IJAS Iranian Journal of Applied Animal Science

Dietary Supplementation of Enzymes: An Approach to Mitigate Ammonia Emission during Broiler Production

Review Article

S. Sugiharto[1*], I. Agusetyaningsih[1], E. Widiastuti[1], H.I. Wahyuni[1], T. Yudiarti[1] and T.A. Sartono[1]

[1] Department of Animal Science, Faculty of Animal and Agricultural Science, Universitas Diponegoro, Semarang, Central Java, Indonesia

Received on: 27 Nov 2022
Revised on: 3 Jan 2023
Accepted on: 6 Jan 2023
Online Published on: Dec 2023

*Correspondence E-mail: sugiharto@lecturer.undip.ac.id

Online version is available on: www.ijas.ir

ABSTRACT

Environmentalists are now becoming very concerned about issues related to air pollutants especially ammonia emissions from the broiler industry. Ammonia produced by broiler farms not only has a detrimental effect on the health and productivity of broiler chickens, but also has a severe influence on the environment. The ammonia emissions from broiler chicken houses can be effectively reduced by lowering the protein content of the feed, although broiler growth performance can suffer as a result. To improve protein digestibility and utilization in broiler chickens, enzymes can be used as feed additives. Hence, enzymes can avoid feeding with excessive protein content to broilers. Due to its ability to improve digestion and retention of N in the body, enzymes especially protease can reduce N excretion through excreta so that it has an impact on reducing ammonia emissions from broiler chicken houses. This present review provides an overview of the role of enzymes in reducing ammonia emissions from broiler farms. The mechanism of how enzymes reduce ammonia emissions from broiler farms is also discussed in this review.

KEY WORDS ammonia emission, broiler, enzyme, greenhouse gas, nitrogen, protein.

INTRODUCTION

Broiler farming is reported to increase the emission of harmful gases into the atmosphere, and increase the risk of odour disturbances in the surrounding environment (Brouček and Čermák, 2015). In addition to ammonia (NH_3), broiler production facilities also emit greenhouse gas (GHG), especially carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (de Sousa *et al.* 2007). Related to odour disturbances, some gases related to broiler farm activityincluding methyl mercaptan, dimethyl trisulfide, dimethyl disulphide, hydrogen sulphide (H_2S), and dimethyl sulphide can cause unpleasant odours in the environment. High concentrations of sulphur, ammonia, and methane gases generated duringbroiler production activitiescan also cause odour pollution in the farm environment (Hidayat and Purwanti, 2021). Ammonia is one of the main gases produced by poultry excreta which can have a negative impact on the environment (Hidayatand Purwanti, 2021). When chickens consume protein, some of the protein that is not utilized by chickens will be converted into uric acid, which is eventually converted into ammonia under favourable conditions (Naseem and King, 2018). In addition to having a negative impact on the environment, high ammonia levels (>25 ppm) in broiler house were reported to compromise body weight gain, feed conversion ratio (FCR), survivability, carcass production, and the poultry immune system (Brouček and Čermák, 2015; Swelum *et al.* 2021). High ammonia levels can also cause pain, eye inflammation, and increased oxidative stress in broiler chickens (Swelum *et al.* 2021). Based on these conditions, efforts to reduce ammonia emissions from the broiler chicken house are very essential. In general, protein digestibility is negatively correlated with ammonia concentration in broiler house (Sugiharto, 2022). The higher of protein digestibility, produces small amount of feed protein excreted, so that the ammonia production will be smaller (Hidayat and Purwanti, 2021; Sugiharto, 2022).

The use of reduced-protein diets has been reported to reduce the potential for ammonia emissions in broiler house (Vilela *et al.* 2020). However, reducing the protein content in the feed can have an impact on decreasing growth rate in broiler chickens (Law *et al.* 2018). To reduce the potential for ammonia emissions from broiler house without reducing growth performance of broilers, the use of feed additives is often carried. This may increase feed utilization so that reduce nutrient excretion by chickens (Bailey *et al.* 2021; Swelum *et al.* 2021). Among the additives that can be given to broilers are enzymes. Malomo *et al.* (2018) confirmed that enzymes can improve the digestive process and use of nutrients so that they contribute positively to the reduction of nutrient excretion, especially protein in chickens. Hence, broiler farmers do not need to provide feed with an excessive protein content since enzymes can maximize the utilization of feed materials, particularly protein, as excess protein can increase the excretion of protein into the environment.

This review provides an overview of the role of enzymes in reducing ammonia emissions from broiler farms. The mechanism of how enzymes reduce ammonia emissions from broiler farms is also discussed in this review.

Ammonia emission during broiler production

Among the air pollutants related to broiler chicken house, ammonia is the main gas and has the most significant impact on the health of both chickens and farmers (Brouček and Čermák, 2015; de Sousa *et al.* 2017; Vilela *et al.* 2020). This gas is the result of the degradation of uric acid and undigested protein by chickens involving special microorganisms, namely decomposition bacteria (Al-Kerwi *et al.* 2022). When consumed by chickens, amino acids or proteins in the feed will be absorbed and converted into other amino acids or degraded to produce energy (de Sousa *et al.* 2017). Because poultry feed generally has a high protein content, some of the protein that is not digested and utilized by chickens will be excreted out of the body and subsequently converted into ammonia (Naseem and King, 2018; Swelum *et al.* 2021). Chickens secrete protein (nitrogen; N) in the form of uric acid which when degraded will release ammonium (NH_4^+), which is the dominant form of N found in chicken excreta. Under certain conditions, for example an increase in temperature, humidity and pH value (alkaline conditions) in the broiler house, ammonium will be quickly converted to ammonia (de Sousa *et al.* 2017).

The decomposition of uric acid is carried out by urease, an enzyme produced by microorganisms, which catalyses the hydrolysis of urea to form ammonia and carbon dioxide. There are five enzymatic steps involved in the aerobic degradation of uric acid, including the conversion of uric acid to allantoin by the enzyme uricase. The second step is the conversion of allantoin to allantoic acid by the enzyme allantoinase. Next is the conversion of allantoic acid to ureidoglycolate by allantoic amidohydrolase, and by ureidoglycolase, ureidoglycolate is finally converted to glyoxylate and urea. The fifth stage is the hydrolysis of urea into ammonia and carbon dioxide catalysed by the enzyme urease (de Sousa *et al.* 2017).

A number of factors have been reported to affect the concentration of ammonia in broiler chicken house, including stocking density and chicken age, nutrition, stress, litter management, ventilation, temperature, and relative humidity (Vilela *et al.* 2020). Abouelenien *et al.* (2016) reported that an increase in stocking density and age of chickens was positively correlated with an increase in ammonia concentration in broiler houses. With increasing stocking density and age, the excreta produced by chickens per unit area increases and simultaneously ammonia emissions also increase in the house. In term of nutrition, the nutritional component that greatly influences the production of ammonia in broiler house is protein. High protein content in broiler feed will have an impact on increasing N excretion along with excreta. In the study of Van Emous *et al.* (2019) it was apparent that compared to broiler fed a high protein diet, the litter and excreta samples of broiler chickens fed a low protein diet have lower total N content and lower ammonia content. Regarding stress, Arifudin *et al.* (2019) reported that stress in chickens can reduce protein digestibility so that some of the undigested protein will be excreted out of the body and in turn will be converted into ammonia. Litter has a major contribution to ammonia emissions in broiler chicken house. Wet litter and alkaline pH greatly contribute to the increase in ammonia emissions in broiler house (Bailey *et al.* 2021). In this case, wet litter can increase the activity of decomposition bacteria so that it can accelerate the conversion of uric acid into ammonia. In relation to pH, ammonia emission will increase significantly when the litter pH is above 8. In this context, urease has a maximum activity at pH 9 so that the breakdown process of uric acid into ammonia becomes faster. Based on the above conditions, keeping the litter dry and has a low pH is very useful for reducing ammonia emissions in broiler chicken house. As already mentioned, high temperature and humidity are factors that can increase ammonia emissions in broiler house (de Sousa *et al.* 2017). High temperatures can cause an increase in the proliferation and activity of decomposition bacteria that play a role in the conversion of uric acid into ammonia. Note that, generally the decomposition bacteria in the litter are included in the group of mesophilic bacteria originating from excreta (faecal origin) so that they can live optimally at a temperature of 34-37 ˚C (Miles *et al.* 2011). In terms of emission of ammonia from litter into the environment, ambient temperature is a very important factor because ambient temperature can affect the convection mass transfer coefficient of ammonia. On the other hand, litter temperature can affect the Henry constant, dissociation constant, and also the diffusion and formation of ammonia in the litter (Liu *et al.* 2007).

Ventilation is a facility in the broiler house that is responsible for the exchange of gases including ammonia. In general, the concentration of ammonia in the boiler house is lower when the air circulation is faster (Liu *et al.* 2007). Ventilation also has a very important role in regulating the temperature and humidity in the room. A room with good ventilation will have a lower temperature and humidity, so that it has a positive impact on reducing the concentration of ammonia in the house.

Negative impact of ammonia on productivity and health of broiler chickens

The presence of ammonia in broiler house has been a concern of the broiler industry for a long time because it can cause potential losses related to decreased chicken production performance, damage to chicken skin and respiratory tract and can decrease chicken welfare (Bailey *et al.* 2021). The same thing was also reported by Swelum *et al.* (2021) that ammonia levels above 25 ppm in the cage can reduce growth rate, increase feed conversion ratio (FCR) and decrease broiler survival ability. Regarding the performance of chickens, increasing the concentration of ammonia in the broiler house can reduce feed consumption, thereby retarding the growth rate of broiler chickens (Brouček and Čermák, 2015).

In line with this, Yahav (2004) reported that high ammonia in the broiler house can affect the ability of chickens to maintain their internal body temperature so that the body temperature of chickens tends to increase. In conditions of high body temperature, chickens will reduce feed consumption so that it has an impact on decreasing growth performance in broiler chickens. Furthermore, Yahav (2004) and Arifudin *et al.* (2019) showed that an increase in body temperature due to high levels of ammonia in the cage could inhibit the activity of the hormones triiodothyronine (T_3) and thyroxine (T_4) which are actually responsible for the metabolic rate. In this case, the disruption of the metabolic rate will have a negative impact on the growth of broiler chickens.

A study by Zhang *et al.* (2015a) reported that an increase in the concentration of ammonia in the broiler house could adversely affect the morphology of the intestine, especially the intestinal villi, thereby reducing the ability to absorb nutrients in chickens. This will have a negative impact on the availability of substrate to carry out metabolic activities and ultimately can impair the growth of broiler chickens. Furthermore, Zhang *et al.* (2015b) and Xing *et al.* (2016) reported that exposure to ammonia in house can cause liver damage in broiler chickens. Considering the function of the liver as a protein producer in the body, liver damage can have a negative impact on protein (meat) deposition in the body of broiler chickens. Furthermore, ammonia at high concentrations can increase lactate dehydrogenase (LDH) levels in the blood, causing kidney damage in broiler chickens (Xing *et al.* 2016). Considering the importance of kidney function in maintaining the physiological condition of chickens, kidney damage due to high ammonia in the cage can result in decreased health performance and growth of broiler chickens.

High concentrations of ammonia in chicken house have been reported to damage the respiratory tract and weaken the immune system of broilers (Brouček and Čermák, 2015; Swelum *et al.* 2021). Furthermore, Brouček and Čermák (2015) confirmed that damage to the respiratory tract due to ammonia can increase the susceptibility of chickens to Newcastle disease virus, increase the incidence of air sacculitis and keratoconjunctivitis and increase the prevalence of *Mycoplasma gallisepticum* (Brouček and Čermák, 2015). In line with the above study, Zhang *et al.* (2015a) reported that an increase in ammonia in the broiler house can result in the disruption of the development of the immune organs so that the immune system in the body becomes less developed. In addition, oxidative stress due to high ammonia in the chicken house can cause disruption of the body's immune response in chickens so that chickens become sick more easily (Zhang *et al.* 2015a; Swelum *et al.* 2021). The study conducted by Chen *et al.* (2021) showed that exposure to ammonia can cause inflammation in the lungs so that it can endanger the health of broiler chickens. Correspondingly, Liu *et al.* (2020) reported inflammation of the lung tissue caused by high levels of ammonia in broiler houses. In this case, ammonia activity can increase the proportion of *Escherichia/Shigella*, activate the NLRP3 inflammasome, and increase the release of IL-1b which can trigger inflammation in lung tissue. Not only in the lungs, exposure to ammoniacan also cause inflammation in the intestines mediated by gut microbiota in broiler chickens through the TLR4/TNF-α signalling pathway (Zhou *et al.* 2021). Thus, high exposure to ammonia in the broiler house can cause damage to intestinal morphology so that it interferes with intestinal function in digestion and health.

Studies show that there is a correlation between the composition of the microbiota in the gut and gut health and at the same time the growth performance of broiler chickens (Sugiharto and Ranjitkar, 2019). The recent study by Han *et al.* (2021) showed that high levels of ammonia in the broiler house can alter the caecal microbiota of broiler chickens, which can have an adverse effect on the health status and growth rate of broilers. In particular, high levels of ammonia in the broiler house can alter the composition/species of microbes that inhabit the digestive tract of chickens. In this context, exposure to ammonia can increase the concentration of ammonia in the blood, which can thereby lead to an increase in intracellular and extracellular pH values. This increase in pH can ultimately inhibit the proliferation of acid-producing bacteria which in fact are good bacteria in the intestines. In line with the above study, Zhou *et al.* (2021) also reported changes in the composition of microbes in the gut (microbiota dysbiosis) when broilers were exposed to high levels of ammonia in the broiler house. Further, Zhou *et al.* (2021) reported an increase in *Streptococcus*, *Escherichia-Shigella*, *Faecalibacterium*, [*Ruminococcus*]_torques_group, *Ruminococcaceae*_UCG-014, unclassified_f_Lachnospiraceae, Rothia, unclassified_f_Ruminococcaceae in broiler intestine exposed to ammonia.

The negative effect of ammonia exposure is not only observed on the growth and health of broiler chickens, but the negative effect of ammonia has also been reported on the carcass and meat quality of broiler chickens (Xing *et al.* 2016; Swelum *et al.* 2021). Xing *et al.* (2016) reported that exposure to ammonia in the house can cause a significant decrease in the percentage of carcass and the percentage of broiler chicken breast meat at the age of 42 days. Regarding meat quality, exposure to ammonia can significantly reduce unsaturated fatty acid content and increase saturated fatty acid content in 42-day-old broiler chicken thighs. The limited movement of broiler chickens due to high levels of ammonia in the cage is associated with a decrease in the content of unsaturated fatty acids and an increase in saturated fatty acids in broiler chicken meat (Xing *et al.* 2016).

Negative impact of ammonia on environmental health

Ammonia emissions can have detrimental effects on ecosystems and the environment in addition to having a severe influence on the health and productivity of chickens (Naseem and King, 2018). Miles *et al.* (2013) reported that emission of ammonia during broiler production activity can cause environmental problems related to the disruption of the biodiversity of the ecosystem around the farm. Another study by von Bobrutzki *et al.* (2012) also showed that ammonia emissions due to broiler production can affect air quality and affect the nitrogen cycle in nature. Another negative impact of ammonia emission is the emergence of an acidification process in the soil which in turn can reduce soil fertility. Furthermore, von Bobrutzki *et al.* (2012) found damage to pine trees at a distance of 200 meters from the broiler farm location which indicates a significant impact on ammonia emissions on the ecosystem around the source of ammonia emissions. Another study by Brouček and Čermák (2015) revealed that ammonia emission is a very serious environmental problem because ammonia in the atmosphere can significantly change the level of oxidation in clouds so that it can cause an increase in acid concentrations in rainwater. Note that, acid rain can acidify the soil and clean water so that it can reduce the fertility level of the soil.

It has been mentioned above that ammonia emissions can cause disruption of biodiversity in nature. In general, Guthrie *et al.* (2018) explained that the impact of ammonia on biodiversity occurs through four main mechanisms, including eutrophication (accumulation of nutrients in ecosystems, especially nitrogen), acidification (acidification of soil and water due to the deposition of nitrogen compounds), direct toxicity (direct damage from ammonia to leaves and plant surfaces), and indirect effects (especially changes in plant species composition due to high nitrogen levels in the environment).

Ammonia is a gas that has a very strong odour. Therefore, the emission of ammonia during broiler production activity can be a source of problems in the environment around the farms related to the pungent smell of ammonia (Carey *et al.* 2004).

Digestion and excretion of protein

Protein is one of the nutritional components that are very important for the growth and development of broiler chickens. Protein plays a very vital role in the synthesis of body tissues so that it determines the overall growth rate of the chicken body. In poultry, protein is the main constituent of biologically active compounds in the body. Proteins, especially in the form of enzymes and hormones (peptide hormones) have important functions in physiological processes that take place in the body of chickens (Beski *et al.* 2015). Because of the important role of protein in the life and growth of the chicken, the adequacy of protein in the body of the chicken must always be met. Feed is a source of protein for broilers, therefore a feed formulation that considers the protein needs of broilers (to live and grow) is absolutely necessary. However, not all the protein in the feed can be utilized by chickens. The amount of protein in feed that can be utilized by chickens really depends on the digestibility of broilers, especially protein digestibility.

In general, protein digestion involves a number of sequential processes, which will eventually produce amino acids that can be absorbed into the body by the small intestine (Beski *et al.* 2015; Bryan *et al.* 2019). Based on the sequence of the protein digestion process, the non-optimal early stages of digestion in the proventriculus and gizzard can affect the digestive process in the small intestine and therefore reduce protein digestibility (Bryan *et al.* 2019). In this case the secretion of hydrochloric acid (HCl) and pepsin activity in the proventriculus will greatly affect the effectiveness of early digestion in broiler chickens. In the intestine, the activity of enzymes produced by the pancreas greatly determines the degradation of protein in feed and protein digestibility (Vilela *et al.* 2020). In addition, the composition of the microflora in the intestine and the morphology of the small intestine (ratio of villi height and crypt depth) greatly determine the effectiveness of digestion and absorption of nutrients (Sugiharto and Ranjitkar, 2019; Shad *et al.* 2022). In general, digestibility is the amount of nutrients from the feed that are not excreted through the excreta or the part of the feed that is lost from the feed after the digestion and absorption process. Several factors greatly affect the value of protein digestibility in broiler chickens. Generally, the level of protein digestibility depends on the protein content in the feed and the amount of protein consumed. In addition, the effectiveness of the digestive process in the digestive tract of chickens and the source of protein used in the ration also greatly determines the value of protein digestibility in broiler chickens (Bryan *et al.* 2019). Qaisrani *et al.* (2022) pointed out that there was a difference in protein digestibility between soybean meal and canola meal, where chickens receiving soybean meal had significantly higher protein and amino acid digestibility values than chickens fed canola meal. There is a close relationship between protein digestibility and N retention in broiler chickens. Maximum protein digestibility in general can increase N retention. The N retention itself is the amount of N that is absorbed and utilized by the body of broiler chickens. Indeed, Nretention shows the difference between N consumption and N excreted in the excreta. Positive N retention indicates that the protein needs of chickens can be met so that in the end it has an impact on increasing body weight of chickens (Tillman *et al.* 2005).

As discussed above, not all protein in the feed can be utilized by the body of broiler chickens. Unlike carbohydrates and fats, proteins do not have an amino acid reserve mechanism in the body, and hence all excess amino acids derived from feed will be catabolized. After being metabolized, this protein derivative becomes a precursor for the formation of uric acid to be excreted together with excreta, which will then be converted into ammonia (Vilela *et al.* 2020). With regard to the digestibility, the level of protein digestibility will determine protein excretion in broiler chickens, where the higher the protein digestibility level will have an impact on decreasing protein excretion and *vice versa*. In addition, the level of protein in the feed will also determine the protein excretion of broilers. In general, chickens have limited ability to digest and utilize protein from feed. Due to these limitations, when giving too much protein to chickens, some of the protein that cannot be digested and utilized by chickens will be excreted out of the body and will eventually be converted into ammonia (Naseem and King, 2018; Swelum *et al.* 2021). Stress (indicated by the increased activity of the corticosterone) is another factor that can affect protein excretion. Sugiharto (2020) showed that heat stress can reduce the value of protein digestibility and increase the degradation of protein tissue in the body. Both of these can have an impact on increasing N excretion from the body of broiler chickens.

Enzymes to reduce protein excretion

Enzymes can be defined as protein molecules that function as catalysts in a biochemical reaction. In poultry, enzymes can be produced in the body of chickens called endogenous enzymes. Enzymes can also be produced outside the body of chickens referred as exogenous enzymes. According to Alabi *et al.* (2019), exogenous enzymes can be produced industrially for commercial purposes including being used in broiler feed as a feed additive. In general, commercial enzymes can be produced from microorganisms, plants and animals. Yet, enzymes produced from microorganisms are the main commercial enzyme products at this time. The application of enzymes in feed is used to increase the energy value of feed ingredients and improve the digestibility, absorption and utilization of carbohydrates, proteins, fats and the release of phosphorus from plant-derived feed ingredients that cannot be digested by poultry. In general, the increase in digestibility due to the use of these exogenous enzymes resulted in a decrease in the excretion of undigested nutrients such as nitrogen and phosphorus. In many cases, the presence of endogenous enzymes is not sufficient to carry out its function as a biocatalyst or to carry out biochemical digestion of feed in the body of broiler chickens. Responding to these conditions, the use of exogenous enzymes to support endogenous enzymes in carrying out their functions becomes very important. dos Santos Andrade *et al.* (2018) reported that the use of exogenous enzymes can maximize the digestive process in the small intestine thereby reducing the amount of undigested feed residue that enters the large intestine.

Various exogenous enzymes have been widely used by broiler producers, including β-glucanase, xylanase, amylase, α-galactosidase, protease, lipase, phytase, and others (Sugiharto, 2016). In its application, the use of enzymes can be carried out singly or in combination with several enzymes (Pessôa *et al.* 2016; Gallardo *et al.* 2018; Al-Qahtani *et al.* 2021). Regarding the application of enzymes in broiler farms, Sugiharto (2016) revealed that to obtain the maximum benefit from enzymes, the use of a combination of several enzymes might have a better impact than the application of single enzyme. The combination of enzymes is reported to produce synergistic effects and can target different anti-nutritional compounds in feed ingredients so as to produce maximum digestibility values.

As stated above, the value of protein digestibility and N retention is inversely proportional to the excretion of protein or nitrogen from the body of broiler chickens. The use of enzymes to maximize protein digestibility and retention and reduce protein or N excretion has been widely practiced. Table 1 shows some examples of using exogenous enzymes to increase protein digestibility/N retention and decrease protein excretion from broilers. Based on the data in Table 1, it can be found that the quantitative increase in protein digestibility and the decrease in protein excretion due to enzyme supplementation in feed varied greatly from one study to another. It is not known for certain the explanation of these differences, but it is very possible that factors such as the natures or types of enzymes (single enzyme or mixture) used, the level of enzymes in the feed, the protein and energy content in the feed, the growth phase of the broiler chickens (e.g., starter or finisher), chicken health, stress, environmental conditions during the study, etc. very influential on protein digestibility and protein excretion of broiler chickens.

The mechanism by which enzymes can increase protein digestibility and N retention in broilers has been widely described in the literature. Nazemzadeh *et al.* (2017) reported that exogenous enzymes can improve the hydrolysis of feed ingredients into simpler components and improve nutrient absorption by broilers. Rehman *et al.* (2017) further showed that the use of exogenous proteases can increase endogenous peptidase activity so that it has a positive impact on increasing feed protein digestibility and hydrolysis of anti-nutritional factors such as antigenic proteins, trypsin inhibitors, and lectins contained in feed ingredients. In line with this, Al-Qahtani *et al.* (2021), Javaid *et al.* (2022) and Yaqoob *et al* (2022) reported that the use of exogenous enzymes in feed can increase the production and activity of endogenous enzymes resulting in better protein digestibility and N retention in broilers. Regarding the ability of enzymes to hydrolyse anti-nutritive factors in feed, Gallardo *et al.* (2018) stated that enzymes, for example phytase, are able to cleave the phytate-protein complex bonds thereby increasing the availability of protein for broiler chickens. Effiong *et al.* (2019) also reported that exogenous enzymes can open the complex matrix of protein so that protein is more maximally digested and absorbed by chickens.

Furthermore, Sugiharto (2016) and Yaqoob *et al.* (2022) showed that the use of exogenous enzymes can improve the ecology (microorganism population balance) of the digestive tract and the morphology of the intestines of broiler chickens. Such conditions will have a positive impact on intestinal function in digesting and absorbing nutrients in broiler chickens. In line with the above study, Shad *et al.* (2022) and Yaqoob *et al.* (2022) reported that the use of protease can increase the ratio of villi height and crypt depth thereby improving the nutrient absorption capacity of broiler chickens. In terms of increasing protein digestibility, Wu *et al.* (2017) reported that the use of enzymes can reduce the viscosity of digesta so that it has an impact on increasing the mixing of protein with pancreatic enzymes and bile acids. The decrease in digesta viscosity also has an impact on the movement of digesta in the digestive tract, thereby increasing the digestion process and protein absorption.

In addition to improving protein digestibility, the use of exogenous enzymes is also reported to increase protein biosynthesis so that it has an impact on decreasing protein excretion out of the chicken body (Javaid *et al.* 2022). Also, exogenous enzymes can reduce the flow of amino acids in the small intestine so that it has an impact on decreasing endogenous amino acid excretion in broiler chickens (Pessôa *et al.* 2016). The same thing was reported by Gallardo *et al.* (2018) that the use of phytase can reduce endogenous N flow in the digestive tract so that it has an impact on increasing N retention and decreasing N excretion from the body of broiler chickens.

Apart from the positive effect of using exogenous enzymes on increasing digestibility and protein retention, the consistency and reproducibility of using enzymes in broiler chickens is still being questioned by poultry nutritionists around the world. In fact, the effectiveness of the use of enzymes in broiler chickens varies greatly depending on the type and dose of enzymes used, the method of mixing enzymes with feed, the composition of feed ingredients in chicken rations, environmental conditions and others (Anjum and Chaudhry, 2010). The study by dos Santos Andrade *et al.* (2018) reported that crude protein digestibility in chickens fed 100 fungal xylanase units (FXU)/kg of xylanase and 300 FXU/kg of amylase did not differ from crude protein digestibility in control chickens. In fact, giving 100 FXU/kg of xylanase and 200 FXU/kg of amylase resulted in lower crude protein digestibility compared to control chickens.

The researcher also reported that there was no effect of giving 100 ppm of endo-1,4-beta-xylanase or 200 ppm of xylanase, amylase and protease on crude protein digestibility when compared to crude protein digestibility in control chickens.

Table 1 Examples of the use of enzymes and their effects on protein digestibility and excretion

Types of enzymes	Level of enzymes in feeds	Protein content of feed between treated *vs.* control group	Impact on broiler growth	Impact on protein digestibility	Impact on protein excretion	References
Enzyme complex containing phytase, protease, xylanase, ß-glucanase, cellulase, amylase, and pectinase	200 g/t of feed	Isonitrogenous	Weight gain increased by 3.77% and FCR[1] reduced by 2.67%	N retention increased by 5.30%	N excretion was reduced by 3.30%	Pessôa *et al.* (2016)
Commercial protease enzyme	30,000 IU/kg of feed	Isonitrogenous	Weight gain increased by 4.31% and FCR reduced by 3.80%	Increased total tract N retention by 3.34%	NM	Jabbar *et al.* (2021)
RONOZYME® (blend of amylase, xylanase, and protease)	80 KNU/kg of amylase, 100 FXU/kg of xylanase, and 15,000 PROT/kg of protease	Isonitrogenous	Weight gain increased by 3.69% and FCR reduced by 3.10% at day 21	Increased coefficient of digestibility of crude protein by 9.63%	NM	Giacobbo *et al.* (2021)
Commercial enzyme (Bio-max) contains phytase (57 U/mL), xylanase (322 U/mL), amylase (89 U/mL) and protease (258 U/mL)	1,000 g per ton diet	Isonitrogenous	Had no significant effect on growth and FCR as compared to non-enzyme-treated birds	NM	Decreased N in excreta by 14.8%	Javaid *et al.* (2022)
Kemzyme® protease	150 g per ton diet	Isonitrogenous	Had no significant effect on growth and FCR as compared to non-enzyme-treated birds	Increased ileal digestibility of crude protein by 1.23%	NM	Cho *et al.* (2020)
Phytase	300 mg/kg feed	Isonitrogenous	Increased weight gain by 3.98%, but had no effect on FCR	Increased ileal protein digestibility by 7.42%	NM	Al-Qahtani *et al.* (2021)
β-glucanase	100 mg/kg feed	Isonitrogenous	Had no substantial effect on weight gain and FCR	Increased ileal protein digestibility by 1.68%	NM	Al-Qahtani *et al.* (2021)
Enzyme blend (cellulase, glucanase, xylanase and phytase)	0.2 g/kg feed	Isonitrogenous	Weight gain increased by 17.2% and FCR reduced by 14.2% during finisher period	Increased apparent nutrient digestibility of crude protein by 28.24%	NM	Effiong *et al.* (2019)
Commercial protease	0.02% of diet	Isonitrogenous	Weight gain increased by 6.59% and FCR reduced by 4.93%	Increased N retention by 27.27%	NM	Rehman *et al.* (2017)
ProAct (CT) Protease enzyme	100 g/ton feed	Isonitrogenous	Had no substantial effect on final weight and FCR	NM	Decreased crude protein content in excreta by 16.67%	Nazemzadeh *et al.* (2017)
Multicarbohydrase	700 U α-galactosidase, 2,200 U galactomannanase, 3,000 U xylanase, and 22,000 U β-glucanase per kg of feed	Isonitrogenous	Weight gain increased by 5.40% and feed efficiency increased by 7.14%	Increased apparent total tract digestibility of N by 3.47%	NM	Gallardo *et al.* (2018)
Phytase	500 FTU per kg of diet	Isonitrogenous	Weight gain increased by 2.82% and feed efficiency increased by 3.42%	Increased apparent total tract digestibility of N by 4.20%	NM	Gallardo *et al.* (2018)
Commercial protease	3,000 protease units/kg of feed	Isonitrogenous	Increased body weight by 2.76% and improved FCR by 3.01%	Increase crude protein digestibility by 5.29% and N retention by 1.93%	NM	Shad *et al.* (2022)
Multi-carbohydrase enzyme complex	NM	Isonitrogenous	Increased body weight by 3.2% and improved FCR by 5.2 points	Enhanced apparent ileal digestibility of crude protein by 4.9%	NM	Wu *et al.* (2017)
Multi-enzymes with composition of 1,4-β-xylanase 20,000 U/g; α-amylase 2,000 U/g and protease 40,000 U/g	100 mg/kg multi-enzymes	Isonitrogenous	Had no substantial effect on weight gain and FCR	Increased n ileal digestibility of protein by 7.34%	NM	Yaqoob *et al.* (2022)

FCR: feed conversion ratio and NM: not measured.

The absence of an enzyme effect was also seen in the study of Al-Qahtani *et al.* (2021), where the administration of the xylanase enzyme could not increase the ileal nutrient digestibility of crude protein in experimental chickens when compared to control chickens. Furthermore, Javaid *et al.* (2022) reported no effect of giving phytezyme (containing xylanase [280 U/mL], phytase [130 U/mL], amylase [168 U/mL] and protease [102 U/mL] on the N content in the excreta.

Enzymes reduce ammonia emissions

Referring to the potential of exogenous enzymes in improving protein digestibility and N retentionin broiler chickens, the use of exogenous enzymes as agents to reduce ammonia emissions during broiler production becomes very logical. Several studies have reported the use of exogenous enzymes and their impact on ammonia emissions from broiler farms. Leinonen and Williams (2015) reported that the use of proteases in feed can reduce ammonia emissions during broiler rearing. The reduction in ammonia emissions was due to a decrease in the N content in chicken excreta and an increase in N retention in the broiler body. In line with the study above, Oxenboll *et al.* (2011) reported that protease administration reduced ammonia emissions throughout the broiler production period. In agreement with Leinonen and Williams (2015), Oxenboll *et al.* (2011) also reported that the reduction in ammonia emissions was closely related to increased protein digestibility and N retention in broiler chickens. The corresponding condition was also reported by Jabbar *et al.* (2021), in which the use of protease enzyme as feed additive improved total tract N retention in broiler chickens. The latter condition has an impact on decreasing the N content in the excreta of broiler chickens. The same condition has also been reported by Shad *et al.* (2022) where the administration of commercial protease enzymes can improve protein digestibility and also N retention so that it has an impact on reducing protein and N waste that is excreted into the environment. Furthermore, da Silva *et al.* (2021) reported the positive impact of using commercial protease enzymes on increasing the coefficient of true ileal digestibility of essential amino acids. Indeed, Park and Kim (2018) noted that the change in the gut microbial community due to enzyme supplementation was responsible for the improvement in N retention, and consequently, excreta ammonia emission. In agreement with this, Giacobbo *et al.* (2021) demonstrated that supplementation with RONOZYME® (blend of amylase, xylanase, and protease) modified the diversity and composition of microbiota in the intestine of broilers. It should be noted that the improvement in intestinal microbial ecosystem may improve gut health and abilities to absorb and digest nutrients, including N (Sugiharto, 2016). Moreover, Law *et al.* (2018) reported that supplementation using protease enzyme can improve the morphology of the small intestine (e.g., increasing intestinal villi height) so that it has a positive impact on the intestinal absorptive surface area. Likewise, Giacobbo *et al.* (2021) revealed that dietary supplementation with RONOZYME® increased villous height, decreased crypt depth and increased villous height to crypt depth ratio. The latter condition may support the intestinal capacity in absorbing nutrients especially N, so that less N is excreted out of the body.

In addition to the improvement in N retention, the improved energy and N partitioning system in broilers as a result of the supplementation with enzymes may also be responsible for the decreased ammonia emissions. In such case, McCafferty *et al.* (2022) reported that protease increased N retention-to-N intake ratio and net energy (NE)-to-apparent metabolizable energy (AME) ratio, while decreasing NE intake-to-N retention ratio. Protease also improved energy utilisation by reducing heat production and heat increment (HI), which hence improved N retention-to-N intake ratio. Overall, the latter investigators suggest that the increase in N retention may not be due to an increase in nutrient digestibility *per se*, but rather a change in the net metric with endogenous enzyme secretion resulting in decreased heat production from N excretion, endogenous loss as well as energy maintenance.

Apart from the positive impact of using exogenous enzymes to reduce ammonia emissions in broiler chicken houses, several studies reported different findings. Lourenco *et al.* (2020) reported that there was no effect of using protease on ammonia emissions. The absence of the effect of using protease on ammonia is in line with the absence of the effect of protease on protein digestibility in broiler chickens. The study by Akter *et al.* (2019) indeed showed that the use of phytase in feed can increase the concentration of ammonia in broiler litter. The reason for the increase in ammonia in the litter is still unclear, but the high Na content in the feed may have an impact on increasing ammonia emissions in broiler chicken house. In this case, the high-water content in the litter (as a result of the high Na in the feed) could explain the increase in ammonia concentration in the broiler house.

CONCLUSION

Ammonia is the main air pollutants related to broiler production activity whose presence not only has a negative impact on the health and productivity of broiler chickens, but also adversely affects environmental health. Reducing protein contents in broiler feed is an effective way in reducing ammonia emissions from broiler chicken house, but decreasing protein in feed can have a negative impact on broiler growth performance. The use of enzymes as feed additives in feed can be a solution to maximize digestibility and utilization of protein in feed so that feeding with excessive protein content can be avoided. Due to its ability to improve digestion and retention of N in the body, enzymes especially protease can reduce N excretion through excreta so that it has an impact on reducing ammonia emissions from broiler chicken houses.

ACKNOWLEDGEMENT

The study was supported by Universitas Diponegoro through “Riset Publikasi Internasional (RPI)”.

REFERENCES

Abouelenien A.F., Khalf-Alla F., Mousa-Balabel T., El-Midany S. and Abd El-Latif Nasser M. (2016). Effect of stocking density and bird age on air ammonia, performance and blood parameters of broilers. *World Vet. J.* **6(3),** 130-136.

Akter M., Graham H. and Iji P.A. (2019). Response of broiler chickens to diets containing different levels of sodium with or without microbial phytase supplementation. *J. Anim. Sci. Technol.* **61(2),** 87-95.

Alabi O.O., Shoyombo A.J., Akpor O.B., Oluba O.M. and Adeyonu A.G. (2019). Exogenous enzymes and the digestibility of nutrients by broilers: A mini review. *Int. J. Poult. Sci.* **18(9),** 404-409.

Al-Kerwi M.S.M., Mardenli O., Jasim M.R.M. and Al-Majeed M.A. (2022). Effects of harmful gases emitted from poultry houses on productive and health performance. *IOP Conf. Ser. Earth Environ. Sci.* **1060(1),** 12082-12097.

Al-Qahtani M., Ahiwe E.U., Abdallh M.E., Changȧ E.P., Gausi H., Bedford M.R. and Iji P.A. (2021). Endogenous enzyme activities and tibia bone development of broiler chickens fed wheat-based diets supplemented with xylanase, β-glucanase and phytase. *Anim. Biosci.* **34(6),** 1049-1057.

Anjum M.S. and Chaudhry A.S. (2010). Using enzymes and organic acids in broiler diets. *J. Poult. Sci.* **47,** 97-105.

Arifudin K., Sarjana T.A., Muryani R., Mahfudz L.D., Sunarti D., Sarengat W. and Angkeke I.P. (2019). Zonation in closed house affecting ammonia emission, immune system and broiler performance in the dry season. *IOP Conf. Ser. Earth Environ. Sci.* **247(1),** 12035-12044.

Bailey M.A., Hess J.B., Krehling J.T. and Macklin K.S. (2021). Broiler performance and litter ammonia levels as affected by sulfur added to the bird's diet. *J. Appl. Poult. Res.* **30(2),** 100159-100167.

Beski S.S., Swick R.A. and Iji P.A. (2015). Specialized protein products in broiler chicken nutrition: A review. *Anim. Nutr.* **1(2),** 47-53.

Brouček J. and Čermák B. (2015). Emission of harmful gases from poultry farms and possibilities of their reduction. *Ekológia*. **34(1),** 89-100.

Bryan D.D.S.L., Abbott D.A., Van Kessel A.G. and Classen H.L. (2019). *In vivo* digestion characteristics of protein sources fed to broilers. *Poult. Sci.* **98(8),** 3313-3325.

Carey J.B., Lacey R.E. and Mukhtar S. (2004). A review of literature concerning odors, ammonia, and dust from broiler production facilities: 2. Flock and house management factors. *J. Appl. Poult. Res.* **13(3),** 509-513.

Chen J., Jin A., Huang L., Zhao Y., Li Y., Zhang H., Yang X. and Sun Q. (2021). Dynamic changes in lung microbiota of broilers in response to aging and ammonia stress. *Front. Microbiol.* **12,** 1-11.

Cho H.M., Hong J.S., Kim Y.B., Nawarathne S.R., Choi I., Yi Y.J., Wu D., Lee H., Han S.E., Nam K.T., Seoung E.I. and Heo J.M. (2020). Responses in growth performance and nutrient digestibility to a multi-protease supplementation in amino acid-deficient broiler diets. *J. Anim. Sci. Technol.* **62(6),** 840-851.

da Silva J.M.S., de Oliveira N.R., Gouveia A.B.V.S., Vieira R.A., dos Santos R.O.F., Minafra C.S. and dos Santos F.R. (2021). Effect of protease supplementation on the digestibility of amino acids in animal-origin meals for broiler diets. *Czech J. Anim. Sci.* **66,** 29-37.

de Sousa F.C., Tinôco I.F.F., Silva J.N., Baptista F.D.J.F., Souza C.F. and Silva A.L. (2017). Gas emission in the poultry production. *J. Anim. Behav. Biometeorol.* **5(2),** 49-55.

dos Santos Andrade T., Nunes R.V., Wachholz L., da Silva I.M. and de Freitas D.M. (2018). The effect of exogenous enzymes on the performance and digestibility of nutrients in broiler. *Semina: Cien. Agrar.* **39(2),** 711-717.

Effiong M.E., Unah U.L., Enyenihi G.E. and Ndelekwute E.K. (2019). Dietary effect of feed grade enzymes on growth and digestive physiology of broiler chickens fed rice bran-based diet. *MOJ Anat. Physiol.* **6,** 74-78.

Gallardo C., Dadalt J.C. and Trindade Neto M.A. (2018). Nitrogen retention, energy, and amino acid digestibility of wheat bran, without or with multicarbohydrase and phytase supplementation, fed to broiler chickens. *J. Anim. Sci.* **96(6),** 2371-2379.

Giacobbo F.C., Eyng C., Nunes R.V., de Souza C., Teixeira L.V., Pilla R., Suchodolski J.S. and Bortoluzzi C. (2021). Influence of enzyme supplementation in the diets of broiler chickens formulated with different corn hybrids dried at various temperatures. *Animals*. **11(3),** 643-654.

Guthrie S., Giles S., Dunkerley F., Tabaqchali H., Harshfield A., Ioppolo B. and Manville C. (2018). The Impact of Ammonia Emissions from Agriculture on Biodiversity. RAND Corporation and the Royal Society, Cambridge, UK. Available at: https://royalsociety.org/-/media/policy/projects/evidence-synthesis/Ammonia/Ammonia-report.pdf Accessed Jan. 2023.

Han H., Zhou Y., Liu Q., Wang G., Feng J. and Zhang M. (2021). Effects of ammonia on gut microbiota and growth performance of broiler chickens. *Animals*. **11(6),** 1716-1725.

Hidayat C. and Purwanti S. (2021). Reducing air pollution from broiler farms. *IOP Conf. Ser. Earth Environ. Sci.* **788(1),** 12150-12159.

Jabbar A., Tahir M., Alhidary I.A., Abdelrahman M.A., Albadani H., Khan R.U., Selvaggi M., Laudadio V. and Tufarelli V. (2021). Impact of microbial protease enzyme and dietary crude protein levels on growth and nutrients digestibility in broilers over 15–28 days. *Animals*. **11(9),** 2499-2507.

Javaid A., Younas F., Ullah I. and Yasinzai M. (2022). Impact of an indigenously produced multienzyme complex from *Bacillus subtilis* KT004404 on growth and blood parameters in broiler chicken. *PLoS ONE*. **17(7),** e0271445.

Law F.L., Zulkifli I., Soleimani A.F., Liang J.B. and Awad E.A. (2018). The effects of low-protein diets and protease supplementation on broiler chickens in a hot and humid tropical environment. *Asian-Australasian J. Anim. Sci.* **31(8),** 1291-1300.

Leinonen I. and Williams A.G. (2015). Effects of dietary protease on nitrogen emissions from broiler production: a holistic comparison using Life Cycle Assessment. *J. Sci. Food Agric.* **95(15),** 3041-3046.

Liu Z., Wang L., Beasley D. and Oviedo E. (2007). Effect of moisture content on ammonia emissions from broiler litter: A laboratory study. *J. Atmos. Chem.* **58(1),** 41-53.

Liu Q.X., Zhou Y., Li X.M., Ma D.D., Xing S., Feng J.H. and Zhang M.H. (2020). Ammonia induce lung tissue injury in broilers by activating NLRP3 inflammasome via *Escherichia/shigella*. *Poult. Sci.* **99(7),** 3402-3410.

Lourenco J.M., Nunn S.C., Lee E.J., Dove C.R., Callaway T.R. and Azain M.J. (2020). Effect of supplemental protease on growth performance and excreta microbiome of broiler chicks. *Microorganisms*. **8(4),** 475-483.

Malomo G.A., Bolu S.A., Madugu A.S. and Usman Z.S. (2018). Nitrogen emissions and mitigation strategies in chicken production. *Anim. Husband. Nutrt*. **43,** 43-62.

McCafferty K.W., Choct M., Musigwa S., Morgan N.K., Cowieson A.J. and Moss A.F. (2022). Protease supplementation reduced the heat increment of feed and improved energy and nitrogen partitioning in broilers fed maize-based diets with supplemental phytase and xylanase. *Anim. Nutr.* **10(2022),** 19-25.

Miles D.M., Brooks J.P., McLaughlin M.R. and Rowe D.E. (2013). Broiler litter ammonia emissions near sidewalls, feeders, and waterers. *Poult. Sci.* **92(7),** 1693-1698.

Miles D.M., Rowe D.E. and Cathcart T.C. (2011). High litter moisture content suppresses litter ammonia volatilization. *Poult. Sci.* **90(7),** 1397-1405.

Naseem S. and King A.J. (2018). Ammonia production in poultry houses can affect health of humans, birds, and the environment-techniques for its reduction during poultry production. *Environ. Sci. Pollut. Res.* **25(16),** 15269-15293.

Nazemzadeh S., Heshmat G. and Ansari H. (2017). Effect of supplemented ProAct (CT) Protease enzyme on performance and the amount of protein excreted in feces of broiler chickens. *J. Livest. Sci.* **8,** 115-121.

Oxenboll K.M., Pontoppidan K. and Fru-Nji F. (2011). Use of a protease in poultry feed offers promising environmental benefits. *Int. J. Poult. Sci.* **10(11),** 842-848.

Park J.H. and Kim I.H. (2018). Effects of a protease and essential oils on growth performance, blood cell profiles, nutrient retention, ileal microbiota, excreta gas emission, and breast meat quality in broiler chicks. *Poult. Sci*. **97(8),** 2854-2860.

Pessôa G.B.S., Ribeiro Junior V., Albino L.F.T., Araújo W.A.G., Silva D.L., Hannas M.I. and Rostagno H.S. (2016). Enzyme complex added to broiler diets: effects on performance, metabolizable energy content, and nitrogen and phosphorus balance. *Brazilian J. Poult. Sci.* **18,** 467-474.

Qaisrani S.N., Hussain A.I., Naveed S., Bibi F., Akram C.A., Pasha T.N., Asif M., Irshad I. and Bilal R.M. (2022). Effects of protein source, whole wheat and butyric acid on live performance, gut health and amino acid digestibility in broiler chickens. *Metabolites*. **12(10),** 989-997.

Rehman Z.U., Kamran J., Abd El-Hack M.E., Alagawany M., Bhatti S.A., Ahmad G., Saleem A., Ullah Z., Yameen R.M.K. and Ding C. (2017). Influence of low-protein and low-amino acid diets with different sources of protease on performance, carcasses and nitrogen retention of broiler chickens. *Anim. Prod. Sci.* **58(9),** 1625-1631.

Shad A.A., Ahmad T., Iqbal M.F. and Asad M.J. (2022). Effects of a novel protease from bacillus subtilis k-5 in low protein corn distiller dried grains with solubles (cDDGS) based diets on performance and nutrients digestibility in broiler chickens. *Brazilian J. Poult. Sci.* **24(2),** 1-12.

Sugiharto S. (2016). Role of nutraceuticals in gut health and growth performance of poultry. *J. Saudi Soc. Agric. Sci.* **15(2),** 99-111.

Sugiharto S. and Ranjitkar S. (2019). Recent advances in fermented feeds towards improved broiler chicken performance, gastrointestinal tract microecology and immune responses: A review. *Anim. Nutr.* **5(1),** 1-10.

Sugiharto S. (2020). Alleviation of heat stress in broiler chicken using turmeric (Curcuma longa)-a short review. *J. Anim. Behav. Biometeorol.* **8(3),** 215-222.

Sugiharto S. (2022). Feeding fermented agricultural byproducts as a potential approach to reduce carbon footprint from broiler production–a brief overview. *Rev. Agric. Sci.* **10,** 90-100.

Swelum A.A., El-Saadony M.T., Abd El-Hack M.E., Ghanima M.M.A., Shukry M., Alhotan R.A., Hussein E.O.S., Suliman G.M., Ba-Awadh H., Ammari A.A., Taha A.E. and El-Tarabily K.A. (2021). Ammonia emissions in poultry houses and microbial nitrification as a promising reduction strategy. *Sci. Total Environ.***781(2021),** 146978-146985.

Tillman A.D., Hartadi H., Reksohadiprodjo S., Prawirokusumo S. and Lebdosoekojo S. (2005). Ilmu Makanan Ternak Dasar. Gadjah Mada University Press, Yogyakarta, Indonesia.

Van Emous R.A., Winkel A. and Aarnink A.J.A. (2019). Effects of dietary crude protein levels on ammonia emission, litter and manure composition, N losses, and water intake in broiler breeders. *Poult. Sci.* **98(12),** 6618-6625.

Vilela M.O., Gates R.S., Souza C.F., Teles Junior C.G.S. and Sousa F.C. (2020). Nitrogen transformation stages into ammonia in broiler production: sources, deposition, transformation, and emission into the environment. *DYNA*. **87(214),** 221-228.

von Bobrutzki K., Ammon C., Berg W., Einert P., Fiedler M., Müller H.J., Scherer D. and Strohbach B. (2012). Ammonia emissions from a broiler farm: spatial variability of airborne concentrations in the vicinity and impact on adjacent woodland. *Environ. Monitor. Asses.* **184(6),** 3775-3787.

Wu D., Choct M., Wu S.B., Liu Y.G. and Swick R.A. (2017). Carbohydrase enzymes improve performance of broilers fed both nutritionally adequate and marginal wheat-based diets. *J. Appl. Anim. Nutr.* **5,** 12-22.

Xing H., Luan S., Sun Y., Sa R. and Zhang H. (2016). Effects of ammonia exposure on carcass traits and fatty acid composition of broiler meat. *Anim. Nutr.* ***2*(4),** 282-287.

Yahav S. (2004). Ammonia affects performance and thermoregulation of male broiler chickens. *Anim. Res.* **53(4),** 289-293.

Yaqoob M.U., Yousaf M., Iftikhar M., Hassan S., Wang G., Imran S., Zahid M.U., Iqbal W. and Wang M. (2022). Effect of multi-enzymes supplementation on growth performance, meat quality, ileal digestibility, digestive enzyme activity and caecal microbiota in broilers fed low-metabolizable energy diet. *Anim. Biosci.* **35(7),** 1059-1068.

Zhang J., Li C., Tang X., Lu Q., Sa R. and Zhang H. (2015a). Proteome changes in the small intestinal mucosa of broilers (*Gallus gallus*) induced by high concentrations of atmospheric ammonia. *Proteome Sci.* **13(1),** 1-14.

Zhang J., Li C., Tang X., Lu Q., Sa R. and Zhang H. (2015b). High concentrations of atmospheric ammonia induce alterations in the hepatic proteome of broilers (*Gallus gallus*): An iTRAQ-based quantitative proteomic analysis. *PLoS One*. **10(4),** e0123596.

Zhou Y., Zhang M., Zhao X. and Feng J. (2021). Ammonia exposure induced intestinal inflammation injury mediated by intestinal microbiota in broiler chickens via TLR4/TNF-α signaling pathway. *Ecotoxicol. Environ. Saf*. **226(2021),** 1-11.