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ORIGINAL PAPER

Extracted and Characterized Humic Substances as Feed Supplement in Rabbit Feeding: Effects on Performance, Blood Metabolites and Caecal Fermentation Activity

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Abstract

This study aimed to evaluate the effect of dietary humic acid (HA) supplementation on the growth performance, nutrient digestibility, carcass characteristics, blood metabolites, and caecal fermentation activity in growing rabbits. Six-week-old New Zealand White (NZW) rabbits (n = 63) with an initial body weight of 726.32 ± 42.85 g, were randomly allocated to three groups of 21 rabbits each. Rabbits were fed on the basal diet with 0 (HA0, control), 5 (HA5) and 10 (HA10) gram/kilogram diet for 70 days in a completely randomised experimental design. A significant heavier ($P < 0.001$) final body weight and a higher average daily gain, with lower feed intake, and a better feed conversion ratio were in HA5 and HA10 rabbits. The digestibility of HA-supplemented diets was greater than that of HA0. Heaviest carcass weights ($P < 0.05$) were observed in the HA5 rabbits, followed by the HA10 rabbits vs. HA0. Protein and ash content of meat increased ($P < 0.001$) in all HA rabbits, while the ether extract decreased ($P < 0.01$) in HA5 and HA10 rabbits. The HA-treated rabbits were distinguished by increased ($P < 0.001$) total protein, albumin, and globulin levels. However, the total cholesterol, triglycerides, and urea levels decreased ($P < 0.001$), and the blood glucose and creatinine levels remained unaffected with HA supplementation. Addition of HA reduced the pH of caecum and sharply decreased ($P < 0.001$) NH_3-N content but it had no significant effect on the levels of total volatile fatty acids and acetic acid. Levels of propionic and butyric acid increased ($P < 0.001$) in response to HA supplementation. In conclusion, supplementation of HA of up to 10 g/kg can be used in diets of growing rabbits, with significant effects on the digestion, growth performance, blood cholesterol concentration, and caecal fermentation activity.

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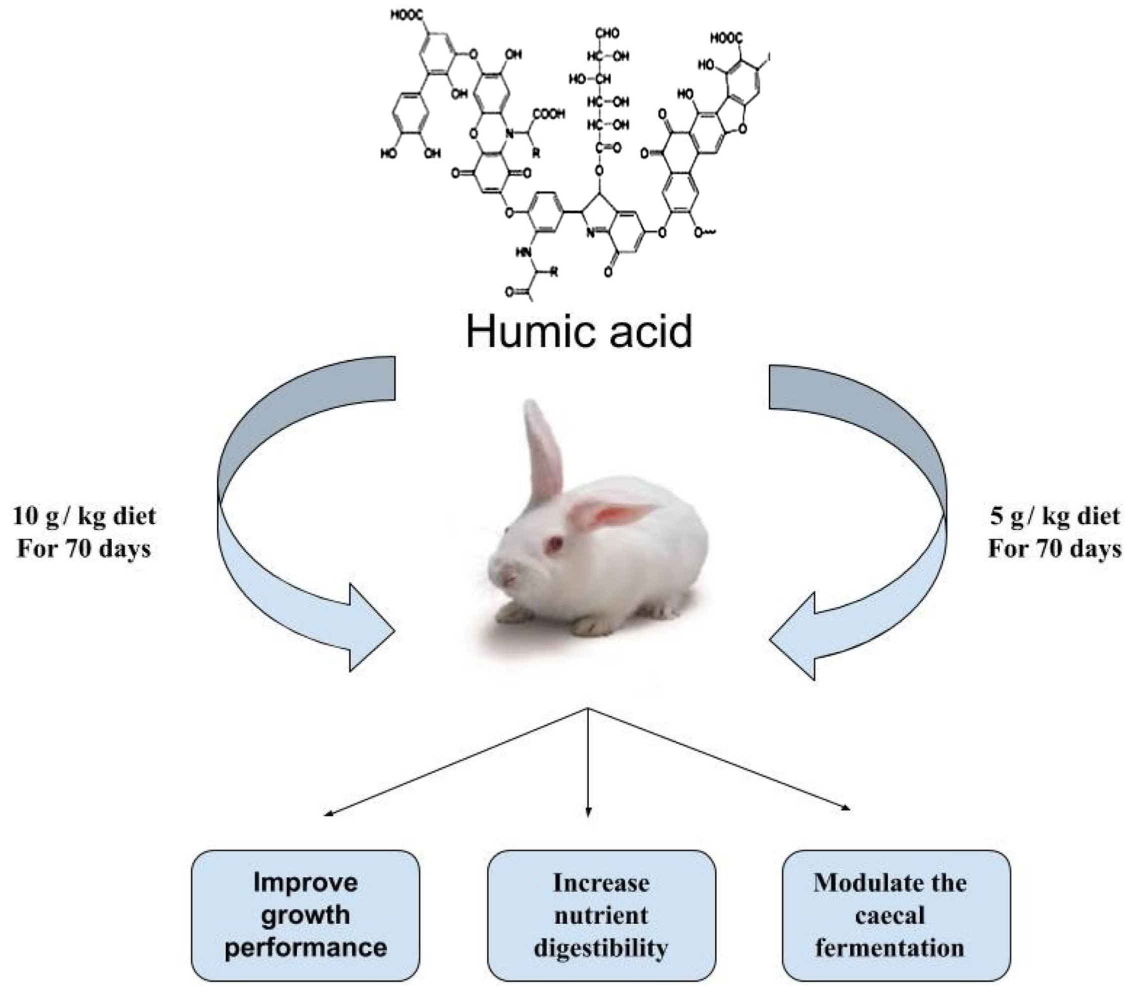
Graphic Abstract



Keywords Humic acid · Blood metabolites · Growth performance · Carcass · Caecum activity · Rabbits

Statement of Novelty

Humic acid (HA) is a widely used alternative growth promoter for antibiotics to improve animal performance and health. The HA in either feed or drinking water can promote growth to increase body weight, feed conversion rate, gastrointestinal motility and decrease the blood cholesterol. It has antimicrobial, absorptive, and detoxifying properties, as well as positively stimulating neutrophil activity and defence mechanisms against pathogens. In the current study, HA treatment was shown to improve body weight, organic matter digestibility, carcass quality and decrease the cholesterol concentration, a novel approach to act as an effective antimicrobial agent for growing rabbits.

Introduction

Research into organic alternatives for feed additives is of integral importance when addressing growth-promoting techniques for livestock production. Humic acid (HA) is a widely used alternative growth promoter for antibiotics to improve animal performance and health. It is the major extractable part of soil humates and is predominantly used to improve soil fertility and enhance nutrient uptake by plants [1]. The HA in either feed or drinking water can promote growth to increase body weight, feed conversion rate, and gastrointestinal motility [2, 3]. It has antimicrobial [4], absorptive, and detoxifying properties [5], as well as positively stimulating neutrophil activity and defence mechanisms against pathogens [6]. Recently, using HA as a growth promoter in animal and poultry farms has become common to improve the body weight and decrease the blood cholesterol in new born kids [7], quail [8] and rabbits [9]. Moreover, heavy metal residue levels declined in the tissues of animals that were fed a HA containing diet [10, 11]. The HA can act as an anti-bacterial, antiviral, and anti-inflammatory agent, reducing mould growth and consequently reducing toxin levels. In addition, HA has an immune stimulant and analgesic effect [2, 10, 12], which is known to have a beneficial effect on the stabilisation of ruminal and intestinal microflora, ensuring enhanced feed efficiency. Moreover, the nitrogen binding capabilities of HA helped to minimise the ammonia nitrogen (NH_3-N) emissions from cattle feedlots [13]. In broilers, Gomez-Rosales et al. [3] found that HA improved the ideal digestibility of energy and the retention of nutrients. Furthermore, HA creates a protective coat in the mucosal membrane of the digestive tract, maintaining the intestinal microflora, and reducing ammonia emission in livestock [5, 14, 15]. Studies have investigated the effect of using HA as a growth promoter in poultry and have obtained positive results [16, 17], with enhanced bird immunity and a reduction in various types of stress [12]. Rabbits fed on a diet supplemented with HA showed a higher body weight gain and a higher feed conversion ratio than rabbits that were fed a basal diet [18]. In addition, HA treatment was shown to improve the organic matter digestibility and decreased the cholesterol concentration in V-line rabbits [9]. Because of the limited information regarding the effects of HA on rabbit production, performance, and fermentation characteristics, the current study was conducted to evaluate these effects on growth performance, nutrient digestion, blood biochemical parameters, and caecal fermentation activity in New Zealand White (NZW) rabbits.

Materials and Methods

This study was conducted at the Rabbit Research Laboratory in Noubaria Experimental Station in compliance with the guidlines approved by the Scientific Ethics Committe of the Animal Production Research Institute, the Agricultural Research Centre, Egypt.

Humic acid Preparation, Extraction, and Characterisation

Humic acid (HA) was extracted from the clayey soil of an experimental farm belonging to the Water and Environment Research Institute at the Agriculture Research Centre, Giza, Egypt. HA extraction and purification were performed according to Aiken [19]. In addition, the carbon, hydrogen, and nitrogen contents of HA were characterised according to [20], while the oxygen was calculated by subtracting (Carbon (C) + Hydrogen (H) + Nitrogen (N))% from 100. The total acidity, carboxyl groups, and phenolic-OH groups were also measured according to [21] (Table 1).

Animals and Diets

A total of 63 growing male New Zealand White (NZW) rabbits aged six weeks, with an average body weight of 726.32 ± 42.85 g, were randomly distributed into three groups (n = 21 rabbits), where each group represented one of the experimental diets. The rabbits were fed a basal diet and subjected to the following dietary rations: the first group was fed a basal diet without supplementation (control; HA0), the second and the third groups were fed the basal diet supplemented with 5 g and 10 g of humic acid (HA) per kg diet, respectively. Rabbits were individually housed in galvanised, metal wire cages and maintained under the same managerial, hygienic, and environmental conditions in rooms with standard air conditioning. Ambient temperature was 23 ± 2 °C, with 55–65% humidity, and a photoperiod of 16 h light: 8 h dark. Each cage was equipped with feeding hoppers and drinking nipples, in which the feed and fresh water were available ad libitum throughout the experimental period of 70 day. The ingredients and the chemical composition of the experimental diets have been summarised in Table 2.

Table 1 Chemical analysis, total acidity, and selected functional groups of humic acid

Elements							Atomic ratio				Ash
C (g/kg)	H (g/kg)	N (g/kg)	P (g/kg)	O (g/kg)	Ca (g/kg)	Fe (mg/kg)	C/H	C/O	C/N	C/P	(%)
461.7	62.1	23.2	8.2	426.9	11.8	307.4	7.4	1.0	19.9	56.4	1.9

Total acidity and selected functional groups (meq/100 g HA)				
Total acidity	COOH	Total—OH	Phenolic—OH	Alcoholic -OH
573.7	257.9	447.1	314.7	133.2

Table 2 The chemical composition of the experimental diet

Ingredients	%
Berseem hay	32.00
Yellow corn	13.10
Barley grains	21.40
Wheat bran	10.30
Soybean meal	18.70
Molasses	3.00
Dicalcium phosphate	0.75
Sodium chloride	0.25
Vitamin and mineral premix	0.30
DL-methionine	0.20
Chemical analysis (% of dry matter)	
Dry matter	90.64
Organic matter	94.84
Crude protein	16.47
Crude fibre	12.33
Neutral detergent fibre	38.13
Acid detergent fibre	17.62
Ether extract	3.25
Nitrogen free extract	62.79
Calcium	0.88
Phosphorus	0.39

Growth Performance

Body weight (BW) of rabbits was recorded weekly, while the feed intake (FI) was recorded daily. The average daily gain (ADG) and the feed conversion ratio (FCR) were calculated, while the mortality rate was recorded as the number of dead animals in each group during the experimental period.

Digestibility Trials

Six rabbits from each group were individually housed in metabolic cages for a digestibility trial of 7 days. The cages facilitated the separate collection of faeces and unconsumed feed from the urine to determine the digestibility. Faeces of each rabbit were collected prior to feeding in the morning. A sample representing 20% of the collected faeces from each rabbit was sprayed with 10% sulfuric acid and 20% formaldehyde and was dried at 60 °C for 24 h. Next, the samples of the feed and faeces were finely ground through a 1 mm screen in a Cyclotec mill (Cyclotec 1093; Foss, Germany) and stored prior to chemical analysis. Similar to the study by Abdl Razek et al. [9] the apparent digestibility coefficients of the dry matter (DM), organic matter (OM), crude protein (CP), crude fibre (CF), neutral detergent fibre (NDF), and acid detergent fibre (ADF) were estimated accordingly [22].

Carcass Characteristics and Meat Quality

At the end of the experiment, six rabbits from each group were randomly selected and starved for 12 h with the provision of water *ad libitum.* Rabbits were individually weighed and then sacrificed by cutting the jugular vein to determine the carcass parameters. To obtain slaughter weight, the rabbits were weighed after complete exsanguination. The dressing percentage (slaughter weight/body weight) was calculated, while the total edible offal (giblets) was weighed, including the heart, liver, and kidneys. Moisture, protein, ether extract, and ash contents were determined from 50 g of the minced meat samples that were obtained from the right half of the carcass, which was dried at 70 °C for 36 h. Subsequently, the dried meat samples were ground and analysed according to [22].

Blood Sampling and Biochemical Analysis

At the end of the experiment, 3 ml of blood from the sacrificed rabbits was collected in labelled clean tubes and immediately centrifuged at 5,000 round per minute (rpm) for 10 min at 20 °C. The serum was separated and kept at − 20 °C until the biochemical analyses to determine the total protein, albumin, glucose, total cholesterol, triglyceride, urea, and creatinine concentrations. Blood biochemicals were colorimetrically determined using the standard kits supplied by Bio-Merieux, France.

Analysis of Caecal Content

After slaughter, the caecal content of each rabbit was evacuated into a clean sterile beaker and immediately strained through two layers of sterile gauze, and the pH of the collected liquid was measured using a digital pH meter. The caecal contents were then centrifuged at 7000 rpm for 12 min at 20 °C, where the resulting supernatant was divided into two parts. The first part was acidified with a 0.2 M hydrochloric acid solution (1 ml-ml^{-1} sample) to determine the ammonia nitrogen (NH_3-N) concentration, while the other part was treated with a solution of 5% orthophosphoric acid (v/v) in addition to 1% mercuric chloride (w/v) (0.1 ml-ml^{-1} sample) to determine the total volatile fatty acid (VFA) concentration and the individual VFA proportions. The caecal NH_3-N concentrations were measured based on calorimetric methods using a spectrophotometer (Shimadzu, Kyoto, Japan) according to the method of Chaney and Marbach [23]. The total VFA concentrations were measured based on steam distillation as described by [24], while the molar concentrations of the acetic, propionic, and butyric acids were analysed using high-performance liquid chromatography (HPLC; Model Water 600; UV detector, Millipore Crop., USA) according to [25].

Statistical Analysis

The collected data were analysed using the linear model (lm) procedure in R software version 3.6.3, where the fitted model included the terms:

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

where Y_{ij} refers to the observation, which consisted of the initial weight (g/rabbit/d), the final weight (g/rabbit/d), the ADG (g), the feed intake (g/rabbit/d), the FCR (g feed/g BW gain), the digestibility of DM, OM, CP, and CF, as well as the carcass, meat quality, caecal fermentation, and blood biochemical parameters, while μ refers to the overall mean, D_i is the effect of ith diet, and e_{ij} is the random residual error, with the diet being the fixed effect in the model.

A Chi-square test was performed to predict the effects of the diet and all the results have been expressed as the least square mean values ± standard error, while the differences were declared significant at $P < 0.5$.

Results and Discussion

Growth Performance

Humic acid (HA) administration produced an increase in the final body weight (FBW) ($P < 0.001$) of the growing NZW rabbits in the HA5 and HA10 groups (Table 3). Rabbits that were fed a 10 g HA/kg diet showed the highest ADG, followed by those that were fed a 5 g HA/kg diet. A lower FI was observed in the rabbits that were fed the HA5 and HA10 diets compared to the HA0 diet ($P < 0.001$), while the rabbits that were fed the HA5 and HA10 diet had a better FCR. The use of humic additives in animal feed has been reported that have a positive influence on growth performance. Such a result was obtained in the present study which reported that final BW improved significantly by 13.61% and 16.1%, while the ADG was higher by 10.55% and 13.82% in the rabbits that were fed HA5 and HA10 rations, respectively, relative to the control group. This concurs with the previous studies which reported that diets supplemented with HA improved BW, ADG, and FCR in Japanese quail [8], NZW rabbits [18], sasso chicken [26], and V-line rabbits [9]. Groups supplemented with 5 g and 10 g of HA in the diet had a better FCR compared to the control group. These positive effects of HA on BW, ADG, and FCR in rabbits could be explained by the ability of HA to facilitate nutrient digestion, absorption, and metabolism, providing sufficient nutrient supply to achieve a higher growth rate [27]. Likewise, Avci et al. [28] and salah et al. [29] reported that supplementing humates to broiler diets improved the FCR. In another study, Arif et al. [30] found that HA in the diet improved the feed efficiency, as organic acids improve the absorption and conversion of nutrients in the body and improve overall gastric function [31]. The same tendency was also noted by Wang et al. [32] who also reported that the HA may stabilise the pig gut microflora and lead to improved nutrient absorption resulting in a beneficial increase in the ratio of body weight gain to feed intake. Selected modes of action have been proposed to clarify the advantages to poultry of humic substance supplementation [33], such as: (i) the capacity to make defensive layers over the epithelial mucosal film of the gut against the passage of toxic and other bacterial-contaminated substances; (ii) the capacity to reduce the pH of the digestive tract may lead to the repression of intestinal microscopic organisms, leading to decreased metabolic needs, and increased metabolism of protein and microbial carbohydrates, thereby increasing the availability of nutrients; (iii) the ability to reduce the absorption of nitrates, fluorites, and heavy metals, thereby detoxifying the gut, and (iv) increase in the immune receptors in the gut lining to protect against pathogens and promote growth.

Table 3 The growth performance of growing rabbits fed a diet supplemented with humic acid (HA)[1]

Items	HA0	HA5	HA10	SEM	*P* value
IBW (g)	725.7	726.5	726.6	58.99	0.940
FBW (g)	2650.9^a	3011.7^b	3077.1^b	60.90	< 0.001
ADG (g/d)	27.5^a	30.4^b	31.3^b	0.60	< 0.003
FI (g/d)	108.6^b	101.3^a	104.6^a	2.11	< 0.001
FCR	4.0^b	3.3^a	3.5^a	0.25	0.031

Least square mean values with different superscript letters in the same row are significantly different at $P \le 0.05$

HA0 No HA, *HA5* HA 5 g/kg diet, *HA10* HA 10 g/kg diet, *IBW* initial body weight, *FBW* final body weight, *ADG* average daily gain, *FI* feed intake, *FCR* feed conversion ratio

Digestibility Trials

Nutrient digestibility was found to be affected by HA treatment (Table 4), where the digestibility coefficients of the DM, OM, CP, CF, NDF, and ADF increased with the dietary HA relative to the basal diet. Rabbits fed on diet supplemented with HA5 had better digestibility value for CP, followed by those in the HA10 group compared to the control group. Likewise, Abdl Razek et al. [9] found that the addition of HA in diets of V-line rabbits significantly increased the digestibility of OM. Thus, the improvement in the digestibility of nutrients in this study could be explained by the ability of HA to maintain the intestinal microflora and ensuring the increased absorption and digestion of nutrients [34], which improved the utilisation of nutrients in the animal feed [13] The current results are consistent with those of Písaříková et al. [35] who reported that supplementation

Table 4 The nutrient digestibility of growing rabbits fed on diets supplemented with humic acid (HA)

Items	HA0	HA5	HA10	SEM	*P* value
Digestibility coefficients (%)					
Dry matter	62.0^{a}	63.0^{b}	62.2^{a}	0.24	0.021
Organic matter	64.0^{a}	65.9^{b}	65.4^{b}	0.20	0.002
Crude protein	63.4^{a}	65.0^{c}	64.3^{b}	0.16	0.001
Crude fibre	41.8^{a}	44.6^{b}	44.5^{b}	0.18	< 0.001
NDF	62.8^{a}	63.5^{b}	63.4^{b}	0.21	< 0.001
ADF	49.4^{a}	51.5^{b}	51.0^{b}	0.13	< 0.001

Least square mean values with different superscript letters in the same row are significantly different at P < 0.05

HA0 No HA, *HA5* HA 5 g/kg diet, *HA10* HA 10 g/kg diet, *NDF* neutral detergent fibre, *ADF* acid detergent fibre, *SEM* standard error of mean

with humic substances improved the ileal digestibility of CP and CF, which tends to increased ileal digestibility of DM, CF, and ash in growing pigs. When humic substances are supplemented in broiler diets, they increase the length of both the mucosal villi of the jejunum and the gut length [33], both of which are associated with improved nutrient digestibility due to the reduction in the passage rate of the intestinal content and the concomitant increase in enzymatic digestion.

Carcass Characteristics and Meat Composition

The heaviest ($P < 0.001$) carcass weights were obtained in the rabbits of the HA5 group, followed by those were found in the HA10 group compared to those in the HA0 group (Table 5). No significant differences were detected with respect to the relative heart, liver, and kidneys weights across the treatment groups. The moisture content of the rabbit meat was not affected by the administration of HA, while the CP and ash contents did increase. However, the ether extract content was reduced ($P < 0.01$) in the HA5 and HA10 rabbits relative to the HA0 rabbits. Supplementing HA in the rabbit diet increased BW, which was reflected in the slaughter and carcass weights. Ozturk et al. [11] reported that the slaughter and carcass weights were higher in broilers that were fed diets supplemented with HA relative to broilers that were fed a basal diet. Abdel-Mageed [8] and Mirnawati and Marlida [36] reported the same effect of humic substances in quail and broilers. Different feed additives have been found to be effective in changing meat protein content in rabbits [37, 38] Consistent with the current study, Miśta et al. [18] observed a slight increase in meat protein in the NZW rabbits due to being fed HA, however changes in meat quality may be attributed to pH changes after slaughter.

Table 5 Characteristics of the carcass and the chemical composition of the rabbit's meat fed on diets supplemented with humic acid

Item	HA0	HA5	HA10	SEM	*P* value
Slaughter weight (g)	2122	2377	2219	136.6	0.861
Carcass weight (%)	1336^{a}	1580^{c}	1485^{b}	26.0	< 0.001
Liver weight (%)	2.62	2.59	2.61	0.023	0.599
Kidney weight (%)	0.79	0.79	0.79	0.011	0.889
Heart weight (%)	0.31	0.30	0.30	0.006	0.617
Chemical composition of the meat (%)					
Moisture	71.16^{b}	70.41^{a}	70.17^{a}	1.253	0.063
Protein	21.18^{a}	22.53^{b}	22.21^{b}	0.362	< 0.001
Ether extract	4.55^{b}	4.18^{a}	4.04^{a}	0.051	< 0.01
Ash	1.28^{a}	1.44^{b}	1.48^{b}	0.018	0.002

The least square mean values with different superscript letters in the same row are significantly different at P < 0.05

HA0 No HA, *HA5* HA 5 g/kg diet, *HA10* HA 10 g/kg diet, *SEM* standard error of mean

Blood Biochemical Parameters

The rabbits that were fed HA had higher levels of blood total protein, albumin, and globulin relative to the rabbits that were fed a basal diet ($P < 0.001$) (Table 6). However, feeding HA did not significantly affect blood glucose and creatinine concentrations. Moreover, feeding rabbits HA rations caused a significant decrease in the concentrations of total cholesterol, triglyceride, and urea. Rabbits fed the HA5 and HA10 diets showed an increase in total blood protein, albumen, and globulin levels, indicating the role of HA in modulating protein metabolism, in addition, increased globulin levels reflect the increased ability of humate substances to stimulate the immune system of animals [39, 40]. Similarly, Rząsa et al. [41] found that supplementing the diets of growing rabbits with 10% humic-fatty acid increased the total protein and albumin levels relative to 5% humic-fatty acid and control diets, which concurs with the current and previous studies on

Table 6 The blood biochemical parameters in growing rabbits fed on diets supplemented with humic acid

Item	HA0	HA5	HA10	SEM	*P* value
Total protein (g/dl)	6.39^{a}	7.35^{b}	7.56^{c}	0.253	< 0.001
Albumin (g/dl)	4.17^{a}	4.58^{b}	4.68^{b}	0.111	< 0.001
Globulin (g/dl)	2.21^{a}	2.76^{b}	2.88^{b}	0.134	< 0.001
Glucose (mg/dl)	79.37	79.88	80.20	5.254	0.547
Total cholesterol (mg/dl)	88.97^{c}	78.88^{b}	77.58^{a}	2.522	< 0.001
Triglyceride (mg/dl)	80.38^{c}	69.93^{ab}	68.07^{a}	1.580	< 0.001
Urea (mg/dl)	35.93^{c}	32.13^{ab}	30.69^{a}	1.493	< 0.001
Creatinine (mg/dl)	0.77	0.78	0.79	0.007	0.899

Least square mean values with different superscript letters in the same row are significantly different at P < 0.05

HA0 No HA, *HA5* HA 5 g/kg diet, *HA10* HA 10 g/kg diet, *SEM* standard error of mean

V-line rabbits [9], goats [42], and Japanese quail [28]. However, glucose levels did not vary across the groups, which could be a useful indicator of the improvement in the energy status of the rabbit. These results are in agreement with those of Abdl Razek et al. [9] who indicated that HA had no effect on V-line rabbit plasma glucose level and with Avci et al. [28] who reported that supplementation of HA has no effect on glucose in Japanese quails, and with Degirmencioglu [42] who confirmed that goats administration with HA identified any difference in serum glucose. In this study, the blood metabolite levels indicated that rabbits supplied with HA were in a normal physiological state [43] and had a normal energy status, reflecting no negative effects by HA, which might explain the lower FI in the HA administered rabbits. In addition, a higher concentration of caecal propionate is associated with high liver gluconeogenesis, raising the concentration of blood glucose. This pattern may have been associated with the rapid flux of caecal propionate into the bloodstream that was converted to glucose in the liver. Furthermore, HA supplementation decreased cholesterol and triglyceride levels in rabbits, which was also observed by Abdl Razek et al. [9] who reported that HAsupplemented diets decreased cholesterol concentration in V-line rabbits. In brown laying hens, supplementation with humic substances reduced blood cholesterol concentration at the age of 22 weeks [44]. Hassan et al. [45] also reported that supplementing with humate substances decreased serum cholesterol levels in dairy cows, while El-Zaiat et al. [39] reported lower blood cholesterol levels in goats treated with HA and Ho et al. [46] concluded that humate substances are known to release iron from ferritin, consequently accelerating lipid peroxidation and decreasing blood cholesterol levels. The low blood cholesterol may also be due to a decrease in the microbial intracellular pH [47], where the inclusion of HA10 caused a decrease in urea concentration. In corroboration, Rath et al. [48] observed a reduction in blood urea N in broilers that were supplemented with humate substances, where the reduced blood urea nitrogen content in humate diets was directly associated with the decreased ruminal NH_3-N content [49, 50]. Finally, the creatinine concentration was similar across treatments. However, Abdl Razek et al. [9] reported a reduction in creatinine concentration in the HA-treated groups in V-line rabbits, and Rath et al. [48] also reported a decline in the blood creatinine levels in HAfed broiler chickens.

Caecal Fermentation Activity

The pH of the caecum was significantly reduced with the administration of HA compared to the control group (Table 7). In addition, the concentration of caecal NH_3-N decreased sharply in rabbits that were fed HA relative to the control, while the administration of HA had no significant effect on the VFA. Finally, the addition of HA to the rabbit diet resulted in increased propionic and butyric concentrations ($P < 0.001$), while the level of acetic acid was not affected by HA administration. The caecal pH was significantly reduced with the administration of HA, which is inconsistent with the results obtained by [9], but the NH_3-N content was decreased with HA supplementation. Because of the ion-complex forming property, the humic substance binds to the ruminal-nitrogen, thus, reducing the NH_3-N content [45, 51]. A decrease in NH_3-N concentration in the present study was a favourable outcome, as the HA diet may promote N utilisation by reducing the loss of NH_3-N due to the decrease in the solubility of N under the inhibiting effect of urease activity [13, 39]. However, higher NH_3-N content may increase the serum NH_3-N content, subsequently increasing N excretion through the urine, thereby causing environmental pollution [49, 50]. In addition, the lower NH_3-N content was accompanied by a decrease in the count of protozoa and the microbial population shift [39, 45]. El-Zaiat et al. [39] reported the ability of HA to alter rumen fermentation by sequestering NH_3-N, and then slowly releasing it for microbial growth by reducing the NH_3-N concentration by 20%. However, Abdl Razek et al. [9] found that higher levels of NH_3-N were in the caecum of rabbits that were fed HA-supplemented diets relative to those fed a control diet. Moreover, the present study showed no significant differences across treatments with respect to the TVFA concentration. This finding was consistent with a few studies reporting the proportion of unaltered TVFA when feeding HA diets [9, 52]. Although the acetate proportion did not differ across treatments, the HA supplementation increased the concentrations of propionic and butyric acids, where El-Zaiat et al. [39] stated that administration of HA increased ruminal acetate and propionate concentrations, but had no significant effect on ruminal butyrate. Thereby, the VFA results obtained in the present study could be explained by the ability of HA to improve nutrient digestion, utilisation, and absorption. Hence, by analysing the beneficial effect of

Table 7 The caecal fermentation activity in growing rabbits fed on diets supplemented with humic acid

Item	HA0	HA5	HA10	SEM	*P* value
pH	6.13^b	6.01^a	5.95^a	0.061	0.012
NH_3-N (mmol/L)	18.3^b	14.32^a	13.92^a	0.390	<0.001
TVFA (mmol/L)	59.61	59.93	59.91	0.242	0.511
Acetic acid (%)	35.63	35.80	35.78	2.233	0.526
Propionic acid (%)	11.22^a	13.48^b	13.5^b	0.193	<0.001
Butyric acid (%)	4.36^a	5.39^b	5.48^b	0.091	<0.001

The least square mean values with different superscript letters in the same row are significantly different at $P < 0.05$

HA0 No HA, *HA5* HA 5 g/kg diet, *HA10* HA 10 g/kg diet, *TVFAs* total volatile fatty acids, *SEM* standard error of mean

HA on the caecal fermentation activity, the use of HA as an organic additive for rabbits is clearly a novel approach to act as an effective antimicrobial agent.

Conclusion

A total of 10 g HA/kg of diet can be used as a natural additive to improve the growth performance, nutrient digestibility, and blood cholesterol concentration, and modulate the caecal fermentation activity without any negative effects on the health of growing rabbits. Overall, using HA is an alternative to antibiotics in animal feeding. However, further investigations are required to evaluate the economic return of the HA dietary supplementation when compared to the typical feeding regimen.

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Compliance with Ethical Standards

Conflict of interest Authors declare that no conflict of interest.

Ethical Approval This study was conducted in compliance with the guidelines approved by the Scientific Ethics Committee of the Animal Production Research Institute, the Agricultural Research Centre, Egypt.

Informed Consent All authors agree to participate in the current work. All authors agree to publish the findings of the current research.

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