



THE INFLUENCE OF SUPPLEMENTING BROILER CHICKENS WITH HUMIC ACID OR BIOCHAR AS NATURAL GROWTH PROMOTERS ON THEIR PRODUCTIVE PERFORMANCE, NUTRIENT DIGESTIBILITY, AND PHYSIOLOGICAL PERFORMANCE

M. I. El-Kelawy¹ Asmaa Sh. ELnaggar² and Enass Abd El-khalek³

¹Dep. of Poult. Prod.Fac. of Agric., New Valley Uni., Kharga, Egypt.

²Dep. of Anim. and Poult. Prod., Fac. of Agric., Damanhour Uni., Damanhour, Egypt.

³Dep. of Poult. Prod.n, Fac. of Agric.e (El-Shatby), Alexandria Uni., Alexandria, Egypt.

Corresponding author: M. I. El-Kelawy Email: m.elkelawy@gmail.com

Received: 03/03/2023

Accepted: 30 /03/2024

ABSTRACT: Study aimed to assess effects of humic acid/biochar on broiler production index, lipid profile, antioxidants, carcass traits, and economic efficiency. A total of 180, 7-day-old, unsexed broiler chicks (Cobb 500) were divided into five experimental groups (36 chicks each), each with six replicates (6 chicks each). The first group was fed the basal diet and served as control; while the 2nd and 3rd groups were fed the same basal diet supplemented with 1.00 and 2.00 g of humic acid (HA) / kg diet, respectively; the 4th and 5th groups were fed the basal diet supplemented with 1.00 and 2.00% of biochar (BC), respectively. The rearing period extended to 35 days. Birds supplemented with HA and BC at varying levels showed significantly higher body weight (BW) and gain (BWG) compared to the control. The group with 1.00 g HA/ kg diet and 2% BC had the best BW and BWG. These supplements also improved feed conversion ratio (FCR) and economic efficiency, with 1 g HA/ kg diet and 2% BC showing the highest production index. Adding HA and BC to the diet improved crude protein (CP) and ether extract (EE) digestibility ($P \leq 0.05$). Additionally, supplementation increased dressing percentage and reduced abdominal fat compared to the control. Dietary supplementation in broiler chickens increased blood parameters (RBCs, hemoglobin, PCV, WBCs and lymphocytes), total protein, globulins, and thyroid hormones. It also reduced serum lipids and oxidative stress markers, while enhancing antioxidant indices and immune response (IgA, IgM, IgG, LTT and phagocytic activity). Moreover, it decreased harmful bacterial count and boosted beneficial bacteria count. In conclusion, supplementation of broilers with HA and BC improved growth performance, nutrient digestibility, immune response, and gut health.

Key words: Broiler, humic acid, biochar, performance, blood biochemical.

INTRODUCTION

To address the challenge of sustaining the burgeoning global population, projected to reach 10 billion by 2050 (FAO, 2019), it is imperative to produce ample nutritious food through sustainable agricultural practices. Within the poultry feed sector, the use of antibiotic growth promoters is widespread for controlling poultry pathogens, reducing mortality rates, and improving meat and egg production efficiency. However, this practice has contributed to the emergence of drug-resistant bacteria (Haque *et al.*, 2020). While antibiotics in animal feed have shown significant benefits such as improved FCR and BWG, potentially increasing gains by up to 4% (Cowieson and Klueener, 2019), the negative repercussions on beneficial intestinal microflora and the escalation of antibiotic resistance have led to the prohibition of antibiotic usage in animal feed across many nations (Andremont, 2000). Consequently, there is a growing preference for natural growth promoters in poultry feed formulations to ensure human health and safety (Andrew *et al.*, 2020). This has spurred a search for natural components that can provide healthful alternatives, maintaining growth and FCR in farm animals without adverse effects (Abudabos *et al.*, 2018; Scicutella *et al.*, 2021).

Humic substances originate from plant decomposition and are naturally present in soil, peat, water, and brown coal. These substances, comprising humin, humic, and fulvic acids, boast a complex structure. Traditionally, they've been utilized to enhance plant growth (van Rensburg, 2015). Additionally, humic acids, derived from decomposed organic matter in soil and lignite (MacCarthy, 2001), are being explored as potential alternatives to antibiotics in poultry diets (Nagaraju *et al.*, 2014).

Humic substances exhibit promising potential in bolstering gastrointestinal health, leading to improved body weight,

feed conversion, and immune response (Taklimi *et al.*, 2012; Ozturk *et al.*, 2009). Notably, humic acids have been integrated into poultry feed and water to stimulate growth (Arif *et al.*, 2016). Research by Arif *et al.* (2016) demonstrated significant enhancements in broiler BWG and FCR with HA supplementation. Moreover, studies by Nagaraju *et al.* (2014) revealed that incorporating HA into broiler diets enhances meat quality, BWG, and immune function. Ozturk *et al.* (2010) further reported improvements in growth, carcass characteristics, blood parameters, and gastrointestinal health with HA supplementation.

Previous research has highlighted the diverse benefits of humic acids, including inhibiting bacterial growth, boosting the immune system, exhibiting antiviral properties, preventing and treating intestinal disorders, enhancing feed nutritive value and trace element utilization, thereby positively impacting growth performance and reducing mortality rates (Mudroňová *et al.*, 2020). Furthermore, humic acids contain trace elements that serve as co-factors for various enzymes, enhancing nutrient digestion and utilization (Hayirli *et al.*, 2005).

Biochar is a carbon rich material produced from the pyrolysis of biomasses under limited oxygen condition. The high surface area and porous structure candidates it as potential sorbent for many pollutants (Abdelhafez *et al.*, 2020). Biochar's potential as a feed additive in animal production remains underexplored. However, studies suggest its inclusion in poultry nutrition can reduce diarrhea incidence, alleviate allergies, counteract mycotoxin effects, and control zoonotic pathogens (Marie, 2013; Yang *et al.*, 2015). Biochar enhances digestibility, FCR and BWG in poultry (Gerlach and Schmidt, 2012). Moreover, it modulates microbial community composition and activity, serving as an electron mediator (Sun *et al.*, 2017), and influencing microbial composition (Terry *et al.*, 2019). Studies

Broiler, humic acid, biochar, performance, blood biochemical.

indicate biochar's positive impact on feed digestibility (Kim *et al.*, 2017).

The objective of this research was to investigate the impact of various concentrations of humic acid or biochar supplementation, acting as natural growth promoters, on the production index, lipid profile, and economic efficiency of broiler chicks.

MATERIALS AND METHODS

The study was conducted at the Poultry Research unit, Damanhour University, Damanhour, Egypt during year 2023.

Birds, treatments, and experimental design:

One hundred and eighty unsexed 7-day-old Cobb-500 broiler chicks, were randomly allocated into five groups. Each group comprised 6 replicates with 6 chicks per replicate. The chicks were raised under identical management conditions and were provided with the same basal diet throughout the study. The dietary treatments administered were as follows: the first group served as the control and received the basal diet without any additional supplementation. The second and third groups received the basal diet supplemented with 1.00 and 2.00 g of humic acid per Kg of diet, respectively. The fourth and fifth groups were given the basal diet supplemented with 1.00 and 2.00% of biochar per Kg of diet, respectively. The experimental diets were formulated in accordance with the breed management guidelines outlined in Table 1.

Housing and husbandry:

The chicks were housed in battery brooders within a semi-opened house that featured two exhaust fans for ventilation. They were provided with *ad-libitum* feeding and continuous access to water throughout the entire experimental duration. A light regimen resembling commercial settings was implemented, consisting of 23 hours of light from days 1 to 7, followed by 20 hours of light from days 8 to 35. The indoor brooding temperature gradually decreased from 32, 30, 27 to 24°C across different age stages (1-7, 8-14, 15-20, and 21-35 days).

Performance traits:

Individual live body weight (LBW), body weight gain (BWG), feed consumption (FC), and feed conversion ratio (FCR) were assessed weekly and computed over the entire experimental duration (7–35 days of age) for each experimental group.

Blood collection and hemato biochemical analyses:

At the conclusion of the experimental period (35 days of age), six fasted birds from each treatment group (one bird per replicate) were randomly chosen for slaughter. Blood samples of approximately 3 ml were drawn from the wing vein before slaughter for hematobiochemical analysis. Heparin was utilized as an anticoagulant; however, a portion of the samples was maintained without heparin to acquire serum. Non-coagulated blood was divided into two portions. The first portion was promptly utilized for determining the complete blood count, while the second portion was used to obtain plasma after separation. Plasma or serum was separated by centrifuging the blood at 3,000 rpm for 20 minutes and then stored at –20°C for subsequent analysis. The red blood cell count (RBC, $10^6/\text{ml}$), white blood cell count (WBC, $10^3/\text{ml}$), and the differential count were analyzed following the methods outlined by Feldman *et al.* (2000). Hemoglobin (Hb) concentration was determined as per the guidelines of Provan *et al.* (2004). The packed cell volume percentage (PCV %) was measured according to the protocols described by Drew *et al.* (2004). Additionally, serum parameters such as total protein, albumin, total lipids, triglycerides, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), glucose, total triiodothyronine (T_3), calcium, and inorganic phosphorus concentrations were quantified. Moreover, aspartate transaminase (AST) and alanine transaminase (ALT) levels, creatinine levels, activities of malondialdehyde (MDA) and total antioxidant capacity (TAC), alkaline phosphatase (ALP) concentration, and

immunoglobulin (Ig) fractions were determined using specific kits obtained from reputable sources such as sentinel CH Milano, Italy; CAL-TECH Diagnostics, Inc., Chino, CA, USA; and Reactivos GPL, Barcelona, Spain.

These analyses were performed using a spectrophotometer, including models like Beckman DU-530 (Hanau, Germany) and equipment from Diagnostic Products Corporation (Los Angeles, USA), in accordance with the recommendations provided by the respective kit manufacturers.

Slaughter traits:

At the conclusion of the experimental period (35 days of age), the six selected birds underwent an overnight fasting period before slaughter. Each bird was individually weighed to determine its pre-slaughter weight. Subsequently, after scalding, feather picking, and evisceration, the carcass along with the organs (liver, gizzard, heart, spleen, bursa and thymus gland) and abdominal fat were weighed separately. The percentage of empty carcass and organs was then calculated based on the pre-slaughter weight of each bird.

Bacterial count:

During slaughtering procedures, six birds from each treatment were selected for the collection of cecal content samples. Then, samples were analyzed for total bacterial count (TBC), *Escherichia coli* and *Lactobacillus* as colony-forming unit (CFU) using modified methods described by Baurhoo *et al.* (2007), which differed only in agars used.

Apparent digestibility of nutrients:

At the end of the trial period, six birds from each treatment group were individually housed in clean metabolic cages to evaluate the digestion coefficient of nutrients. Each cage was equipped with a clean tray placed underneath for easy collection of excreta. Over a span of 4 days, the quantities of feed intake and fecal matter were measured for each experimental group. For nitrogen fixation analysis, excreta were treated with

Boric acid (4%) before drying. Samples of both feed and dried excreta were analyzed following the protocols outlined by the Association of Official Analytical Chemists (AOAC, 2004). The nitrogen-free extract of feed and dried excreta was calculated as per the method described by Abou-Raya and Galal (1971), while fecal nitrogen was determined according to Jackobsen (1960). These analyses provide valuable insights into nutrient utilization and digestive efficiency in broiler chickens under different dietary treatments.

Digestibility was then calculated as:

$$\text{Nutrient Digestibility (\%)} = \frac{(\text{Nutrient in Feed} - \text{Nutrient in Excreta})}{\text{Nutrient in Feed}} \times 100$$

The economic evaluation for all experimental treatments was made (Zeweil, 1996) as below:

Economic efficiency (EEF)

$$= \frac{\text{Total revenue} - \text{Total cost}}{\text{Total cost}} \times 100$$

Where:

Total revenue = BW × Meat Price

Total cost = Feed cost + Addition cost + other cost

Relative economic efficiency (REEF);

Assuming the REEF of the control = 100

Statistical analysis:

Data were subjected to the one-way ANOVA procedure using a statistical analysis system (SAS, 2006) with the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} = is the dependent variable; μ = the general mean; T_i = the fixed effect of treatment and e_{ij} = random error. The difference among means was determined using Duncan's new multiple range test (Duncan, 1955) at $P < 0.05$.

RESULTS AND DISCUSSION

Productive performance:

The productive performance of broiler fed diet with HA and BC during days 7-35 d of age are shown in Table 2. Broiler fed diet with either HA and BC at different levels had significantly ($p \leq 0.05$) greater BW and BWG than the control group. Groups fed 1.00 g HA and 2% BC had significantly

Broiler, humic acid, biochar, performance, blood biochemical.

($p \leq 0.05$) higher BW and BWG as compared to the other groups. Broiler fed diet with HA and BC at different levels recorded lower FC and better FCR as compared to the control group. Furthermore, broiler fed diet with 1.00 g HA and 2% BC had significantly lower FC and better FCR than other groups. Furthermore, broilers fed diet with 1 g HA and 2% BC recorded the best EEF, REEF (%) and production index compared to the other groups.

The recent findings indicate significant performance enhancements across all treatments compared to the control group, possibly attributed to the antimicrobial properties of biochar, which inhibit bacterial growth and enhance intestinal flora function. This leads to improved digestion, energy utilization, and ultimately, better growth. These outcomes align with Bakr (2008) findings, where a 2% biochar inclusion resulted in higher chick feed intake, BWG, and FCR. Similar benefits were observed in broiler chicks with up to 1.0% inclusion of maize cob biochar (Kana *et al.*, 2011), as well as in studies utilizing hardwood biochar (Majewska and Zaborowski, 2003 and Majewska *et al.*, 2011). El-Ghalid *et al.* (2022) who found that treatment groups that added BC levels of 1, 2, 4, and 6 % outperformed the control group in terms of productivity, EEF, and production index. The mechanisms proposed for these benefits include biochar's detoxification potential in feed, reduction of surface tension in the digestive tract, and binding of anti-nutritional factors (Kutlu, 1998).

Consistent with previous research (Kutlu *et al.*, 2001; Kana *et al.*, 2011), our study confirms improved broiler performance with dietary BC inclusion levels of 0.2 - 0.6%. Prasai *et al.* (2016) suggested that BC may enhance FCR by altering the bird's gut microbiota. Additionally, Monica (2019) demonstrated enhanced growth rates in broiler chicks with low concentrations of BC in feed during the initial three weeks of the experiment.

As a feed supplement, biochar shows promise in improving BWG, and FCR in broiler and ducks (Kana *et al.*, 2014; Louis *et al.*, 2018). The notably superior growth performance observed in birds fed 4 and 6% dietary BC kg^{-1} compared to the control suggests that BC inclusion enhances overall performance. This improvement could be attributed to biochar's adsorbent properties in the gut, which mitigate toxins and anti-nutritional factors, ensuring efficient nutrient absorption. These findings contrast with studies reporting growth rate depression and lower final body weights in broiler chickens with BC inclusion levels of 2% and higher (Jiya *et al.*, 2013).

The current study's findings align with those of Ozturk *et al.* (2014), who observed a positive impact on broiler growth performance with daily HA supplementation. Additionally, Arif *et al.* (2016) noted that incorporating HA into chick diets improved both starter and finisher BWG as well as feed efficiency. Similarly, Salah *et al.* (2015) reported enhanced BWG and FCR in broilers supplemented with humates. Moreover, our results coincide with several studies indicating that adding HA to broiler diets did not significantly affect feed consumption compared to control diets (Esenbuga *et al.*, 2008).

Our findings on economic efficiency were in line with the observations of Elnaggar and El-Kelawy (2018), who noted that Sasso chicks fed diet with HA exhibited significantly higher EEF compared to the control group. Similarly, El-Ghalid *et al.* (2022) demonstrated that incorporating BC into broiler diets enhanced EEF, REEF (%), and production index.

Humic acid may stabilize the gut microflora, enhancing BWG (Pistova *et al.*, 2016), which is consistent with the improved FCR observed in studies where HA were supplemented in broiler drinking water (Ozturk *et al.*, 2010) or feed (Ghazalah *et al.*, 2022). Additionally, Taklimi *et al.* (2012) proposed four potential modes of

action for humic substances: forming protective layers over the gut epithelial mucosal membrane, reducing digestive tract pH to suppress intestinal bacteria and increase nutrient availability, and enhancing immune receptors in the gut lining to defend against pathogens and promote growth. Moreover, the improved FCR could be attributed to the reduction in total bacterial count, *Salmonella*, *E. coli*, and *Proteus* due to HA supplementation, leading to better nutrient utilization and increased BW (Lala *et al.*, 2016)..

Apparent digestibility of nutrients:

The data presented in Table 3 illustrate the impact of HA and BC supplementation on nutrient digestibility and ash retention in broiler chickens. When added to the basal diet at varying levels, HA and BC enhanced the digestibility of CP and EE in comparison to the control diet. However, there were no notable effects observed on CF, DM digestibility, or apparent ash retention across the different levels of HA and BC supplementation.

The studies by Sheikh *et al.* (2010) have demonstrated that the inclusion of HA in broiler diets leads to enhancements in the apparent digestibility of nutrients. This improvement is attributed to several factors, including the elongation of mucosal villi in the jejunum and overall gut length, as observed in the research by Taklimi *et al.* (2012). These physiological changes are associated with a reduction in intestinal content passage rate and an increase in enzymatic digestion extension, resulting in improved nutrient digestibility. The mechanism behind these effects can be attributed to the stabilizing effect of HA on the intestinal microflora. By maintaining a balanced microbial environment, HA promotes enhanced nutrient absorption and utilization in animal feed, as outlined in the work by ELnaggar and El-Kelawy (2018). Moreover, the absorption capability of humic acid leads to a slower passage of gut contents, prolongs the digestion period, and enhances anabolic processes. This ultimately

results in an increase in the live weight of animals without requiring additional feed, as demonstrated in studies by Karaoglu *et al.* (2004), and Islam *et al.* (2005). Furthermore, the improved digestibility of CP can be attributed to the reduction of ammonia emissions in the environment due to humate applications, as reported by Herzig *et al.* (2001) in broiler studies. This indicates a multifaceted benefit of HA supplementation in animal diets, leading to both improved growth performance and environmental sustainability.

The available literature regarding the impact of BC on digestibility is limited. Mui and Ledin (2006) conducted a study on goats, evaluating various levels of BC (0, 0.5, 1.0, and 1.5% of dry matter) in diets composed of concentrate and forage. The results indicated significantly higher protein digestion and dry matter intake at 0.5% compared to 0% and 1.5%. The authors proposed that the reduced intake and protein digestibility at 1.5% might be attributed to impaired rumen activity. The reason for the positive performance of the treatment (1% BC) was an increase in the bacterial population, which led to the formation of several colonies called biofilm, which supported increased digestion of DM and OM causing the benefit of the diet. Studies have demonstrated that BC can enhance feedstuff digestibility. Kim *et al.* (2017) specifically showed a 4.9% increase in DM and a 3.9% increase in OM digestibility in pig feed with 0.25% organic medicinal charcoal compared to a basal diet without charcoal in an in-vitro study. Mui and Ledin (2006) also observed enhanced DM, OM, and CP digestibility in growing goats with bamboo charcoal supplementation in-vivo.

Slaughter traits:

Dietary supplementation increased percentage of dressing and decreased abdominal fat compared with the control. While summarizes the effects of HA or BC supplementation on the percentage of lymphoid organs that increased non-significantly (Table 4). These findings are

Broiler, humic acid, biochar, performance, blood biochemical.

consistent with those of El-Ghalid *et al.* (2022) who discovered that biochar treatments increased the carcass percentage compared to the control group. Furthermore, with respect to biochar, the overall percentage of abdominal fat in chicks was significantly reduced compared to the control group. Also, Kana *et al.* (2011) found that incorporating 0.2, 0.4, and 0.6% charcoal in bird diets did not significantly impact carcass yield, and abdominal fat. This aligns with Abdel-Fattah *et al.* (2008), who similarly observed that dietary organic acids did not affect carcass yield or live weight in broiler chickens. Similarly, Abdel-Mageed (2012) observed significant increases in dressing, breast, and thigh percentages, along with significant decreases in abdominal fat percentage in birds fed diets containing humic substances compared to those on control diets. Consistency is seen with Hanafy and El-Sheikh (2008) proposed a significant increase in spleen relative weight for hens fed a high level of HA (200 mg) compared to the control group, suggesting a potential improvement in immune function. Joone *et al.* (2003) also suggested immunostimulatory, anti-inflammatory, and antiviral effects of humic acid. Nevertheless, Islam *et al.* (2005) found no effect on spleen weights with dietary HA addition,

Hematological parameters:

Feeding diet with different supplements increased RBCs, hemoglobin, PCV, WBCs, and lymphocyte as compared to control group (Table 5). Increased red blood cell counts may be attributed to the effect of humic acid feed additives on trace elements such as iron, which interfere in red blood cell formation (Islam *et al.*, 2005). Humic acid has been found to stimulate the body's resistance forces and increase phagocytic activity (Terratol, 2002). The results of this study align with Ipek *et al.* (2008) who found that the addition of humic substances to the diets of Japanese quails increased Hb, RBC, and PCV. Similarly, Hanafy and El-Sheikh (2008) reported a significant increase in

RBCs, WBCs, and hemoglobin in laying hens fed high levels of humic acid. However, Rath *et al.* (2006) did not find any differences in RBC or PCV index between control animals and broilers given humic acids. Also, Arif *et al.* (2016) reported no significant influence of humate on RBCs, WBCs, and hemoglobin. These discrepancies might be attributed to differences in ages and strains of broilers (Elnaggar and El-Kelawy, 2018; Ghazalah *et al.*, 2022). On the other hand, Majewska *et al.* (2009) found no significant impact on turkeys' hematological indices with 0.3% charcoal supplementation. On the contrary, Boonanuntasarn *et al.* (2014) linked activated charcoal's (biochar) immune-enhancing properties to its role as a non-specific detoxifier, improving overall animal health. However, Dim *et al.* (2018) observed a notable decrease in RBC values in birds fed 2% biochar kg^{-1} diets compared to other treatments, while PCV and WBC values remained stable during the finisher phase. Hemoglobin concentration and red blood cell counts showed significant differences, aligning with trends observed in the starter phase.

Biochemical constituents of blood:

Protein profile:

The total serum protein levels of broilers fed diet with HA and BC from days 7 to 35 of age are presented in Table 6. Broilers fed diet with either HA or BC at various levels exhibited significantly increased concentrations of total protein, total globulin, and γ -globulin compared to the control group. Specifically, supplementation with 2 grams of HA resulted in the highest levels of γ -globulin in chicks. However, the impact of different supplementation levels on serum albumin, α -globulin, and β -globulin in chicks was not significant. The blood protein profile is known to be influenced by factors such as fodder quality, alimentary tract efficiency, and the condition of the liver and kidneys (Kłyszczko-Stefanowicz, 2005). These findings align with those of Hanafy and El-Shikh (2008), who observed a significant increase in total

protein levels in blood with the addition of HA to the laying hen diet at a dosage of 200 mg/kg. However, Rzasa *et al.* (2014) reported significantly lower serum total protein levels with the addition of 5 or 10 % humic-fatty acid to the diets of NZW rabbits. Additionally, Rath *et al.* (2006) indicated a trend toward decreased protein and albumin levels in serum with high concentrations of HA.

profile:

Additionally, all dietary supplements led to a reduction in serum total lipids, cholesterol (Chol.), LDL and VLDL compared to the control group. Moreover, broilers fed diet with 1 gram of HA and 2% BC exhibited significantly lower levels of total lipids and Chol. compared to the other groups. Furthermore, all supplementation resulted in an increase in HDL levels compared to the control group, as indicated in Table 7. These results are coincident with the results of Neuvonen *et al.* (1989) who observed a significant decrease in Chol. levels in birds fed the highest BC inclusion levels (6%) compared to other treatments. This suggests that BC intake can interfere with Chol. circulation, lowering serum Chol. levels in hypercholesterolemic conditions. Similar findings were seen by Boonanuntanasarn *et al.* (2014) in Nile Tilapia fed activated charcoal diets, with Chol. values decreasing as activated charcoal levels increased. Dim *et al.* (2018) found no significant differences in HDL and TG levels across treatment means during the starter phase, but significant differences existed in Chol. and LDL values. Alena and Maria (2010) observed significantly lower Chol. concentrations in sodium humate groups, while Sakine *et al.* (2006) showed that HA supplementation reduced egg Chol. in laying hens. On the contrary, Rath *et al.* (2006) found no effect of HA on cholesterol and TG in broiler chicks when supplemented in water, and Can and Sakir (2009) saw no statistical difference in serum Chol. and TG with HA supplementation in broilers. Avci *et al.* (2007) reported no effect on

triglycerides and VLDL in Japanese quails with humic acid supplementation, but Hakan *et al.* (2012) noted a slight increase in blood Chol. levels with humate addition to hen's diet without significance. ELnaggar and El-Kelawy (2018) demonstrated that HA decreased serum total lipids, TG, Chol., HDL, and LDL compared to the control group.

Blood glucose and thyroid hormones:

Blood glucose and thyroid hormone levels were significantly higher in chicks fed diet with either HA or BC at varying levels compared to the control group. The highest concentrations were observed for T3 in groups supplemented with 1 gram of HA and 2% BC in the basal diet. These findings are detailed in Table 8. In our study, we observed a consistent increase in blood plasma glucose levels with higher dietary levels of biochar. These findings align with previous studies by Kalus *et al.* (2020). The hormone triiodothyronine (T3) is essential for regulating growth by managing energy levels and protein metabolism in the body. Therefore, the rise in thyroid hormones due to HA supplementation may be attributed to biochar's protective role in safeguarding the thyroid gland from oxidative damage caused by excess hydrogen peroxide during thyroid hormone synthesis (Arthur *et al.*, 1999). Glucose, being intricately involved in metabolic processes, undergoes precise regulation in the blood (Braun and Sweazea, 2008). Interestingly, some studies have shown no significant changes in plasma glucose levels with HA supplementation. For instance, Avci *et al.* (2007) found that HA supplementation had no effect on glucose levels in Japanese quails. In contrast, Rath *et al.* (2006) observed a decreasing trend in glucose levels in broiler chickens under the influence of HA.

Liver and renal functions:

Liver enzyme tests, previously known as liver function tests, are a set of blood tests used to identify inflammation and damage in the liver. These tests typically include AST, ALT, and ALP levels. The results from

Broiler, humic acid, biochar, performance, blood biochemical.

Table 9 indicated that broiler chicks treated with various levels of HA or BC showed non-significant differences in serum AST, ALT, ALP, uric acid, and creatinine levels compared to the control group. These findings align with the research of Baral *et al.* (2006), who noted that supplementing broilers with HA led to non-significant decreases in creatinine concentrations. Additionally, they observed a trend towards decreased serum levels of ALP and ALT with a 2.5% HA supplementation in broilers. Similarly, Celik *et al.* (2008) found that organic acids (OA) in broilers affected all serum chemistry, with a trend towards decreased ALP concentrations.

In a study by, Rath *et al.* (2006) reported non-significant decreases in creatinine concentrations with humic acid supplementation in broilers, along with a trend towards decreased serum ALP and ALT concentrations with a 2.5% HA supplementation. Hanafy and El-Sheikh (2008) found no significant effect of HA supplementation on AST and ALT in laying hens. The combination of HA and HA-glucan notably decreased the levels of these enzymes in the serum, indicating a substantial reduction in liver damage. This liver-protective effect may be attributed to an increase in the antioxidant enzyme protective system, leading to elevated Glutathione (GSH) levels, which are often compromised due to liver toxicity (ELnaggar and El-Kelawy, 2018; Ghazalah *et al.*, 2022).

Antioxidative defense indicators:

The impact of supplements containing HA or BC on enhancing antioxidants in the blood serum of treated broiler chicks is depicted in Table 10. Serum antioxidant indices and enzymes such as TAC, GSH, GPX, and SOD were notably higher in broiler chicks fed diet with varying levels of HA or BC compared to the control group. Additionally, levels of serum MAD, a marker of oxidative stress, were reduced in broiler chicks fed diet with HA or BC compared to the control group. The results

obtained could be attributed to the presence of antioxidants in biochar, which act by inhibiting free radicals and thereby maintaining normal enzyme levels. These findings are consistent with studies by Dim *et al.* (2018) and Kalus *et al.* (2020). Glutathione is widely recognized for its role in detoxifying hydrogen peroxide and shielding cells from peroxide-induced damage. GSH-Px's primary function is to eliminate excess peroxide and hydrogen peroxide from fatty acids, stemming from the oxidative breakdown of lipids (Almeina *et al.*, 2012). This discovery aligns with research by Ghazalah *et al.* (2022), which also underscore the importance of GSH in neutralizing hydrogen peroxide and safeguarding cells from oxidative harm. It is hypothesized that HA acts as an antioxidant through various proposed mechanisms, possibly by stabilizing lipid membranes and preventing lipid peroxidation caused by free radicals. Conversely, malondialdehyde, shows an inverse relationship with TAC in organisms. MAD is a byproduct of peroxidation of polyunsaturated fatty acids in cells, and an increase in free radicals leads to heightened MAD production (Gawel *et al.*, 2004).

Immune response indices:

Dietary supplementation led to increased levels of IgA, IgM, IgG, lymphocyte transformation test (LTT), phagocytic activity (PA), and phagocytic index (PI) compared to the control group. However, there was no significant effect of different supplement levels on IgA levels (Table 11). Chicks fed diet with HA showed a significant increase in PA, PI, LTT, bacteriocide activity (BA), and lysosome activity (LA) in blood compared to those fed the control diet. These findings align with those of El-Ghalid *et al.* (2022), demonstrating a significant increase in serum IgG and IgM concentrations under various dietary biochar levels compared to the control group. Moreover, there was a notable increase in LA, BA, LTT, PA, and PI under different dietary biochar levels

compared to the control group. Furthermore, Hanafy and El-Sheikh (2008) corroborated our results by showing a notable rise ($P \leq 0.05$) in plasma total protein levels among hens receiving a high dose of HA, surpassing other groups. Similarly, Salah *et al.* (2015) observed substantial improvements in leukocyte count, lymphocyte levels, phagocytosis, PI, and total proteins in broiler chickens with HA supplementation. Ertas *et al.* (2006) noted improved protein digestion in Japanese quail with HA supplementation.

Bacterial count:

Our study revealed that all dietary supplements contributed to a reduction in the total bacterial count, *E. coli*, and *Proteus* spp. when compared to the control group. Conversely, they led to an elevation in *Lactobacillus* levels compared to the control group (Table 12). These findings underscore the significant decrease in pathogenic bacteria populations, emphasizing the beneficial role of HA in mitigating bacterial imbalances within the broiler chickens' digestive tract. It's widely recognized that maintaining a healthy and diverse intestinal microbial community is pivotal for preserving mucosal structure and function, impacting the immune system, and preventing intestinal diseases in chickens. In

line with our research, Abdel-Mageed (2012) suggested that incorporating humic substances into diets significantly decreased *coliform*, *Escherichia coli*, and *Clostridium perfringens* populations in intestinal content, alongside a reduction in intestinal pH compared to the control diet. Humic substances are known to create a protective barrier on the gastrointestinal tract's mucous epithelium, shielding it from infections and toxins, thereby enhancing nutrient utilization in animal feed (Islam *et al.*, 2005). According to Gerlach and Schmidt (2012), incorporating BC into the diet of broiler can deactivate toxins present in the digestive tract, stimulate the growth of beneficial intestinal bacteria, and enhance the overall health of the birds. The gastrointestinal tract (GIT) microbiota of chickens plays a crucial role in bolstering the immune system, including intestinal immune cells such as T cells and epithelial monolayers (Engberg *et al.*, 2002). This heightened immune response allows the birds to effectively combat diseases. For instance, the secretion of immunoglobulins like IgA, which binds to bacterial epitopes, helps regulate the bacterial population in the gut (Gerlach and Schmidt, 2012). However, it's worth noting that the commensal microbiota competes with the host for energy and nutrients.

Broiler, humic acid, biochar, performance, blood biochemical.

Table (1): Composition and calculated analysis of basal and experimental diets of broiler's ingredients (%).

Diets	Starter period (1-21 days)	Grower period (22-35 days)
Ingredients, %		
Yellow corn.	55.50	60.50
Soybean meal (46%).	26.25	21.50
Full fat soya.	5.00	6.00
Glutein (60%).	7.00	6.00
Soya oil.	1.75	1.50
Mono calcium phosphate.	1.65	1.65
Limestone.	1.75	1.75
L-lysine.	0.25	0.25
DL-methionine.	0.20	0.20
Salt (Na Cl).	0.35	0.35
Premix*.	0.30	0.30
Total	100	100
Calculated analysis		
Crude protein, %.	23.00	21.14
ME (kcal/kg)	3059	3101
Crude fiber, %.	3.83	3.59
Ether extract, %.	5.23	5.28
Calcium, %.	1.06	1.05
Phosphorus available, %.	0.45	0.45
Methionine, %.	0.56	0.54
Lysine, %.	1.23	1.13
Methionine + Cystine, %.	0.95	0.90

*Each kg of vitamin and mineral mixture contains: 12 M IU vitamin A; 5 M IU D₃; 80000 mg E; 4000 mg K; 4000 mg B₁; 9000 mg B₂; 4000 mg B₆; 20 mg B₁₂; 15000 mg pantothenic acid; 60000 mg Nicotinic acid; 2000 mg Folic acid; 150 mg Biotin; 400000 mg Choline Chloride; 15000 mg Copper sulphate; 1000 mg calcium Iodide; 40000 mg ferrous sulphate ; 100000 mg Manganese oxide ; 100000 mg Zinc oxide and 300 mg Selenium selenite.

Table (2): Effect of supplementation with different levels of humic acid and biochar on productive performance and economic efficiency of Cobb broiler chicks.

Dietary supplementations	BW (7 d.)	BW (35 d.)	BWG (7-35 d.)	FC (7-35 d.)	FCR (7-35 d.)	EEF	REEF	Production index
Control	180.1	1880 ^c	1700.1 ^c	3142.9 ^b	1.85 ^a	0.326 ^c	100	290.34 ^c
HA (1 g)	184.2	2280 ^a	2095.8 ^a	3290.0 ^a	1.57 ^c	0.568 ^a	174.22	414.9 ^a
HA (2 g)	185.7	2090 ^b	1904.3 ^b	3300.1 ^a	1.73 ^b	0.416 ^b	127.63	345.169 ^b
BC (1%)	182.2	2080 ^b	1897.8 ^b	3412.7 ^a	1.80 ^b	0.393 ^b	119.81	330.15 ^b
BC (2%)	183.4	2290 ^a	2106.6 ^a	3330.2 ^a	1.58 ^c	0.593 ^a	181.86	414.10 ^a
SEM	0.321	9.10	18.31	9.08	0.021	0.054	--	3.04
<i>P value</i>	0.180	0.0001	0.002	0.001	0.0001	0.001	--	0.0001

^{a, b, c}Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of means, BC, Biochar; HA, Humic acid; EEF, Economic efficiency; REEF, Relative economic efficiency (%).

Table (3): Effect of supplementation with different levels of humic acid and biochar on the apparent digestibility of the nutrients and ash retention of Cobb broiler chicks (%).

Dietary supplementations	OM	DM	CP	EE	CF	Apparent ash retention
Control	60.21	68.90	71.61 ^c	70.18 ^b	17.80	37.50
HA (1 g)	65.84	69.30	81.03 ^a	88.10 ^a	18.20	33.60
HA (2 g)	63.35	66.11	76.15 ^b	86.85 ^a	17.58	32.58
BC (1%)	66.77	67.90	75.90 ^b	89.19 ^a	18.19	36.13
BC (2%)	65.88	69.76	82.37 ^a	80.81 ^a	19.81	33.32
SEM	4.01	1.90	1.90	1.96	1.66	1.19
P value	0.091	0.056	0.001	0.001	0.087	0.087

^{a, b}Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of means. OM= Organic matter, DM= Dry matter, CF= Crude fiber, EE= Ether extract, CP= Crude protein,

Table (4): Effect of supplementation with different levels of humic acid and biochar on carcass traits and lymphoid organs of broiler chicks.

Dietary supplementations	Carcass traits (%)		Lymphoid organs (%)		
	Dressing	Abdominal fat	Spleen	Thymus	Bursa
Control	64.4 ^c	0.876 ^a	0.145	0.397	0.137
HA (1 g)	75.2 ^a	0.575 ^b	0.158	0.389	0.122
HA (2 g)	73.6 ^a	0.491 ^b	0.166	0.396	0.125
BC (1%)	69.4 ^b	0.340 ^c	0.169	0.328	0.130
BC (2%)	74.2 ^a	0.612 ^b	0.171	0.304	0.140
SEM	1.21	0.119	0.121	0.041	0.090
P value	0.0001	0.0001	0.019	0.069	0.067

^{a, b, c}Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of means.

Table (5): Effect of supplementation with different levels of humic acid and biochar hematological parameters and white blood cells and differential leukocytes counts of broiler chicks.

Dietary supplementations	Hematological parameters			White blood cells and differential leukocytes counts			
	RBC's ($10^6/\text{mm}^3$)	Hb (g/dl)	PCV %	WBC's ($10^3/\text{mm}^3$)	Lympho . %	Hetero. %	H/L ratio
Control	3.34 ^b	9.13 ^c	28.13 ^c	18.86 ^c	50.73 ^c	32.53	0.641
HA (1 g)	5.16 ^a	12.96 ^a	36.54 ^a	25.69 ^a	64.97 ^a	31.93	0.491
HA (2 g)	4.99 ^a	11.07 ^b	32.81 ^b	22.40 ^b	63.87 ^a	32.13	0.503
BC (1%)	4.98 ^a	11.12 ^b	33.76 ^b	25.45 ^a	59.53 ^b	29.87	0.502
BC (2%)	4.88 ^a	12.70 ^a	38.73 ^a	29.38 ^a	62.57 ^a	30.29	0.484
SEM	0.780	1.54	3.01	0.760	0.007	2.07	0.065
P value	0.001	0.002	0.001	0.0001	0.001	0.061	0.078

^{a, b, c}Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of means. HB= Hemoglobin; RBC's=red blood cell; PCV=packed cell volume; WBC's= white blood cells.

Broiler, humic acid, biochar, performance, blood biochemical.

Table (6): Effect of supplementation with different levels of humic acid and biochar on protein profile (g/dl) of broiler chicks.

Dietary supplementations	Protein profile (g/dl)			different types of globulin		
	T. protein	Albumin	Globulin	α -globulin (μ g/dl)	β -globulin (μ g/dl)	γ -globulin (μ g/dl)
Control	5.26 ^b	2.68	2.58 ^b	0.907	0.881	0.792 ^c
HA (1 g)	6.36 ^a	2.98	3.38 ^a	0.711	1.129	1.54 ^b
HA (2 g)	6.41 ^a	3.09	3.31 ^a	0.709	0.691	1.91 ^a
BC (1%)	6.55 ^a	3.02	3.53 ^a	0.701	1.109	1.72 ^b
BC (2%)	6.43 ^a	3.04	3.39 ^a	0.778	0.942	1.67 ^b
SEM	0.069	0.701	0.090	0.005	0.006	0.004
P value	0.001	0.071	0.0001	0.098	0.081	0.001

^{a, b, c}Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of means.

Table (7): Effect of supplementation with different levels of humic acid and biochar on lipids profile (mg/dl) of broiler chicks.

Dietary supplementations	Lipids profile (mg/dl)					
	T. Lipid	Chol.	TG.	HDL	LDL	VLDL
Control	498.2 ^a	198.6 ^a	85.6	45.67 ^b	135.81 ^a	17.12 ^a
HA (1 g)	300.6 ^c	144.3 ^c	83.5	55.9 ^a	71.70 ^b	16.71 ^b
HA (2 g)	332.1 ^b	151.4 ^b	84.33	56.07 ^a	78.46 ^b	16.86 ^b
BC (1%)	365.4 ^b	166.2 ^b	83.1	57.23 ^a	92.38 ^b	16.62 ^b
BC (2%)	299.2 ^c	140.5 ^c	84.4	58.37 ^a	65.25 ^b	16.88 ^b
SEM	1.98	9.00	1.98	3.11	7.11	6.01
P value	0.001	0.001	0.075	0.002	0.001	0.002

^{a, b} Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of means. Chol.= total cholesterol; TG= triglycerides; HDL=high-density lipoprotein; LDL=low-density lipoprotein, VLDL= very low density lipoprotein

Table (8): Effect of supplementation with different levels of humic acid and biochar on blood glucose , thyroid hormones and immunological status of broiler chicks.

Dietary supplementations	Glucose (mg/dl)	Thyroid hormones	
		T3(ng/dl)	T4 (ng/dl)
Control	174.2 ^b	3.11 ^c	9.86 ^b
HA (1 g)	265.3 ^a	4.23 ^a	11.53 ^a
HA (2 g)	242.0 ^a	3.88 ^b	12.13 ^a
BC (1%)	260.9 ^a	3.90 ^b	12.21 ^a
BC (2%)	274.6 ^a	4.14 ^a	11.85 ^a
SEM	2.98	0.416	0.716
P value	0.001	0.001	0.001

^{a, b, c}Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of means. T3= triiodothyronine; T4=thyroxine.

Table (9): Effect of supplementation with different levels of humic acid and biochar on liver and renal functions of broiler chicks.

Dietary supplementations	Liver functions			Renal functions	
	AST (U/L)	ALT (U/L)	Alk. P. (IU/L)	Uric acid (mg/dl)	Creatinine (mg/dl)
Control	64.7	35.42	9.28	3.89	0.816
Control	69.9	32.89	10.67	3.14	0.670
HA (1 g)	64.11	31.87	9.21	2.98	0.673
HA (2 g)	66.7	32.49	9.78	2.96	0.612
BC (1%)	64.9	33.86	12.90	3.07	0.701
SEM	1.17	2.18	2.82	0.209	0.098
P value	0.071	0.097	0.071	0.081	0.076

SEM, Standard error of means. AST=aspartate amino transferase; ALT=alanine amino transferase; Alk. P =Alkaline phosphatase.

Table (10): Effect of supplementation with different levels of humic acid and biochar on indicators of antioxidative status of broiler chicks.

Dietary supplementations	Indicators of antioxidative status in blood (mg/dl)				
	TAC	GSH	GPX	SOD	MAD
Control	245.2 ^b	698.0 ^b	31.91 ^b	300.1 ^b	196.6 ^a
HA (1 g)	388.1 ^a	836.1 ^a	38.9 ^a	362.7 ^a	140.4 ^c
HA (2 g)	377.5 ^a	869.3 ^a	40.2 ^a	370.5 ^a	142.9 ^c
BC (1%)	386.6 ^a	870.0 ^a	37.8 ^a	369.6 ^a	156.7 ^b
BC (2%)	385.0 ^a	869.6 ^a	41.9 ^a	328.5 ^b	143.8 ^c
SEM	2.18	7.96	2.19	8.29	11.09
P value	0.002	0.0001	0.0002	0.007	0.001

^{a, b, c}Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of means. TAC=total antioxidant capacity; ; GSH-Px =glutathione peroxidase; SOD=superoxide dismutase, MDA= malondialdehyde

Table (11): Effect of supplementation with different levels of humic acid and biochar on blood plasma immune indices of broiler chicks.

Dietary supplementations	Immunological status (%)			Immunological status		
	LTT	PA	PI	IgG (mg/100 ml)	IgM (mg/100 ml)	IgA (mg/100 ml)
Control	18.8 ^c	17.9 ^b	1.65 ^b	737.4 ^c	200.2 ^b	50.91
HA (1 g)	27.6 ^a	20.8 ^a	2.38 ^a	852.6 ^a	250.1 ^a	59.95
HA (2 g)	29.3 ^a	23.3 ^a	2.41 ^a	893.7 ^a	261.1 ^a	51.87
BC (1%)	23.2 ^b	24.7 ^a	2.65 ^a	807.7 ^b	241.3 ^a	61.0
BC (2%)	28.4 ^a	29.6 ^a	2.98 ^a	880.6 ^a	230.8 ^a	59.9
SEM	0.763	0.870	0.187	11.90	4.10	2.10
P value	0.001	0.001	0.002	0.0001	0.001	0.076

^{a, b, c}Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of means. LTT= Lymphocyte transformation test; PA= Phagocyte activity; PI = Phagocytic index.

Table (12): Effect of supplementation with different levels of humic acid and biochar on bacterial count of broiler chicks.

Dietary supplementations	TBC (cfu x 10⁶)	Lactobacillus (cfu x 10³)	<i>E.Coli</i> (cfu x 10³)	<i>Proteus.</i> (cfu x 10³)
Control	4.39 ^a	1.67 ^b	1.82 ^a	0.853 ^a
HA (1 g)	3.21 ^b	2.62 ^a	0.701 ^b	0.500 ^b
HA (2 g)	3.28 ^b	2.70 ^a	0.743 ^b	0.456 ^b
BC (1%)	3.12 ^b	2.84 ^a	0.696 ^b	0.363 ^c
BC (2%)	3.07 ^b	2.90 ^a	0.829 ^b	0.256 ^c
SEM	0.078	0.676	0.076	0.0890
P value	0.001	0.001	0.001	0.0005

^{a, b, c}Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM,

Standard error of means. TBC = Total Bacterial Count.

REFERENCES

- A.O.A.C 2004.** Association of Official Analytical Chemists International: Official Methods of Analysis of AOAC International. 18th edition, AOAC International, Gaithersburg, MD, USA.
- Abdelhafez, A. A., Zhang, X., Zhou, L., Zou, G., Cui, N., Abbas, M. H.H. and Hamed, M. H. 2020.** Introductory Chapter: Is Biochar Safe?. In Applications of Biochar for Environmental Safety, Intech Open, pp 1-6. Doi:10.5772/intechopen.91996
- Abdel-Fattah, S. A., El-Mednay, N. M., and Abdel-Azeem, F. 2008.** Thyroid activity, some blood constituents, organs morphology, and performance of broiler chicks fed supplemental organic acids. International Journal of Poultry Science, 7, 215-222.
- Abdel-Mageed, M. A. A. 2012.** Effect of dietary humic substances supplementation on performance and immunity of Japanese quail. Egyptian Poultry Science, 32, 645-660.
- Abou-Raya, A. K., and Galal, A. G. 1971.** Evaluation of poultry feeds in digestion trials with reference to some factors involved. Journal of Animal Production, United Arab Republic.
- Abudabos, A. M., Alyemni, A. H., Dafalla, Y. M., and Khan, R. U. 2018.** The effect of phytogenics on growth traits, blood biochemical, and intestinal histology in broiler chickens exposed to Clostridium perfringens challenge. Journal of Applied Animal Research, 46, 691-695.
- Alena, S., and Mária, D. 2010.** Effect of Diet Supplemented with Natural Humic Compounds and Sodium Humate on Performance and Selected Metabolic Variables in Broiler Chickens. Acta Veterinaria Brno, 79, 385–393.
- Almeina, A., Saeerens, L., De Beer, T., Remon, J. P., and Vervaet, C. 2012.** Upscaling and in-line process monitoring via spectroscopic techniques of ethylene vinyl acetate hot-melt extruded formulations. International Journal of Pharmaceutics, 439(1-2), 223.
- Andremont, A. 2000.** Consequences of antibiotic therapy to the intestinal ecosystem. Annals of French Anesthesia and Intensive Care, 19, 395–402.
- Andrew, S. L., Mohammed, H. Z., Manyelo, T. G., and Mabelebele, M. 2020.** The current status of the alternative use to antibiotics in poultry production–An African Perspective. Antibiotics, 9, 594.

- Arif, M., Rehman, A., Saeed, M., Abd El-Hack, M. E., Arain, M. A., Haseebbarshad, M., Zakria, H. M., and Abbasi, I. M. 2016. Impacts of dietary humic acid supplementation on growth performance, some blood metabolites and carcass traits of broiler chicks. *Indian Journal of Animal Sciences*, 86(9), 1073–1078.
- Arthur, M., Inkson, K., and Pringle, J. 1999. *The new careers: Individual action and economic change*. Sage.
- Avcı, M., Denek, N., and Kaplan, O. 2007. Effects of Humic Acid at Different Levels on Growth Performance, Carcass Yield and Some Biochemical Parameters of Quails. *Journal of Animal and Veterinary Advances*, 6(1-4), 1-4.
- Bakr, B. E. 2008. The effect of using citrus wood charcoal in broiler rations on the performance of broilers.
- Baral, N., Luitel, K., and Khanal, P. 2006. Effects of humic acid on growth performance and serum biochemical profiles of broiler chickens. *International Journal of Poultry Science*, 5(9), 860-864.
- Baurhoo, B., Phillip, L., and Ruiz-Feria, C. A. 2007. Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. *Poultry Science*, 86(5), 1070–1078.
- Boonanuntanasarn, S., Khaomek, P., Pitaksong, T., and Hua, Y. 2014. The effects of the supplementation of activated charcoal on the growth, health status, and fillet composition-odor of Nile tilapia (*Oreochromis niloticus*) before harvesting. *Aquaculture International*, 22(4), 1417-1436.
- Braun, E. J., and Sweazea, K. L. 2008. Glucose regulation in birds. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 151(1), 1-9.
- Can, A. K., and Sakir, D. T. 2009. The effect of humates on fattening performance, carcass quality, and some blood parameters of broilers. *Journal of Animal and Veterinary Advances*, 8, 281-284.
- Celik, K., Ugur, K., and Uzatici, A. 2008. Effect of Supplementing Broilers Diets with Organic Acid and Whole Grain. *Asian Journal of Animal and Veterinary Advances*, 3, 328-333.
- Cowieson, A. J., and Kluentner, A. M. 2019. Contribution of exogenous enzymes to potentiate the removal of antibiotic growth promoters in poultry production. *Anim Feed Sci Technol*, 250, 81-92.
- Dim, C. E., Akuru, E. A., Egom, M. A., Nnajifor, N. W., Ossai, O. K., Ukaigwe, C. G., and Onyimonyi, A. E. 2018. Effect of dietary inclusion of biochar on growth performance, haematology, and serum lipid profile of broiler birds. *Agro-Science*, 17(2), 9-17.
- Drew, P., Charles, C. R. J. S., Trevor, B., and John, L. 2004. *Oxford Handbook of Clinical Haematology*. Second Edition. Oxford University Press, USA.
- Duncan, D.B., 1955. Multiple Range and Multiple F- test. *Biometrics*, 11:1- 42.
- EL nagggar, A. S., and El-Kelawy, M. I. 2018. Effect of humic acid supplementation on productive performance, blood constituents, immune response, and carcass characteristics of sasso chicken. *Egyptian Journal of Animal Production*, 55(1), 75-84.
- El-Ghalid, O. A., Abdel-Hamid, A. E., Harfoush, A. S., and ELnagggar, A. S. 2022. Effect of graded levels of biochar supplementation as a growth promoter on productive and physiological performance of broiler chicks. *Egyptian Poultry Science Journal*, 42(3), 243–263.
- Engberg, R., Hedemann, M., and Jensen, B. 2002. The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. *British poultry science*, 43(4), 569-579.
- Ertas, O. N., Ciftci, M., Guler, T., and Dalkilic, B. 2006. The use of the possibility of mussel shell

Broiler, humic acid, biochar, performance, blood biochemical.

- supplementation as a calcium source in Japanese quails raised under heat stress conditions: The effect of mussel shell on egg yield and some blood parameters. *Fýrat Üiversitesi Sađlýk Bilimleri Veteriner Dergisi*, 20(1), 15-20.
- Esenbuga, N., Macit, M., Karaoglu, M., Aksu, M. I., and Bilgin, O. C. 2008.** Effects of dietary humate supplementation to broilers on performance, slaughter, carcass, and meat color. *J Sci Food Agric*, 88, 1201-1207.
- Feldman, B. F., Zinkl, J. G., and Jain, N. C. 2000.** Schalm's Veterinary Hematology. Lippincott Williams and Wilkins, Philadelphia, USA.
- Food and Agriculture Organization (FAO). 2019.** The future of food and agriculture: Alternative pathways to 2050. Rome. Retrieved from <http://www.fao.org/3/ca6640en/ca6640en.pdf>.
- Gawel, S., Wardas, M., Niedworok, E., and Wardas, P. 2004.** Malonaldehyde (MDA) as a lipid peroxidation marker. *Wiadomosci lekarskie (Warsaw, Poland: 1960)*, 57(9-10), 453-455.
- Gerlach, A., and Schmidt, H. P. 2012.** The use of biochar in cattle farming. *Ithaka Journal*, 2012, 281-285.
- Ghazalah, A. A.; El-Tahawy W. S.; Asmaa A., Ghalwash and Asmaa Sh. ELnaggar 2022.** Prouductive and physiologiçl response of broiler chicks to dietary humic acid. *Egypt. Poult. Sci. Vol. (42) (II): (157-170)*
- Hakan, K. B., Gultekin, Y., and Ozge, S.2012.** Effects of boric acid and humate supplementation on performance and egg quality parameters of laying hens. *Braz J Poult Sci*, 14, 233-304.
- Hanafy Maysa, M., and El-Sheikh, A. M. H. 2008.** The effect of dietary humic acid supplementation on some productive and physiological traits of laying hens. *Egyptian Poultry Science Journal*, 28(4), 1043-1058.
- Haque, M., Sarker, S., Islam, M., Karim, M., Kayesh, M. E. H., Shiddiky, M. J., and Anwer, M. S. 2020.** Sustainable antibiotic-free broiler meat production—Current trends, challenges, and possibilities in a developing country perspective. *Biol*, 9, 411. doi:10.3390/biology9110411.
- Hayirli, A., Esenbuga, N., Macit, M., Lacin, E., Karaoglu, M., Karaca, H., and Yildiz, L. 2005.** Nutrition practice to alleviate the adverse effects of stress on laying performance, metabolic profile, and egg quality in peak-producing hens: I. The humate supplementation. *Asian-Australasian Journal of Animal Sciences*, 18, 1310-1319.
- Herzig, I., Kozler, J., Písaříková, B., Fengl, M., and Jursa, V. 2001.** Effects of a humine acid-based sorbent on the concentration of ammonia in broiler houses. *Archiv Geflug*, 65, 246-250.
- Ipek, H., Avci, M., Iriadam, M., Kaplan, O., and Denek, N. 2008.** Effects of humic acid on some hematological parameters, total antioxidant capacity, and laying performance in Japanese quails. *Arch Geflügelk*, 72, 56-60.
- Islam, K. M., Schuhmacher, S. A., and Gropp, M. J. 2005.** Humic acid substances in animal agriculture. *Pakistan Journal of Nutrition.*, 4, 126–134.
- Jakobsen, P.E., Getov, K., and Nilsen, S.H. 1960.** Frdjelighed frogmed fierbrae. "Digestibility trials with poultry." Bereting fra for sogstabortoiriet, Kabenhaven, 56, 1-34.
- Jiya, E., Ayanwale, B., Ijaiya, A., Ugochukwu, A., and Tsado, D. 2013.** Effect of activated coconut shell charcoal meal on growth performance and nutrient digestibility of broiler chickens. *British Journal of Applied Science and Technology*, 3(2), 268-276.
- Joone, G. K., Dekker, J., and Van Rensburg, C. E. 2003.** Investigation of the immunostimulatory properties of oxihumate. *Zeitschrift für Naturforschung*, 58(3-4), 263–267.
- Kalus, K., Konkol, D., Korczyński, M., Koziel, J. A., and Opaliński, S. 2020.**

- Effect of biochar diet supplementation on chicken broilers performance, NH₃ and odor emissions, and meat consumer acceptance. *Animals*, 10(9), 1539.
- Kana, J. R., Tegua, A., Mungfu, B. M., and Tchoumboue, J. 2011.** Growth performance and carcass characteristics of broiler chickens fed diets supplemented with graded levels of charcoal from maize cob or seed of *Canarium schweinfurthii* Engl. *Tropical animal health and production*, 43(1), 51-56.
- Kana, J. R., Ngoula, F., Tchoffo, H., Tadondjou, C. D., Sadjo, Y. R., Tegua, A., and Gnonlonfin Gbemenou, J. B. 2014.** Effect of biocharcoals on hematological, serum biochemical, and histological parameters in broiler chickens fed aflatoxin B₁-contaminated diets. *Journal of Animal Science Advances*, 4(7), 939-948.
- Karaoglu, M., Macit, M., Esenbuga, N., Durdag, H., Bilgin, O. C., and Turgut, L. 2004.** Effect of supplemental humate at different levels on the growth performance, slaughter, and carcass traits of broilers. *International Journal of Poultry Science*, 3, 406-410.
- Kim, K. S., Kim, Y. H., Park, J. C., Yun, W., Jang, K. I., Yoo, D. I., and Cho, J. H. 2017.** Effect of organic medicinal charcoal supplementation in finishing pig diets. *Korean Journal of Agricultural Science*, 44(1), 50-59.
- Klyszejko-Stefanowicz, L. 2005.** *Ćwiczenia z biochemii*, wyd. I, Wyd. Naukowe PWN, Warszawa.
- Kutlu, H. 1998.** Effects of dietary wood charcoal on performance and fatness of broiler chicks. *British Poultry Science*, 39(S1), 31-32.
- Kutlu, H. R., Ünsal, I., and Görgülü, M. 2001.** Effects of providing dietary wood (oak) charcoal to broiler chicks and laying hens. *Animal feed science and technology*, 90(3-4), 213-226.
- Lala, A. O., Okwelum, N., Irekhore, O. T., Ogunlade, B. A., Adigun, A. A., Elegbede, L. A., and Oyedeji, M. M. 2016.** Haematological and Biochemical Parameters of Broiler Chicken Supplemented with Humic Acid in the Drinking Water. *Applied Tropical Agriculture*, 21(2), 74-78.
- Louis, A., Mohammed, A., Andreas, B., and Regina, R. 2018.** Influence of dietary wood charcoal on growth performance, nutrient efficiency, and excreta quality of male broiler chickens. *International Journal of Livestock Production*, 9(10), 286-292.
- MacCarthy P. 2001.** The principles of humic substances. *Soil Science*, 166(11), 738-751.
- Majewska, T., and Zaborowski, M. 2003.** Charcoal in the nutrition of broiler chickens. *Medycyna weterynaryjna*, 59(1), 81-83.
- Majewska, T., Mikulski, D., and Siwik, T. 2009.** Silica grit, charcoal, and hardwood ash in turkey nutrition. *Journal of Elementology*, 14(3), 489-500.
- Majewska, T., Pudyszak, K., and Kozłowski, K. 2011.** The effect of charcoal addition to diets for broilers on performance and carcass parameters. *Veterinarija ir Zootechnika (Vet Med Zoot)*, 55, 10-12.
- Marie, C. I. 2013.** Biochar as a feed supplement. An overview. *International Journal of Livestock Research*, 9(1), 58-72.
- Monica, C. 2019.** Evaluation of the effects of biochar inclusion in broiler chicken feeds and litter on growth performance and fecal bacterial count [PhD Thesis]. Bindura University of Science Education, Zimbabwe.
- Mudroňová, D., Karaffová, V., Pešulová, T., Koščová, J., Maruščáková, I. C., Bartkovský, M., and Marcinčák, S. 2020.** The effect of humic substances on gut microbiota and immune response of broilers. *Food and Agricultural Immunology*, 31(1), 137-149.
- Mui, N. T., and Ledin, I. 2006.** Effect of the method of processing foliage of *Acacia*

Broiler, humic acid, biochar, performance, blood biochemical.

- mangium and inclusion of bamboo charcoal in the diet on the performance of growing goats. *Animal Feed Science and Technology*, 130(3-4), 242-256.
- Nagaraju, R., Reddy, B. S., Gloridoss, R., Suresh, B. N., Ramesh, C. 2014.** Effect of dietary supplementation of humic acids on the performance of broilers, *Indian Journal of Animal Sciences*, 84(4), 447–452.
- Neuvonen, P. J., Kuusisto, P., Vapaatalo, H., and Manninen, V. 1989.** Activated charcoal in the treatment of hypercholesterolemia: dose-response relationships and comparison with cholestyramine. *European Journal of Clinical Pharmacology*, 37(3), 225-230.
- Ozturk, E., Coskun, I., Ocak, N., and Erener, G. 2009.** Effects of dietary humic substances on egg production and egg shell quality of hens after the peak laying period. *African Journal of Biotechnology*, 8, 1155–1159.
- Ozturk, E., N. Ocak, I. Coskun, S. Turhan, and E. Erener. 2010.** Effects of humic substances supplementation provided through drinking water on performance, carcass traits, and meat quality of broilers. *Journal of Animal Physiology and Animal Nutrition*, 94(1), 78-85.
- Ozturk, E., I. Coskun, N. Ocak, G. Erener, M. Dervisoglu, and S. Turhan. 2014.** Performance, meat quality, meat mineral contents, and caecal microbial population responses to humic substances administered in drinking water in broilers. *British Poultry Science*, 55(5), 668-674.
- Pistova, V., Arpášová, H., Hrněár, C., Kačániová, M., and Haščík, P. 2016.** The Effect of Humic Acid and Herbal Additive Supplement on Production Parameters of Broiler Chicken. *Scientific Papers: Animal Science and Biotechnologies*, 49(2).
- Prasai, T. P., Walsh, K. B., Bhattarai, S. P., Midmore, D. J., Van, T. T., Moore, R. J., and Stanley, D. 2016.** Biochar, bentonite, and zeolite-supplemented feeding of layer chickens alters intestinal microbiota and reduces *Campylobacter* load. *PLoS One*, 11(4), e0154061.
- Provan, D., Singer, C. R. J., Baglin, T., and Lilleyman, J. 2004.** *Oxford Handbook of Clinical Hematology*, 2nd edition. Oxford University Press, USA.
- Rath, N. C., Huff, W. E., and Huff, G. R. 2006.** Effects of humic acid on broiler chickens. *Poultry Science*, 85(3), 410–414.
- Rzasa, A., Mišta, D., Wincewicz, E., Zawadzki, W., Dobrzański, Z., and Gelles, A. 2014.** Humic-Fatty Acid Preparation in Growing Rabbits Nutrition. *EJPAU*, 17(3).
- Sakine Y., Ahmet E., Bülent Ö., Suzan Y., Handan E., and İlyas O. 2006.** The Effects of Dietary Supplementation of L-carnitine and Humic Substances on Performance, Egg Traits, and Blood Parameters in Laying Hens. *Asian-Australasian Journal of Animal Sciences*, 19, 1478–1483.
- Salah H., El Sayed M., Reham R. R., and Abd El Hamid E. S. 2015.** Study on the Effect of Humic Acid on Growth Performance, Immunological, Some Blood Parameters, and Control Intestinal *Clostridium* in Broiler Chickens. *Zagazig Veterinary Journal*, 43(1), 102–109.
- SAS, 2006.** SAS/STAT User's guide statistics. SAS institute INC., Cary. NC, USA.
- Scicutella F., Mannelli F., Daghighi M., Viti C., and Buccioni A. 2021.** Poly-phenols and Organic Acids as Alternatives to Antimicrobials in Poultry Rearing: A Review. *Antibiotics*, 10(8), 1010.
- Sheikh A., Tufail B., Gulam A. B., Masood S. M., and Manzoor R. 2010.** Effect of Dietary Supplementation of Organic Acids on Performance, Intestinal Histomorphology, and Serum Biochemistry of Broiler Chicken. *Veterinary Medicine International*, 2010, Article ID 479485, 7 pages. <http://dx.doi.org/10.4061/2010/479485>

- Sun T., Levin B. D. A., Guzman J. J. L., Enders A., Muller D. A., Angenent L. T., and Lehmann J. 2017. Rapid Electron Transfer by the Carbon Matrix in Natural Pyrogenic Carbon. *Nature Communications*, 8(1), 14873.
- Taklimi S. M., Ghahri H., and Isakan M. A. 2012. Influence of Different Levels of Humic Acid and Esterified Glucomannan on Growth Performance and Intestinal Morphology of Broiler Chickens. *Agricultural Sciences*, 3(5), 663–668.
- Terratol L. L. C. 2002. Effects of Humic Acid on Animals and Humans. Retrieved from <http://www.terratol.com/sitebuildercontent/sitebuilderfiles/EffectsofHumicAnimalsandHumans.pdf>
- Terry S. A., Ribeiro G. O., Gruninger R. J., Chaves A. V., Beauchemin K. A., Okine E., and McAllister T. A. 2019. A Pine Enhanced Biochar Does Not Decrease Enteric CH₄ Emissions, but Alters the Rumen Microbiota. *Frontiers in Veterinary Science*, 6, 308.
- van Rensburg C. E. 2015. The Anti-inflammatory Properties of Humic Substances: A Mini Review. *Phytotherapy Research*, 29, 791–795.
- Yang H., Chen Z. Q., and Ou W. 2015. Microconchids from Microbialites Near the Permian-Triassic Boundary in the Zuodeng Section, Baise Area, Guangxi Zhuang Autonomous Region, South China and Their Paleoenvironmental Implications. *Journal of Earth Science*, 26(2), 157–165.
- Zewel, H. S. 1996. Enzyme supplements to diets growing Japanese quails. *Egypt. Poult. Sci. J.*, 16: 535-557.

الملخص العربي

تأثير إضافة حمض الهيوميك والفحم الحيوي كمنشطات نمو طبيعية على الأداء الإنتاجي والفسولوجي وهضم العناصر الغذائية لكتاكيت التسمين

محمود إبراهيم الكيلوي¹؛ أسماء شوقي النجار²؛ إيناس عبد الخالق محمود³

¹قسم إنتاج الدواجن - كلية الزراعة - جامعة الوادي الجديد

²قسم الإنتاج الحيواني والداجني - كلية الزراعة - جامعة دمنهور

³قسم إنتاج الدواجن - كلية الزراعة (الشاطبي) - جامعة الإسكندرية

أجريت هذه التجربة بهدف دراسة تأثيرات إضافة كلا من حمض الهيوميك أو الفحم الحيوي على الصفات الإنتاجية، خصائص الدم، الخصائص المضادة للأكسدة، صفات الذبيحة والكفاءة الاقتصادية لكتاكيت اللحم. تم استخدام كتكوت تسمين كوب 500 - عمر 7 أيام تم تقسيمها إلى خمس معاملات تجريبية بكل معاملة 36 طائر في ستة مكررات بكل منها 6 كتاكيت على النحو التالي: المجموعة الأولى هي الكنترول وكانت بدون إضافات؛ والمعاملات الثانية والثالثة تغذت على العليقة الأساسية مع إضافة حمض الهيوميك بمستويات 1، 2 جم/كجم عليقة على التوالي، بينما المعاملات الرابعة والخامسة تغذت على العليقة الأساسية مع إضافة الفحم الحيوي بمستويات 1، 2٪ على التوالي. واستمرت التجربة حتى عمر 35 يوم.

أظهرت الطيور المغذاة على عليقة مضاف لها حمض الهيوميك (HA) أو الفحم الحيوي (BC) بمستويات مختلفة زيادة معنوية في الوزن الحي (BW) والزيادة في الوزن (BWG) مقارنة بمجموعة الكنترول. أظهرت المجموعة المغذاة بـ 1,00 جم حمض الهيوميك/كجم عليقة و 2٪ الفحم الحيوي أفضل وزن حي وزيادة في الوزن. كما أدت هذه الإضافات إلى تحسين معدل تحويل العلف والكفاءة الاقتصادية، مع ملاحظة أن 1 جم حمض الهيوميك/كجم عليقة و 2٪ الفحم الحيوي أظهرت أعلى دليل إنتاج. أدت إضافة حمض الهيوميك والفحم الحيوي إلى العليقة الأساسية إلى تحسين معاملات هضم البروتين الخام والمستخلص الإثيري ($P \leq 0.05$) ولكن لم تكن لها تأثيرات معنوية على معاملات هضم الألياف والبروتين الخام. علاوة على ذلك، أدت الإضافات إلى زيادة نسبة التصافي وقللت من دهون البطن مقارنة بمجموعة الكنترول. زادت الإضافات الغذائية في دجاج اللحم من قياسات الدم (RBCs، hemoglobin، PCV، WBCs و lymphocytes)، البروتين الكلي، الجلوبيولينات، وهرمونات الغدة الدرقية. كما خفضت مستويات الدهون في الدم، بينما عززت مؤشرات الأكسدة والاستجابة المناعية (IgG، IgM، IgA، LTT، نشاط البلعم). علاوة على ذلك، خفضت عدد البكتيريا الضارة وزادت مستويات البكتيريا النافعة. عموماً، أدت الإضافات (حمض الهيوميك أو الفحم الحيوي) إلى تحسين أداء النمو، وهضم العناصر الغذائية، وقياسات الدم، والاستجابة المناعية، وصحة الجهاز الهضمي في دجاج اللحم.