability, enhancing immunity, lowering inflammation, and improving intestinal health. It also enhances growth performance and feed efficiency.

2.5.5. Effect of lysozyme supplementation

As previously mentioned, lysozymes, also called 1,4-β-N-acetylmuramidase, are enzymes secreted in the broiler's intestines as part of the innate immunity, that cause the loss of cellular permeability and cellular death by cleaving the glycosidic connection between the N-acetylglucosamine and N-acetylmuramic acid in the bacterial peptidoglycan of the cellular wall. Lysozyme supplementation can have many beneficial effects on broiler chicken including boosting the immune system of chickens, improving the intestinal microbiota, and promoting growth. For these reasons, lysozymes are being used as natural antibiotic substitutes [60].

Even on the genetic level, lysozyme supplementation results in a notable increase in the presence of genes related to the degradation of the external membrane and cell wall of bacteria [61]. Significant changes in gene expression can be seen in response to lysozyme supplementation and are confirmed by an increase in the expression of certain mRNAs. A wide range of genes are affected by these modifications, most notably genes producing enzymes such as copper and zinc-superoxide dismutase, and glutathione peroxidase, which are crucial parts of the antioxidant defense system. Additionally, the increased levels of interferon-gamma, interleukin-10, and interleukin-18 after lysozyme supplementation prove that lysozyme positively affects immune response-related gene expression. The higher expression of these immune-related genes suggests an improvement in immune system performance, possibly making the chicken more resistant to pathogens and inflammatory stimuli [62].

Lysozymes are also known for directly decreasing the severity of necrotic enteritis lesions. Indeed, many experiments have proven that lysozyme is capable of significantly reducing the ileal *Clostridium* counts and several butyrate-producing clostridia. Lysozymes are also proven to have an antibacterial effect by reducing several types of clostridia such as *Clostridium perfringens*, *Clostridium botulism*, and even *Listeria monocytogenes* [36, 63].

In conclusion, supplementing lysozyme has a wide range of advantages for broiler chicks. Notably, it strengthens the immune system and favorably affects the production of genes linked to bacterial destruction, antioxidative defense, and immunity. This improves the chicken's overall health by making it more capable of fighting pathogens and reacting to inflammatory triggers. Additionally, lysozymes exhibit a direct antibacterial impact, significantly lowering the incidence of dangerous bacteria including *Listeria monocytogenes* and *Clostridium* species. Lysozyme supplementation greatly impacts broiler chicken performance overall [61, 62, 63].

2.5.6. Effect of multi-enzyme supplementation on the immunity

While we demonstrated that the supplementation of specific enzymes can play a key role in the development of the skeletal, metabolic, and immune systems of broiler chickens, the supplementation of a multi-enzyme mixture can also have positive impacts on the same levels in broiler chickens, and especially on the immune system.

Indeed, it has been demonstrated that enzyme supplementation can be beneficial in reducing viral infections such as the Newcastle disease virus. The Newcastle disease virus is a highly pathogenic viral disease of poultry that belongs to the family of Paramyxoviridae and results in huge morbidity and mortality levels, which causes significant economic losses every year among farms in third-world countries [64]. Broiler chicken's hemagglutination inhibition antibody titer against the Newcastle disease virus can be considerably improved by supplementing enzymes. In parallel, supplementing enzymes can also slightly improve the hemagglutination inhibition antibody titer of broiler chicken receiving avian influenza vaccines [65].

Not only multi-enzyme supplementation is effective against bacteria and viruses, but also against parasites. In fact, enzymes can play an important role in reducing necrotic lesions induced by coccidiosis, a devastating disease caused by the *Eimeria* parasites that are responsible for over 3 billion dollars of losses in morbidity and mortality every year [66]. It has been demonstrated that supplementing an enzyme mixture containing amylase, protease, and xylanase to broiler chicks reduced lesion scores of *Eimeria acervulina* and *Eimeria maxima* in the intestines [67].

2.6. Negative effects of enzyme supplementations and contradictions

While the supplementation of enzymes has been demonstrated to have numerous positive impacts and advantages on broiler chickens, several studies have been conducted to try to prove the opposite. In fact, many researchers still believe that the supplementation of enzymes to broiler chickens does not have any beneficial effects, while some others think that enzyme supplementation to broiler chickens may have a negative impact on broilers. Although the negative impacts of enzyme supplementation are negligible compared to the positive impacts, they are still significant in order to understand how to correctly supplement enzymes to broilers.

Firstly, different studies suggested that the supplementation of proteases has no impact on the nutrient digestibility, feed conversion ratio, body weight gain, and average feed intake in the first 14 days of life of broilers [68]. In addition, there is evidence that supplementing external protease to broiler chicken's diet may cause the pancreas to secrete its natural proteolytic enzymes less frequently, which can cause the digestibility of proteins to decrease as a result of this action, while in parallel, there was no evidence of a protease effect or an interaction between feed and protease on the amount of ammonia in the excreta [50].

Secondly, some enzymes available in commercial blends have been demonstrated to have significantly negative impacts on all levels of broiler chicken’s health. For instance, alpha-galactosidase enzyme supplementation has been shown to a significant decrease in the body weight, body weight gain, and relative growth of broiler chickens [69].

Thirdly and most importantly, the limitation of the use of enzyme supplementations is due to the different physiological nature and environment of the broiler chicken’s intestinal tract. The first physiological limitation to the beneficial effects of enzymes is the pH. The majority of exogenous enzymes require a pH of 4 to 6 in order to have full efficacy [70], while the pH of the proventriculus and gizzard fluctuate between 2.5 and 3.5, the pH of the jejunum between 6.5 to 7 and the pH of the ileum between 7.0 and 7.5 [71]. This pH variety can cause the loss of functionality of enzymes partially or totally, depending on the specific location within the intestinal tract.

The second natural physiologic limitation for the efficacy of enzymes is time. The average retention time of feed within the intestinal tract from the esophagus to the end of the ileum is on average 3 to 4 hours [70]. Meanwhile, the digesta does not take more than 60 to 90 minutes from the crop to the gizzard, which will provide only a brief timeframe for enzyme activity [71]. This process emphasizes the importance of adapting better feeding strategies for the broilers in order to increase the passage time in a way to get a full mode of action of enzymes without having an extremely long passage time that can encourage bacterial growth.

3. Method

This literature review uses a systematic and thorough methodology to assess the body of academic literature already existing. The main goals are to find and evaluate important articles, summarize results, and provide support that enzyme supplementation has positive effects on broiler chicken's health status. I used multiple data sources including journal articles and books found on different scientific websites such as “PubMed” and “ScienceDirect”, using a detailed and specific search strategy. Ethical considerations such as proper citation were carefully addressed throughout the process.

4. Results

The different enzyme supplementation has great benefits for broiler chickens. Non-starch polysaccharide degrading enzymes could improve the feed conversion ratio, decrease the viscosity of the digesta, and decrease pathogenic bacterial counts in the ileum such as *E. coli* and *Salmonella*, while increasing the number of beneficial bacteria in the gut such as *Lactobacillus*. Meanwhile, Protease enzymes were found to improve body weight gain, boosting the immune system by increasing the levels of IgM and improving the intestinal structure by increasing the villus height in both the duodenum and the jejunum. Phytase enzymes also had important benefits such as increasing calcium and phosphorus absorption and improving the structure of certain bones in the skeletal system such as the tibia, by increasing its length, ash content, and bone marrow density. Phytases have also been demonstrated to reduce litter moisture and footpad dermatitis. Some enzymes demonstrated to have purely anti-inflammatory and anti-bacterial properties such as mannanase enzymes that could increase blood serum levels of IgA, IgG, and IgM. In parallel, lysozyme enzyme supplementation showed to increase in the presence of genes related to the degradation of the external membrane of the cell wall of bacteria, while decreasing the severity of necrotic enteritis and showing a direct antibacterial effect on different types of *Clostridium* bacteria. Finally, multi-enzyme supplementation could reduce the severity of certain viral infections such as the Newcastle disease virus, and reduce necrotic lesions caused by coccidiosis, while also improving the antibody titer after certain vaccines.

Although exogenous enzyme supplementation showed great benefits and impacts on broiler's health, some limitations due to the physiological nature of enzymes could be noted.

5. Conclusions

This study could demonstrate that the exogenous enzyme supplementation of broiler chicken had numerous positive effects and showed great impact on different critical parameters of broiler chickens such as improving their nutrient absorption and body conditioning, promoting their intestinal microflora to a rich and active state, and boosting their immunity. Additionally, this study proved that enzyme supplementation can help fight against different bacteria and viruses and decrease the severity of certain lesions such as necrotic enteritis. Although it has significant beneficial effects, enzyme supplementation still has certain limitations especially physiological ones that can reduce their effects, but these limitations are negligible compared to the benefits. These results emphasize that enzyme supplementation can play a key role in the broiler chicken industry by promoting the growth of a healthy chicken and contributing to a natural decrease in the number of pathogens affecting poultry.

6. Summary

Enzyme supplementation to broiler chicken could have great benefits to the industry and could help increase production while maintaining a healthy chicken.

In the first part of the work, we discussed the evolution of the broiler chicken breeding, before moving to the nutritional requirements of broiler chicken and all the essential parts of the diet of broilers that could be affected by enzymes and related to our topic.

In the second part of the work, we showed the nutritional and immunological importance of the intestinal microflora of broilers, which plays a great role in both the digestion of the feed particles and the provision of an additional line of defense for the boiler.

In the third part of our work, we demonstrated how different enzymes can affect nutrition, the intestinal microflora, and different other parameters of the body. Each of the enzymes selected is of high value for chicken, and we demonstrated how each one of those enzymes and their combination could be considered beneficial in its own way to the different parameters and body functions of the broiler chicken.

In the last part of our work, we demonstrated the limitations of enzyme use in the broiler chicken industry and showed why exogenous enzymes cannot always be beneficial.

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