Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 20 No. 3 Ser. No. 68 2019

IRANIAN JOURNAL OF VETERINARY RESEARCH

Review Article

Humic acid as a feed additive in poultry diets: a review

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(Received 12 Apr 2018; revised version 6 Jan 2019; accepted 21 Jan 2019)

Abstract

Many studies tested different feed additives, among these additives, humic substances (HS) have been used in livestock and poultry diets. Humic substances commonly present in nature as they are created from the organic matter decomposition, and are normally found in the soil and natural water. Active components of HS consist of humic acid (HA), humus, ulmic acid, fulvic acid, humin and certain microelements. Humic acid is widely used as an alternative growth promoter for antibiotics in improving poultry performance and health. Moreover, supplementation of a commercial substance as a source of HS through the drinking water or diet improved the feed consumption, feed efficiency and weight gain of broiler chickens, and also improved egg weight, egg mass, and egg production of laying hens. This review describes the useful applications and recent facets of HA including its modes of action and various valuable uses in improving the production and health safeguarding of livestock and poultry.

Key words: Growth promoter, Health, Humic acid, Performance, Poultry

Introduction

Antibiotics are widely used to improve growth of animal (Dibner and Winter, 2002). However, it is observed that antibiotics have a negative effect because its residual effect in poultry products causes many problems related to human health (Donoghue, 2003). So, antibiotics are banned in the European Union as growth promoters because they cause bacterial resistance in birds (Mutus *et al*., 2006). There is a dire need to explore alternative additives of antibiotics to improve poultry production. Nowadays organic acids, plant extracts, enzymes, probiotics and prebiotics are used as a growth promoter (Griggs and Jacob, 2005; Abd El-Hack and Alagawany, 2015; Alagawany and Abd El-Hack, 2015; Dhama *et al*., 2015; Abdi *et al*., 2018; Alagawany *et al*., 2018). The use of organic acids has gained importance as a growth promoter and as a substitute of antibiotics to promote poultry performance (Mutus *et al*., 2006; Ur Rehman *et al*., 2016).

Humates or humic substances (HS), have been shown to induce rates of seed germination, transfer micro-nutrients from soil to plants, improve water retention and enhance microbial counts in soils and are composed of humic acid (HA), humus, ulmic acid, fulvic acid and other minerals (Peña-Méndez *et al*., 2005; Arif *et al*., 2016). Further uses in animal diets are still being found. The use of HA isimportant as a growth promoter to promote growth rate of poultry (Mutus *et al*., 2006; Arafat *et al*., 2017). Also, HA is widely used as an alternative growth promoter for antibiotics in improving bird performance and health (Ceylan *et al*., 2003).

Some studies investigated the effect of using HA as growth promoter in poultry and obtained positive results (Kamel *et al*., 2015; Sahin *et al*., 2016). It could enhance the bird immunity and reduce various kinds of stress (Humin, 2004). In broilers, Gomez-Rosales and Angeles (2015) found that HA improved the ileal digestibility of energy and the retention of nutrient. Moreover, HA can act as an antibacterial agent and could reduce mold growth and consequently reduces the toxin level (Humin, 2004). In this review, we have described the modes of actions, beneficial applications and biological activities of HS including HA in poultry health, nutrition and production. The information presented here would be helpful for the nutritionists, veterinarians, students, researchers, and poultry producers.

Structure and mechanisms of action of HA

Humic acid could be defined as an organic substance derived from the decomposition of organic matter and having a long molecular chain high in its molecular weight. Humic acid is insoluble in strong acids and has a pH below 2 and it could be soluble in alkaline media (Islam *et al*., 2005). Substances like HA have a medium molecular size and their molecular weight ranges from 5,000-100,000 Da. In this substance, the proportion of oxygen represents 33-36%, and nitrogen represents 4% (Islam *et al*., 2005). The chemical HA structure is presented in Fig. 1. The mechanism by which HS affects performance of poultry is largely unidentified. There are limited numbers of researches such as Abdel-Mageed (2012) and Taklimi *et al.* (2012), which displayed that HS enhance growth through modifying partitioning of nutrient metabolism. Humic acid has an essential role in poultry productivity due to its chemical compositions such as proteins, vitamins, digestive enzyme, water solubility and many other antibacterial substances and immune stimulating agents. Also, HS has ability to alter the intestinal microflora by increasing the counts of beneficial bacteria (Schepetkin *et al.*, 2003). As stated previously in the study of Taklimi *et al.* (2012), HA had an important impact on the crypt depth in the jejunum villi of broiler. Although villi growth generally depends on toxic substances, pH and microflora in the intestine, the HA has the potential to reduce pH and the count of pathogenic bacteria in the intestine. Thus, HA could have a favorable impact on performance of poultry via ecosystems in the gastrointestinal tract (Taklimi *et al.*, 2012).

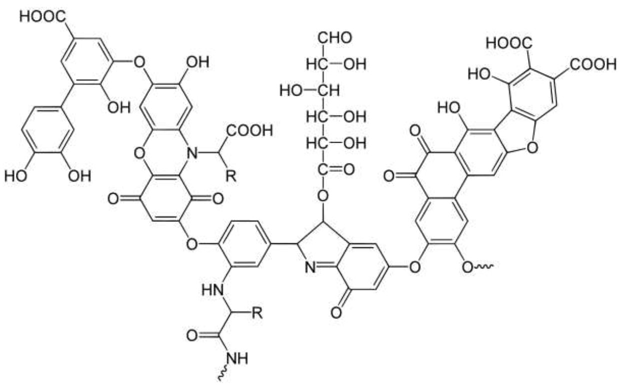

Fig. 1: The average chemical formula of humic acid ($C_{187}H_{186}O_{89}N_9S_1$)

Humic acid as a growth promoter

Humic substances as natural growth enhancers are used for their antioxidant, antifungal, detoxifying, and antiseptic properties (Rath *et al.*, 2005). In broilers and layers, a growth-promoting impact of HA or HS when added to the drinking water or feed has been documented (Ozturk *et al.*, 2010; Cetin *et al.*, 2011). In broilers, the benefits in body weight gain, feed efficiency and feed utilization as well as increasing in the length of villi of the jejunal mucosa and reduction in depth of crypt due to the inclusion of HA have been observed (Ozturk and Coskun, 2006; Taklimi *et al.*, 2012). Also, in layers, improvements in egg production, feed intake (FI), egg mass, egg quality have been described (Dobrzanski *et al.*, 2009; Ergin *et al.*, 2009).

The approach of using humates in poultry nutrition as an alternative feed additive has gained increasing importance (Ceylan *et al.*, 2003), especially after banning the use of antibiotics in feed as growth enhancers. Humic acid increased the FI and nutrient digestibility by increasing villus length and subsequently when villus length increases the area for absorption of nutrients increases. The nutrients absorption ultimately improves growth performance. It has ability to maintain gut micro flora. It provides a protective layer against the penetration of microbes and other toxic substances and stops their entry to intestine (Taklimi *et al.*, 2012). A study by Windisch *et al.* (2008) evaluated the effect of HA as a growth promoter on broiler and they proved that HA is a good growth promoter and it also improved nutrient digestibility by maintaining gut microbiota. Weight gain was also highest in broiler fed HA at 2.25 g/kg diet (Arif *et al.*, 2016). The improvement in growth rate may be due to the role of HA in improving the feed conversion ratio (FCR) at the same level. On the other hand, Kaya *et al.* (2009) stated that there were no differences due to supplementation of humates on weight gain in broilers. Karaoğlu *et al.* (2004) found that supplementation of humates and HA in broiler diet showed no impacts on body weight gain. Furthermore, Arafat *et al.* (2015) confirmed that FCR of layers was improved with the addition of HA in drinking water. Because it could stabilize the gut microbiota and enhance nutrients utilization, the enhancement in live body weight without augmentating the quantity of feed given to the chicks it led to better FCR (Humin, 2004). Growth rate and viability were markedly improved in broiler chickens exposed to high ambient temperatures when fed a diet containing HA (Edmonds *et al.*, 2014).

Effect of HA on productive performance

Egg weight and production for hens fed diet containing HA at 0.1, 0.2 or 0.3% were significantly improved compared with the control hens (Abo-Egla El-Samra *et al.*, 2011). Arpášová *et al.* (2016) illustrated that dietary HA supplementation at a level of 0.5% improved egg weight and production (P>0.05). Shermer *et al.* (1998) stated that HA increased egg weight and production of laying hens. On the contrary, Wang *et al.* (2007) stated that the dietary HS supplementation at 5 or 10% improved egg weight but egg production was decreased. In broilers, Kocabagli *et al.* (2002) found that no significant impact on FI was detected when chicks group was fed diet supplemented with HS. Furthermore, HS did not affect FI, feed efficiency, metabolizable energy intake, egg weight, egg production, mortality rate, egg quality traits such as shape index, shell thickness, breaking strength, yolk index, albumen index, Haugh unit score and the percentages of shell, yolk and albumen (Yalcin *et al.*, 2006). Yörük *et al.* (2004) reported that 0.1 or 0.2% humate had no significant influence on FI in late production stage, but these levels increased egg production during the late laying period in comparison with the control. Contrarily, Kucukersan *et al.* (2005) pointed out that FI of hen fed diets supplemented with HA was statistically (P≤0.05) declined in comparison with the control hens.

The results obtained by Ergin *et al.* (2009) showed that HA addition at a level of 30 mg/kg diet increased the egg shell strength without affecting feed efficiency and egg production compared with the control group. While, egg production in the birds fed diet supplemented with HA (90 mg/kg) was significantly higher than the control, but FCR, yolk weight and egg weight were not affected by supplementation of HS (Ergin *et al*., 2009). On the other hand, Sopoliga *et al*. (2016) did not confirm a positive impact of dietary HS supplementation at a level of 0.5% on FI and FCR as well as egg weight and production of pheasant laying hens. Also, HA had a positive effect on production performance of broiler chickens (Nagaraju *et al*., 2014; Pistova *et al*., 2016). Humic acid at a level of 1.7 ppm enhanced live weight gain without any harmful effects on FCR (Ozturk and Coskun, 2006).

Effect of HA on nutrient digestibility and utilization

Stepchenko *et al*. (1991) designed an experiment to observe the impact of sodium humate on the metabolism and productivity of poultry. They found that the different levels of sodium humate improved the digestibility and nutrient metabolism by altering partitioning of nutrient in the feed. Similarly, Parks *et al*. (1996) also conducted an experiment to find out the effect of Menefee humate with low and high crude protein on the immunity and growth performance of turkey and they observed that humate improved digestibility of nutrient by modifying partitioning of nutrient metabolism.

Ertas *et al*. (2006) used mussel shell as a source of HA and calcium in Japanese quails reared under heat stress and they reported that HA enhanced calcium level and also improved digestibility of protein. The effect of HA as feed additive in broiler was evaluated by Windisch *et al*. (2008) who found that HA improved digestibility of nutrient by maintaining gut microflora. Islam *et al*. (2008) also stated that supplementation of HA in broiler diets improved the utilization of nutrients and growth performance by improving the gut health. Moreover, Ozturk *et al*. (2014) examined the effect of HS (7.5, 15 and 22.5 g/kg) on performance and nutrients utilization in Ross chicks. The authors pointed out that 15 and 22.5 g/kg of HS significantly increased nutrients digestibility.

Ceylan *et al*. (2003) assessed the effect of mixture of antibiotic, probiotic, prebiotics and HA on performance of broiler and its gut microflora. By the statistical analysis they proved that using the mixture of all above mentioned growth promoters significantly improved feed efficiency and nutrients digestibility. Similarly, Ozturk *et al*. (2010) evaluated the effect of utilization of HA in drinking water on nutrient digestibility or utilization in broiler and they concluded that HA (1.7, 5.1, and 8.1 ppm) in drinking water improved gut health and it also improved nutrient utilization. Kocabagli *et al*. (2010) designed an experiment to evaluate the dietary effect of humate on feed utilization in broilers and the latest authors showed that addition of humate (2.5 kg/Ton) in feed significantly improved body weight gain by increasing nutrient utilization. The ileal digestibility of energy reported a quadratic response ($P<0.05$) by increasing the dose of HA in the drinking water, but the ileal digestibility of N was not affected (Gomez-Rosales and Angeles, 2015). The increased gut length and the increased length of jejunal villi due to the dietary HA inclusion in broiler chickens (Taklimi *et al*., 2012) have been correlated with the improved digestion coefficients of nutrients, due to the augmented extension of enzymatic digestion and the lessening in passage rate of the intestinal content.

Effect of HA on some health-related blood parameters

Humic acid has positive impacts on poultry health-related parameters in the blood such as cholesterol level, antioxidant and hematological parameters as shown by previous authors. Determination of blood cholesterol level is very important for quality of poultry products and consumer health. It is observed that high cholesterol level in blood increases the chance of heart attack. It also causes atherosclerosis and cardiovascular diseases based on clinical and epidemiological studies. A survey in year 1980 showed that in USA higher blood cholesterol level up to 17% increased the risk of heart diseases (National Cholesterol Program in 2004). There are two types of cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL). High density lipoprotein is a good type, and helps to remove LDL from blood vessels. The optimal level of HDL is 150 mg/dl for good health (Shao and Heinecke, 2009).

There was non-significant impact of treatment on blood cholesterol in laying hens when Hakan *et al*. (2012) evaluated the impact of boric acid and HA on blood parameters of laying hens. Arif *et al*. (2016) clarified that levels of blood cholesterol and LDL were significantly declined in groups of HA (2.25 and 3 g/kg diet). Reduction in blood lipids and cholesterol might be due to reductions in microbial intracellular pH (Abdo, 2004). By inhibition of microbial enzymes, bacterial cell membrane is forced to use energy to release acidic protons which lead to lower intracellular pH (Young and Foegeding, 1993). On the other hand, there were no impacts of humate on levels of blood cholesterol of laying hens (Hakan *et al*., 2012). Köksal and Küçükersan (2012) investigated the effects of dietary supplementation of humate on laying hens and broiler. The author found that humate addition slightly increased blood cholesterol levels ($P>0.05$).

Dietary HA addition augmented the hematological parameters such as hemoglobin (Hb), red blood cell (RBC) and paked cell volume (PCV) (Ipek *et al*., 2008; Miśta *et al*., 2012), and may be due to its impact in binding inorganic ions and transporting the minerals to cells (Islam *et al*., 2005). Humic acid presented protective impacts against liver damage (Ghahri *et al*., 2010). Lower levels of HA did not have any influence on total antioxidant capacity, but high levels of HA reduced total antioxidant capacity. Thus, high levels of HA should not be supplemented because of increasing oxidative stress (Ipek *et al*., 2008). Additionally, HA had a sturdy antioxidant activity and protected the cells from oxidative damage and stress by inducing the total antioxidant, catalase and glutathione reductase activity, and also decreasing the malondialdehyde levels protect the cells from lipid peroxidation and synthesis of the toxic free radicals (Kamel *et al*., 2015).

Effect of HA on viable microbial count

Scientific reports about the impacts of HS on gut health and composition of microbiota in the gastrointestinal tract are rather scarce (Aksu and Bozkurt, 2009). Shermer *et al*. (1998) and Islam *et al*. (2005) stated that HS might positively influence the animal performance by modifying the ecosystem in the gut with following stabilization of flora in the small intestine, better utilisation of nutrients and improvement of gut health.

Humic acid plays a vital role in the protection of gut against infections and favorably affect its functions. Humic substances affect the microbes' carbohydrates and proteins metabolism destroying the pathogenic viruses and bacteria. Humic substances have good buffering capacity and can modulate the gut pH (Rath *et al*., 2005; Arpášová *et al*., 2016). Humates are known to stimulate microbial activity. Humates supplemented to poultry diets stimulate the microbial growth and the large extent depend upon the environment, the culture medium and species (Huck *et al*., 1991). Humic derivatives have been recognized to show anti-microbial aspects. Species for which natural humic derivatives have been revealed to be inhibitory include *Enterobacter cloacac*, *Candida albicans*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Strpyogenes* and *Staphylococcus epidermidis* (Riede *et al*., 1991). In the body, humates suppress the bad microbes but stimulate the good microbes. Shermer *et al*. (1998) stated that HA stabilizes the intestinal microbiota and consequently ensures an enhanced nutrients utilization in poultry feed.

Effect of HA on the mold growth and immune response

Humic acid possessed potential to inhibit mold and bacterial growth, by reducing the mold growth, toxin level can be reduced (Humin, 2004). The macro-colloidal structure of HA has a protecting influence on the mucous membrane of the gut and stomach. As a result, the toxic metabolites absorption is decreased or wholly prevented. It also helps to stop excessive water loss via the intestine (Humin, 2004). Arafat *et al*. (2017) clarified that 0.1, 0.2 or 0.3% HA reduced the effects of aflatoxin growth and also decreased the aflatoxin residues in the liver, resulting an apparent protection for the liver organ. These results refer that HA provides significant reduction in the immunotoxic effects of aflatoxin. As well, 0.2% or 0.4% HA might be sufficient to counteract the adverse effects of aflatoxin in broilers (Ghahri *et al*., 2010).

Immunostimulatory properties of HA have been investigated to augment immune potency and then the health status of animal. Rath *et al*. (2006) stated that white blood cell and monocyte cell were not affected, however, there was a significant reduction in blood heterophil counts of HA-treated birds. There was a significant improvement in lymphocyte counts and reduction in heterophil counts and heterophil to lymphocyte ratio due to humate treatments as compared to control group (Ebru *et al*., 2011). Inclusion of HA in diets that improved immune development in broilers may be due to the important role HA plays in the growth of immune organs, mainly the thymus and bursa of Fabricius, as major elements of the avian immune system (Disetlhe *et al*., 2017). Humic acid showed protective effects against bursa of Fabricius damage (Ghahri *et al*., 2010). Also, broiler chickens supplemented with HA revealed significant increases in lymphocyte, leukocytic count, globulin (α, β and γ), phagocytosis and phagocytic index (Salah *et al*., 2015). Addition of HA (up to 0.1%) mainly in low nutrient density antibiotic free diets can improve the immune status (Nagaraju *et al*., 2014). Humic acid has a nutriceutical property that improved activity of neutrophil which might protect against pathogenic bacteria and decrease mortality rate during acute bacterial infection (Dabovich *et al*., 2003). Finally, the impacts of HA in improving immune functions may be due to its antiviral properties, activation of neutrophils, phagocytic activity of leukocytes (Chang-Hua *et al*., 2003), ability to prevent the colonization of intestinal pathogens (Klocking, 1994), and improve nutritive value of feed (Kocubagli *et al*., 2002).

Conclusion

The advantages of dietary HA supplementation as a feed additive are promising. Humic acid has a favorable role in boosting productive performance due to its useful impact on nutrient utilization and absorption. Besides, HA exhibit many nutritional features by declining the total cholesterol and LDL in serum. Advances in the field of biotechnology need to be explored further to achieve well production and attempt useful *in vivo* applications of HA for safeguarding health of animals. It is hard to compare the actual impacts of HA products due to various preparations and sources, in addition to animal rearing in different areas of the world.

Acknowledgements

The authors thank and acknowledge the support of their respective Institutes and Universities.

Conflict of interest

The authors declare that there is no conflict of interests that could possibly arise.

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