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Chapter

Mechanisms of Action of Humic Substances as Growth Promoters in Animals

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Abstract

A review of the latest research on the addition of humic substances (HSs) in the drinking water or feed of farm animals including poultry, pigs, dairy cows and calves, goats, and rabbits was carried out. The results reinforce the promoted protective action of HS on the digestive mucosa, their antioxidant properties, immunomodulatory, and anti-inflammatory attributes, the suggested microbial shift to a probiotic-type bacteria in the gut, including antiparasitic and antifungal effects, as well as their influence on the higher efficient of minerals, proteins, and lipids utilization within the body. The outstanding improvements in health, productivity, and meat and milk quality obtained from animals supplemented with HS are common features in the majority of available research. The main benefits in the gastrointestinal tract stem from the formation of protective barriers on the epithelial mucosa due to its colloidal properties and stimulation of mucin production. HS also promotes the development of probiotic microbiota and positive changes in bacterial fermentation patterns, which results in improved intestinal health and integrity. As a result, a cascade of benefits is derived within the body, reinforcing antioxidant protection responses, immunomodulation, and anti-inflammation mechanisms, as well as improving nutrient utilization efficiency. However, the truly molecular mechanisms of action of HS in the intestine and throughout the body remain unknown.

Keywords: humic substances, farm animals, mucosal protection layers, microbiota, antioxidant and immune responses, nutrient utilization, performance

1. Introduction

Humic substances (HSs) are organic compounds derived during the humification process of decaying plants and animals and are mainly composed of humic acid (HA) and fulvic acid (FA). HSs have been utilized in humans for ages as nutritional supplements and therapeutic purposes. The first mentions of the therapeutic applications of HS may be found in Sanskrit and ancient Chinese and Roman texts, where they were assigned magical properties [1, 2]. Some of the therapeutic characteristics of HS are mentioned in the Chinese Materia Medica Pharmacology Compendium, which
dates back to the Ming Dynasty in the fifteenth century; their medicinal usage was permitted by the China Drug Administration, and they were known as “the gold of medicine” in China [3]. Another important medical application of HS is its usage in balneotherapy in ancient Babylon, in Lower Mesopotamia and in the Roman Empire [4]. Information about the use of HSs in humans has recently been published, including medical issues, preclinical trial outcomes, and successful clinical therapies [5–7].

HSs are molecules with anti-inflammatory, antioxidant, antiparasitic, antibacterial, and antiviral properties, according to the European Agency for the Evaluation of Medicinal Products’ Committee for the Evaluation of Veterinary Products [5, 8], and the feasibility of using them orally in horses, dogs, pigs, and birds in doses of 500 to 2000 mg/kg of live weight for the treatment of diarrhea, dyspepsia, and acute poisoning was documented; it was also indicated that HSs exert a protective action on the mucosa of the intestine and have antiphlogistic, adsorbent, antitoxic, and antimicrobial properties. The various structures and functional groups give HS properties such as colloidal, spectral, electrochemical, and ion exchange, which confers significant adsorption capacity [5, 9]. HS has been shown in animals to be able to modulate the harmful effects of a variety of xenobiotics and unwanted compounds that enter the digestive tract through feed and drinking water due to their colloidal properties and propensity to form chelates [10, 11]. HS has also been shown to minimize the accumulation of some heavy metals in tissues, including mercury, cadmium, and zinc in fish [12], lead and cadmium in rats [13], and cadmium, zinc, and lead in chickens [9–11]. The main research findings and some proposed theories about the mechanism of action of
HS in animals are discussed in the following sections. Figure 1 summarizes the main benefits observed in animals provided HS.

2. Formation of protective layers in the digestive mucosa

Due to their colloidal characteristics and high capacity to form aggregates within solutions, it has been proposed that HSs have the ability to create protective layers on the epithelial mucous membrane of the digestive tract, preventing the penetration of pathogenic bacteria or toxic substances produced by bacteria [8, 14, 15]. The ability of HS to form polymers in a media with a slightly alkaline pH, such as the intestine, is credited with the creation of protective barriers [16]. HS also interacts with biomolecules like collagen, promoting the resistance and maturity of its fibers, resulting in an increase in the intestinal villi’s integrity [17].

HSs were found to increase intestinal viscosity, inhibit bacterial translocation from the intestine to the liver, and lower the serum levels of an intestinal permeability marker, fluorescein isothiocyanate dextran (FITCd), in a recent study [15]. HS has also been demonstrated to positively regulate mucine-2 (MUC-2) gene expression in the cecum mucosa [18]. MUC-2 is a main gel-forming mucin that serves as the principal barrier component of mucus layers as well as a storage location for secretory immunoglobulin A (IgA). The number of goblet cells in the villi of the jejunum of broiler chickens fed with HS was similarly found to be increased 24 hours following a diet change challenge [19]. HS may increase the protective mucus layer in the small intestine, allowing the villi to maintain their integrity against pathogenic agents, toxins, and dietary changes, according to this research. In addition, the development of gels causes increased viscosity in the small intestine, which results in slower rate of feed passage through the gut [20, 21], which increases the time of exposure of nutrients to digestive enzymes [22, 23].

The protective effect of HS on the digestive epithelium has been associated with increases in villus height, crypt depth, and epithelial surface area in mice [17], chickens [24–26], Japanese quails [27], and rabbits [28], and increased activity of digestive enzymes in the digestive mucosa of chickens [26, 29] and freshwater fish [30], and also to increased energy and protein digestibility in chickens [31, 32], organic matter, protein, and fiber in rabbits [28, 33] and protein in milking cows [34]. In addition, an increased weight of the gizzard and length of the duodenum and jejunum has been reported in chickens fed canola-based diets and added with HS [35], as well as the size and weight of the cecum of rabbits [28].

It has been proven that peat-based treatments can be used to relieve gastric, intestinal, and liver diseases [36]. Peat moss has been proven to minimize the size of ethanol-induced stomach ulcers in rats, speeding up the healing process of both gastric and duodenal ulcers [37]. Orally administered HS to rats has been shown to bind, presumably metabolize, and resorb toxins in the gastrointestinal system [38]. These results supported the orally administration of HS to animals for the treatment of diarrhea, dyspepsia, and acute poisoning [8, 39]. In recent research, pigs [40–42] and calves [43–45] fed with HS showed less severe diarrhea.

In addition to the benefits of HS observed on the mucosal surface of the digestive tract, healing and protective effects have also been reported on other types of epithelia. The restorative effect of HS on the epidermis has been demonstrated with balneotherapy, which is used for the treatment of various skin diseases such as chronic eczema, neurodermatitis, and psoriasis in various parts of the world [2], in which the activation of skin metabolism and regenerative processes has been observed [5].
On the other hand, the plethoric benefits that have been widely documented in the immune response, antioxidant status, digestive microbiology, and nutrient metabolism in humans and animals added with HS suggest that the mechanisms of action go far beyond its mucosal protective effect. It has previously been suggested that the absorption of orally administered HS is very low, at 0.05 to 0.07% [8]. But in subsequent evaluations, it has been reported that HS can be absorbed and transported to other tissues of the body. In young pigs, HS particles were observed in all segments of the small intestine and in lymph nodes associated with the intestine, and in urinary bladder and trachea [46]. In adult pigs, it was not possible to confirm these findings [47]. However, in older studies using 125I-HA, distribution of HA was observed in several tissues, notably the skin, blood serum, liver, muscle, and digestive tract of rats [48].

In several studies in which hepatotoxicity was induced using lipopolysaccharide (LPS), carbon tetrachloride, and ethanol in rats given HS orally by gavage, the protection against liver injury was confirmed, due to reduced serum levels of aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) [49–51]. The medical benefits of HS offered orally against different types of musculoskeletal and gynecological diseases as well as for the treatment of heart and liver conditions have been reviewed in other documents [2, 5]. In the following sections, the main results of the use of HS on the antioxidant status, immune response and inflammation, digestive microbiota, use of minerals, proteins, and energy, and the growth performance parameters in animals are reviewed. For information about the benefits of HS in aquaculture, it is recommended to review other scientific publications in this area [52–54].

3. Antioxidant status

The redox properties of HS have been investigated in in vitro and in vivo studies, with quinones being classified as reducible fractions and phenols being classified as electron-donating fractions with antioxidant properties in comparison with electron-accepting quinones, respectively [55, 56]. The neuroprotective, cardioprotective, and renoprotective properties of HS in rats have been already described [2]. In all cases, the total oxidative status and oxidative stress index levels were significantly decreased, and total antioxidant status was increased in the HS experimental groups. The hepatoprotective properties of HS have also been long recognized. In studies with rats in which hepatotoxicity was created using different challenge models, such as administration of LPS, carbon tetrachloride and ethanol, the oral feeding of HS elicited increased glutathione (GSH) and superoxide dismutase (SOD) activities and decreased a marker of lipid peroxidation, malondialdehyde activity (MDA) [49–51].

In broiler chickens supplemented with HS, a potent antioxidant activity such as increased glutathione reductase (GSH-Rx), total antioxidant capacity (T-AOC), and catalase activity in the blood has been reported [57]. In broilers supplemented with increasing dietary FA, increased SOD and glutathione peroxidase (GSH-Px) activity and decreased MDA levels in the blood were also found in a recent study [29]. On the contrary, in the liver of HS-added broilers, reductions of the SOD were found, whereas in the mitochondria of the muscle lower MAD, SOD and higher catalase were found [58]. It was suggested that the difference of SOD activity in serum versus the liver and mitochondria from muscles may be due to their ability to recombine with intermediate free radicals that may lead to partly inactivation of the enzyme [59]. In broilers supplemented with HS and subjected to transportation stress, increased...
SOD and GSH-Rx activities in the mitochondria of the liver were observed [60]. In breast meat and thighs of HS-added chicken, decreased lipid oxidation was reported, especially after the fourth day of storage after slaughter [61]. Similarly, an increase of MDA, lightness and yellowness were observed in breast meat after 7 days of storage [62]. In red blood cells of HS-added hens, reduced levels of MDA, GSH-Px, glutathione-S-transferase, γ-glutamyltransferase and oxidized glutathione and increased levels of GSH-Rx, reduced glutathione and the ratio of reduced glutathione/oxidized glutathione were found [58].

In HS-added weaned pigs, reduced T-AOC and MDA were observed [63]. In piglets born from HS-added sows 2 weeks before and 1 week after farrowing, reduced TBA-active products concentration, lipid hydroperoxides, and protein carbonyl groups and increased SOD and catalase activity were found; after re-feeding HS in the older piglets, reinforced positive impact on the antioxidant defense system and free-radical processes were reported [64]. In HS-added weaning pigs, a decrease in oxidative stress was also reported after a challenge with an *Escherichia* (*E.*) *coli* LPS [65]. In HS-added calves, higher serum GSH-Px and T-AOC activities and lower diamine oxidase (DAO) and MDA concentrations were observed [44]. Later on, higher total SOD and T-AOC activities and lower MDA concentrations were confirmed in HS-added calves [45].

It has been suggested that decreased activity of SOD was connected with the antioxidant properties of HS that decreased the concentration of oxidized products, including superoxide anion [58]. Another possible way to activate the enzymatic activity of the antioxidant system is to increase the concentration of metals in the liver, which are part of the active centers of metal proteins: Zn—catalase, Mn (Cu/Zn)—superoxide dismutase [59]. The lower fat content in the meat of HS-added broilers and pigs may elicit a higher proportion of antioxidant components and could have an effect on the higher oxidative stability [62].

### 4. Immune response and inflammation

HSs have been shown to exhibit different immunostimulatory and immunomodulatory effects in patients with different infectious diseases, which have been associated with their anti-inflammatory properties [2, 5, 6]. It seems that HSs form solid complexes with carbohydrates, which allow the formation of glycoproteins with the ability to bind to natural killer cells and T lymphocytes and allow subsequent communication between these cells [66].

HS has been shown to stimulate the immune response in broilers [8, 67, 68]. For example, elevated lymphoid tissue distribution and density in the bursa of Fabricius and thyme [25] increased concentration of antibodies against infectious bursal disease [69], avian influenza [70], and Newcastle disease virus (NDV) [71, 72], and greater lymphocytes and leukocytes counts, globulins (α, β, and γ), phagocytosis, and phagocytic index have been found in HS-added broilers [73]. In laying hens, supplementation HS significantly increased the serum IgG and IgM level [74].

In weaned and growing pigs supplemented with HS, increased percentage of lymphocytes and activity of neutrophils has been reported [41, 75]. In HS-added weaned pigs, increased level of IgM and IgG and reduced concentration of inflammatory factors such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and IL-1, myeloperoxidase, and DAO were observed [63]. In HS-supplemented calves, higher IgA and IgG concentrations, lower TNF-α [43, 44] and higher serum IgA, IgG, and IL-4 concentrations, and lower IL-6 and TNF-α were observed [45].
The enhanced immune response in HS-added animals may be the cause of the reduced severity of diarrhea reported in pigs [40–42] and calves [43–45] and to the strong tendency of lower mortality reported in broilers [25, 67, 76, 77], Japanese quails [27], and pigs [41, 78].

5. Digestive microbiota

Reports from the microbial, geo, soil, and environmental sciences indicate that HS stimulates the growth and diversity of soil and environmental fungi and bacterial communities [79, 80]. A review of the main ways of action of HS on biota was previously reported [81]. The most outstanding mechanisms of HS on the activity of microorganisms are a) as a source of substrates, providing carbon, nitrogen, phosphorus, trace elements, and vitamins [82], and b) as natural surfactants, increasing the permeability of cell membranes in bacteria, due to their amphiphilic character, which enhances the absorption of nitrogen and other micronutrients [83]. Examples of some aerobic HS-degrading bacteria are *Pseudomonas* spp., *Streptomyces* spp., *Bacillus (B.) brevis*, *B. cereus* and *Alcaligenes faecalis*, among others [81, 84]. In one report, the anaerobic decomposition of HS by *Clostridium (C.)* spp. was also reported [79]. The recovered HS from the bacterial cultures show some modifications such as losses of aliphatic structural units and gains of aromatic structures under aerobic conditions [85], while under anaerobic conditions removal of carboxyl groups and polysaccharide-related substances and gains of aliphatic components, amide and aromatic groups have been observed [79]. Furthermore, it has been suggested that differences of the microbial degradability of HS are associated with differences of their chemical composition and the bacterial species.

All these factors are probably associated with the contrasting microbiological results reported under *in vitro* experiments in which different types of bacteria are cultured with HS or in *in vivo* experiments in which the feed or water of broiler chickens are added with HS. In an *in vitro* study, in which natural and synthetic HS were tested, the spectrum and degree of antimicrobial activity against many human pathogenic bacteria varied according to the origin, extraction mode of the HS, and the tested bacterial strain [86]. In another *in vitro* report, natural HS showed insignificant inhibition of the growth of bacteria such as *E. coli* and *Salmonella (S.) Enteritidis*, but the modified HS caused reductions from 78 to 80% and 58 to 70% of the number of colonies of *E. coli* and *S. Enteritidis*, respectively [87].

In HS-added broiler, no significant effects of HS have been reported on the total Gram-negative bacteria [15] or the anaerobic bacterial populations in the ceca [88, 89], while reductions of enterobacteria in the small intestine and cecum were found in one study [18]. Lower *E. coli* counts in the digesta content from the small intestine and ceca [71] and higher *E. coli* counts between 10 and 100 times) in the ceca content [88] have been also reported. Furthermore, the addition of HS in broilers did not reduce the counts of *Clostridium perfringens*, *S. Enteritidis*, and *E. coli* in the gut [15, 90–92]. Opposite to this, in Japanese HS-added quails, reductions in *E. coli*, coliforms, and *C. perfringens* of the intestinal content were found [27].

Some differences observed on the antimicrobial effects of HS in *in vitro* studies may be due to the use of different sources and dosages of the tested products, the length of the incubation period, and hence, the duration of the bacterial exposure to the products, the temperature and pH conditions, and the nutritional composition of the culture broth. In contrast to this, the total passage time of feed through the
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The gastrointestinal tract in chickens lasts between 3 and 4 h [93], and only during this short period of time, the bacteria is exposed to HS; the core temperature of chickens is about 40°C and the pH varies in the range 4.5–5.9, 2.0–3.0, and 5.5–7.0 in the crop, proventriculus, and intestine, respectively [94]. The microbiota that resides in the digestive tract feeds on the nutrients released from the feed as digestion proceeds and on the endogenous secretions associated with the different compartments. In in vitro experiments, a cytotoxic effect of HS against many mammalian and bacterial cells has been demonstrated due to the accumulation of free radicals during long-term culture times, inducing lower oxygen uptake, lower electron transfer to acceptors, and lipid peroxidation in cell membranes [95]. It is probably that in some long-term in vitro experiments, the antimicrobial activity of HS was due to the accumulation of toxic metabolites in the culture. This effect may be precluded in the gastrointestinal tract.

In a recent study, the addition of HA extracted from worm compost in an in vitro chicken digestive system caused, in the simulated crop, increasing counts of E. coli, C. perfringens, Lactobacillus (L.) salivarius, and B. subtilis as the concentration of HA increased. In the simulated proventriculus, the counts of S. Enteritidis, E. coli, and B. subtilis were enhanced at the higher level of HA. In the simulated intestine, HA increased the counts of S. Enteritidis, E. coli, C. perfringens, and B. subtilis. The results suggest that HA can be used by bacteria as substrates, since they are organic sources of carbon, nitrogen, phosphorus, and other nutrients. HA can also improve nutrient assimilation, as probably occurred during the experiments as bacterial counts were increased. It is probably that in the simulated digestive system the rapid transit time through the digestive compartments, the addition of the buffering solutions and the presence of several dietary components in the intestine overcome the possible accumulation of toxic metabolites [96]. In two recent in vivo studies, increased lactic acid bacteria counts were reported in the gut of HS-added broilers [77, 91].

In weaning pigs supplemented with HS, lower relative abundance of Firmicutes, Bacteroides, Anaerovibrio, Oscillospira, and Ruminococcus and a trend toward higher abundance of Prevotella in feces were reported compared to control pigs [41]. Also in HS-added weaned pigs, reduced short-chain fatty acids (SCFAs), gas formation, and microbial alpha diversity and no changes in the microbiome of the cecal and colon content were found [97].

In weaned Holstein HS-added calves, increased ratio of Firmicutes to Bacteroidetes and relative abundance of Firmicutes and decreased relative abundance of Bacteroidetes were reported [43]. In addition, a correlation analysis indicated that Bifidobacterium, Lactobacillus, and Olsenella were positively correlated with IgG, weight gain, and GSH-Px and negatively correlated with MDA, DAO, and fecal score, respectively, which might explain that HS inclusion could improve growth performance, anti-inflammatory, and antioxidative status and alleviate diarrhea of weaned calves via increasing the abundance of intestinal beneficial microbiota, and that upregulated fatty acid metabolites were positively correlated with increased beneficial intestinal microbiota [43]. In two additional research, HS supplementation increased the abundances of Bifidobacterium and Lactobacillus but decreased the abundance of E. coli in feces of calves [44, 45]. In HS-added goats, for 14 days prepartum and 56 days postpartum, increased ruminal acetate and propionate concentrations but reduced protozoa counts were observed [34, 98]. In growing HS-added rabbits, the total bacteria and E. coli counts in cecum were decreased [28]. Also in HS-added rabbits, increased concentrations of propionic and butyric acids were found [33].
It was previously suggested that within the body, HSs stimulate the good microbes while suppressing the bad microbes, and that HSs stabilize the intestinal flora thus ensuring an improved utilization of nutrients in animal [67]. Recent results in broiler chickens, pigs, calves, goat and rabbits seem to confirm this suggestion. Several of the aforementioned authors agree in that HSs cause a shift in the digestive microbiota by stimulating the growth of probiotic-type bacteria and modifying the microbial fermentation in nonruminant and ruminant animals, leading to the formation of a greater amount of SCFA, which reduces the pH of the medium and probably inhibits the growth of potentially pathogenic bacteria. In addition, some of the SCFAs found have a trophic effect on the digestive mucosa. The increased beneficial flora, reduced potentially pathogenic bacteria, and stimulated growth of intestinal villi have been associated with better intestinal health and improvements in the processes of digestion, including increased activity of different digestive enzymes. This provides excellent nutritional feed conversion efficiency which aids in body weight gain without increasing the amount of feed consumed.

5.1 Antiparasitic effects

In the area of aquaculture, experiments with goldfish (Carassius auratus) and common carp (Cyprinus carpio) under pond culture conditions, a HS bath caused reduction of infections of skin and gills by protozoan parasites and dropping of infections of the gills caused by Ichthyophthirius spp. [52]. In other studies, similar findings in HS exposed fish, ornamental fishes, and Nile tilapia were reported [53, 99]. In experimentally challenged mice with Trypanosoma (T.) brucei brucei and T. brucei gambiense, the administration of HS for 21 days in the drinking water induced adequate protection and significantly reduced the mortality rate, while all non-treated control mice died within 10 days after the challenge [100]. In HS-added goats and dairy cows, reduced ruminal protozoa counts were observed [34, 98]. Using liquid or solid extracts of HS from worm compost, the count of coccidia eggs in the excreta of broilers was reduced, but the results were generally inconsistent [91, 101]. More information is required on this topic.

5.2 Antifungal effects

It has been reported that HSs have a fungicidal effect on Candida (C.) albicans under in vitro conditions [86] and over that inhabiting the gastrointestinal tract of animals and humans [102]. HS was recently found to decrease the toxic effects of aflatoxicosis in broilers [103, 104]. Results of in vitro binding studies showed that HS has a high mycotoxin adsorption capacity; in HS-added broilers and fed aflatoxin-contaminated feeds, a protective effects against liver damage, stomach, and heart enlargement as well as reduction of some of the hematological and serum biochemical changes associated with aflatoxin toxicity were shown [103]. In HS-added broilers, provided with aflatoxin-contaminated feeds, improved feed efficiency and reduced liver and bursa damage as well as reduction of serum AST, ã-glutamyl transferase, and lactate dehydrogenase were observed [104, 105]. Reduced aflatoxin B1 residues in liver and increased NDV antibody titers in 28- and 35-day-old birds [105, 106] were also reported in HS-added broilers. In HS-added weaned pigs, improved weight gain and increased elimination of zearalenone in fecal samples (from 64, 77, and 92%) in a dose-dependent manner as well as recovery of the secretion of β-estradiol were observed [107].
The protective effect of HS against mycotoxicoses seems to involve the sequestration of aflatoxins in the gastrointestinal tract, reducing their bioavailability and increasing their excretion through feces, which reduces damage to the liver and other vital organs, thus promoting better immune response and greater growth in animals that consume feed contaminated with mycotoxins.

6. Metabolism of minerals, proteins, and lipids

6.1 Minerals

HSs are considered as the natural ligands with the highest complexation capacity, giving them a strong potential to form chelates with various ions, which have been linked to better mineral utilization in plants and animals [108]. Increases in ash and Ca content in tibia bone have been reported in broilers fed HS [25, 109, 110]. In the same way, supplementation with liquid extracts of HS from worm compost caused an increase in ash retention in growing chickens [31] and increments in the tibia ashes, Ca and P percentage in 21-day-old broilers as well as in tibia dry matter percentage, and Ca and P content in 42-day-old broilers [111].

The plasma concentrations of Cu, Mn, and Cu and the liver concentration of Cu, Fe, and Se were higher in under normal rearing conditions in HS-added broilers [112]; however after the transportation to the slaughter house, the plasma concentrations of Zn and Mn were decreased, while the Fe and Se were increased as well as the liver concentrations of Zn Cu, Mn, and Se [112]. In HS-added broilers, increases in Ca, P, Fe, and Cu concentrations in the meat [113–115] and increases in Ca, Mg, Zn, and Fe in the thigh and breast have been reported [116]. These results also agree with the increased percentage, thickness, and hardness of eggshells reported in HS-supplemented laying hens and pheasants [117–120]. In HS-fed weaned pigs increased serum phosphatase alkaline, Ca, P, and Mg [121]. In the milk of HS-added cows, increased Ca and Fe content [122] and serum Ca levels [123] were found.

In HS-added rabbits, an early increased content of Fe and Ca and late increased activity of ALP and Cu content in serum were observed; additionally, in HS-added rabbits, increased number of layers of osteons and osteoblasts in the bone tissue of the femur, number of osteons and osteoblasts in the bone tissue of the sternum, number of columns of chondrocytes, and number of chondrocytes in the column in the cartilaginous tissue of the sternum were found [124].

6.2 Proteins

It has been suggested that protein utilization efficiency is improved in nonruminant and ruminant animals supplemented with HS. In HS-added broiler chickens, increased total body mass [125, 126] and serum and tissue protein contents [127] were previously reported. These results agree with the higher protein efficiency ratio [128], breast and thigh meat and total protein content [62, 102, 129], and higher carcass and breast yield [35, 91, 102] recently found in HS-added broilers. The addition of HS has also caused increases in protein retention and digestibility in broiler chickens [31, 32] and protein digestibility rabbits [28, 33] and milking cows [34]. In laying hens, HS linearly increased serum total protein, albumin, and globulin [130]. In Holstein-Friesian HS-added cows, the protein and casein contents in milk were increased [122].
In HS-added rabbits, a significant decrease in the concentrations of urea and increase in total blood protein, albumen, and globulin levels have been observed [33, 131].

6.3 Ammonia emissions

Nitrogenous waste, ammonia emissions, and bad odors are reduced in animals supplemented with HS due to improved feed efficiency, digestibility, and nitrogen retention [15, 78, 132]. In HS-added broilers and pigs, reductions of aerial and feces ammonia were observed [15, 78, 133]. It was suggested that the reduction of aerial ammonia in pigs supplemented with HS could be attributed to the inhibition of the urease activity in manure [78]; it is possible that the reduction of the urease activity inside the intestine may have contributed to the greater nitrogen retention and digestibility in HS-added animals. In aquaculture systems, the addition of HS improved the water quality by decreasing the total ammonia nitrogen and nitrate-nitrogen concentration and increased the nitrogen utilization efficiency by changing the microbial communities and strengthening nitrification [54].

Using a rumen stimulation technique and in HS-added goat and dairy cows, linear reductions in ruminal ammonia concentration have been reported [34, 98, 134]. In addition, in HS-added rabbits, the concentration of ceca ammonia decreased sharply in a dose response manner [33]. The lower ruminal or ceca ammonia concentrations may be linked with the effectiveness of HS to reduce ammonia accumulation and also to their strong nitrogen-binding properties of HS. In this way, in ruminant animals, HS may enhance rumen crude protein utilization by decreasing ammonia loss owing to reduced solubility under the inhibitory effects of HS on urease activity [78]. This might cause a shift in nitrogen excretion from urine to feces due to reduced solubility. The ability of HS to alter ruminal fermentability may be linked to ammonia sequestration and then slow release for microbial growth; additionally, reducing protozoa number may increase microbial crude protein flow to the small intestine [34, 98].

6.4 Lipids

In humans, HA and FA preparations are promoted as supplements to increase energy and to reduce fatigue. In a rodent model, a product containing 60–80% HS reversed the negative behavioral symptoms of depression and anxiety caused by chronic fatigue syndrome and also stabilized the HPA axis stress response by reversing the drop in corticosterone levels and adrenal gland weight; it was suggested that the effects of HS were partially due to the regulation of mitochondrial bioenergetics by increasing the activities of mitochondrial NADH dehydrogenase, succinate dehydrogenase, cytochrome oxidase, and ATP synthase.

In the majority of the publish research, it is evident that HS modulates the use of energy toward reductions of the lipid content in chicken and pig meat but increases the milk fat content in cows and goat. In HS–added broilers, reductions of the total lipid content of liver [125] fat content in the breast meat [62, 114, 129] and blood cholesterol levels [68, 110, 135–137] have been reported. In HS-added broilers and laying hens, HS linearly decreased serum triglycerides and very low-density lipoproteins concentrations [68, 130] and also reduced the cholesterol content in the eggs yolk [138]. In HS-supplemented pigs, reduced backfat has been observed [75, 78, 139]. The findings in pigs seem to be associated with increased activity of the hormone-sensible lipase and reduced activity of lipoprotein lipase in adipose tissue, as has been shown in pigs added with increasing dietary FA [140].
Opposite to this, in dairy cows, increased fat milk content has been found [122, 123, 141, 142] but decreased serum nonesterified fatty acids and blood beta-hydroxybutyric acid levels [123] and serum cholesterol [34] have been seen. In HS-added goats, significantly reduced serum cholesterol concentrations [98, 143] have been reported. Also in HS-added rabbits, reduced total and low-density lipoproteins cholesterol and triglycerides [28, 33, 144, 145] but increased high density lipoproteins [28] have been observed.

7. Productive response in animals supplemented with humic substances

7.1 Poultry

In two recent published articles, more descriptive effects of HS in poultry have been given [68, 96]. In several experiments, enhanced final body weight and weight gain and reduced feed conversion ratio have been reported in HS-added broilers using different commercial sources of HS [24, 68, 113, 114]. In agreement with these results, using a liquid or dry extract of HS from worm compost, higher final body weight and weight gain and lower feed conversion ratio have been reported in broiler chickens [19, 31, 77, 146].

Greater carcass weight and yield have been also observed in HS-added broilers [35, 113, 114, 128], including those supplemented with worm compost-derived HS [91, 146]. In breast and thigh meat, lower water loss and increased lightness and yellowness were observed [62, 129].

In laying hens and partridges supplemented with HS, benefits in feed intake, egg production, egg weight, and feed conversion ratio have been observed [118–120, 130, 147]. Improved Haugh unit values and egg yolk color in HS-added laying hens [74] and increased albumen height, albumen index, and Haugh units of the egg in partridge [148] were observed. A strong tendency of lower mortality have been reported in HS-added broilers [25, 67, 77] and Japanese quails [27].

7.2 Pigs

In HS-added weaned pigs, improved body weight, weight gain, feed intake, and feed conversion ratio have been reported [41, 42, 63, 121, 149, 150]. In HS-added growing pigs, higher weight gains and feed efficiency have also been reported [75, 78, 139]. Reduced severity of diarrhea [40–42] and lower mortality [41, 78] were reported in HS-added pigs.

7.3 Ruminants

In HS-added calves, higher body weight and weight gain [43–45, 142] while in HS-added dairy cows, increased milk production, milk fat, and milk protein were found [122, 123, 141], as well as in dairy goats [145]. Reduced severity of diarrhea has been reported in HS-added calf [43–45].

7.4 Rabbits

In HS-added rabbits, enhanced body weight, weight gain, and carcass weight but reduced feed conversion ratio have been found [28, 33, 144].
8. Conclusions

The present review reinforces the promoted protective action of HS on the digestive mucosa, their antioxidant properties, immunomodulatory, and anti-inflammatory attributes, the suggested microbial shift to a probiotic-type bacteria in the gut, including antiparasitic and antifungal effects, as well as their influence on the higher efficient of minerals, proteins, and lipids utilization within the body. The sum of all of these effects result in improved health, lower diarrhea and mortality rates, and increased growth rate, feed conversion, meat and milk production, as well as improved animal product quality.

The information reviewed does not allow to clearly define the main mechanism(s) of action of HS due to the plethoric benefits found. It is likely that, in order of importance, the main benefits of HS derive from their ability to form protective barriers on the digestive mucosa, blocking the passage of pathogenic bacteria and bacterial toxins or metabolites that could otherwise damage or invade the mucosal cells of the digestive tract. This also includes other compounds such as mycotoxins, anti-nutritional factors, and other contaminants that could enter the intestine through the diet.

The other no less important benefit is the ability of HS to stimulate the establishment of a beneficial microflora, and a pattern of microbial fermentation that promotes intestinal health, which could reduce the development of potentially pathogenic bacteria, creating a hostile environment, for example, through the production of SCFA, such as lactic, butyric, and propionic acid, which may acidify the intestinal content.

The rest of the benefits of HS within the body, such as enhanced antioxidant and immune status, increased efficiency of mineral, protein, and lipid utilization and improved health and growth and are likely to be dependent on the proposed effects of the digestive tract. However, since the effects on the gut may vary depending on the factors associated with the animal (species, age, and physiological and health status) to the tested HS (origin, age, concentration of functional groups, and composition and length of the side chains) and the experimental design and treatments (dosage, route and form of administration, length of supplementation, type of facilities, and sanitary conditions), the additional effects within the body may significantly differ.

The current research also demonstrates that numerous theories have been presented to account for the infinite number of effects of HS reviewed in this literature survey. With the existing evidence, it is not possible to reach consensus conclusions. The truly molecular mechanisms of action of HS in the intestine and throughout the body remain unknown. However, outstanding improvements in health, productivity, and meat and milk quality obtained from animals supplemented with HS are common features in the majority of available research.

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Conflict of interest

The authors declare no conflict of interest.

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