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The Use of Humic Acid in Improving Roughages and to Enhance the Performance of Iraqi Local Black Goats

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Abstract | The present aim to study the effect of humic acid (HA) supplementation on growth performance, nutrient utilization, rumen fermentation and microbial ecology in local Iraqi black goats. Eighteen male goats (4-month-old, 18.6 -0.4 kg BW) were randomly placed in three diets control (no HA), 3kg HA t -1DM, and 5kg HA t -1DM for 105 days. Comparison and control with HA supplementation showed that there are significant ($p < 0.05$) differences in average daily gain (ADG), feed conversion ratio (FCR), and total volatile fatty acids (VFAs). It also altered the molar ratios of major VFAs to raise the propionate and lower the acetate thus causing a lower acetate: propionate ratio and less methane production. The upregulation of cellulolytic bacteria *(Ruminococcus albus, Fibrobacter succinogenes, Prevotella ruminicola) and inhibition of methanogenic archaea (Methanobrevibacter smithii, Methanobacterium formicicum)* by HA were proved by quantitative PCR and 16S rRNA sequencing. Also, alpha-diversity indices (Chao1, Shannon, Simpson) were significantly higher suggesting a more diverse and stable rumen ecosystem. When combined all these effects of humic on the rumen fermentation, VFA profile and microbial ecology means that it can be used to increase the efficiency of the ruminant digestion and thus the optimum energy efficiency by increasing the fermentations and reducing the generation of enteric methane. Taken together, HA is an eco-friendly additive that enhances productive efficiency, as well as environmental efficiency of small-ruminant systems.

Keywords | Humic acid, Goats, Rumen microbiota, Volatile fatty acid, Microbial diversity, Growth performance, Reduction of methane

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INTRODUCTION

Humic compounds are recent subjects of interest as bioactive feed additives in animal nutrition, as a by-product of natural decomposition of residues in plants and animals. Such compounds possess broad physiological and microbiological effects including an advantage in intestinal health, enhancement of nutrient absorption and gut microbial population regulation (de Lourdes *et al*., 2022; Hu *et al*., 2022; Maffia *et al*., 2025). They are multitasking in nature since they have functional groups active in the intestine such as carboxyl and phenolics hence chelate minerals, stabilize intestinal microflora and facilitate enzyme activity in the digestion process.

Humic substances have been suggested as natural antibiotic

growth stimulant alternatives in ruminant production, which is in line with the global transition to residue-free animal feeds and sustainable feeds (Malyugina and Horky, 2024). Ruminants are able to break down fibrous feeds and generate volatile fatty acids and microbial protein by a unique set of microbes inhabiting the rumen (Xu *et al*., 2025; Du *et al*., 2025). Nevertheless, inadvertent fermentation practices or low-fiber diets have the ability to decrease microbial productivity, resulting in decreased efficiency in the use of the feed and performance of animals. Having humic acid in the ruminant diet could potentially improve the equilibrium of the microbes, enhance the proliferation of cellulolytic bacteria such as *Ruminococcus albus and Fibrobacter succinogenes*, prevent the increase of the number of methanogenic archaea, improve its fermentation efficiency, and curtail the excretion of energy in the form of methane (Terry *et al*., 2018).

Iraqi local black goats as a great natural resource in Iraq are well suitable to the arid and semi-arid regions of the country but have difficulties in growing with a low-quality roughage base and limited nutrient production (Alkass and Mustafa, 2023). Increasing their nutritional efficiency and sustainability of production by feeding them with eco-friendly feed additives. Humic acid that is naturally constructed and biodegradable may serve as a fiber improver to increase bacterial penetration within the cell wall and help extract nutrients from the poor-quality forages (Sheng *et al*., 2019; Dwatmadji *et al*., 2025).

The present study sought to answer these questions by establishing how dietary humic acid influenced growth performance, nutrient digestibility and the rumen microbial community structure amongst Iraqi local black goats. The hypothesis of the study was that supplementing goat diets with humic acid would enable the ruminant metabolism, microbial community balance, and indirectly help to achieve improved feed efficiency and animal performance under the conditions of local fodder.

MATERIALS AND METHODS

Experimental design and animals

The experiment was carried out in Animal Field, College of Agriculture, University of Basrah, Iraq. There were three dietary treatments (n= 6) of eight male Iraqi local black goats (4 months, initial body weight 18.6 ± 0.4 kg). The feeding experiment took 105 days inclusive of a 15-day adaptation period.

Dietary treatments

Control (T1): Basal diet (40% concentrate, 60% alfalfa hay), T2: Basal diet + 3 kg humic acid per ton of feed (DM basis) and T3: Basal diet + 5 kg humic acid per ton of feed (DM basis). The concentrate was a mixture of 50 percent grains of barley, 37 percent wheat bran, 10 percent yellow corn, and 3 percent vitamin-mineral premix. The diet was completely mixed with humic acid (commercial grade, 85 percent purity). Goats were fed on basal diet on maintenance and moderate growth according to the recommendations by NRC (2007). The basic diet consisted of 40 percent concentrate and 60 percent DM basis alfalfa hay. The concentrate mixture consisted of barley (45%), wheat bran (37%), yellow corn (10%), soybean meal (7%), and a vitamin -mineral premix (1%). AOAC (2016) was used to acquire the feeding diet in terms of dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), ash, and nitrogen-free extract (NFE). The chemical composition was used to measure metabolizable energy (ME) using the NRC (2007) equation: ME (MJ/kg DM) = 0.01551 × DCP + 0.03431 × TDN where DCP = digestible crude protein (%) and TDN = total digestible nutrients (%) were used. Chemical and energy constituents of the feed ingredients and the mix of concentrate are presented in Table 1.

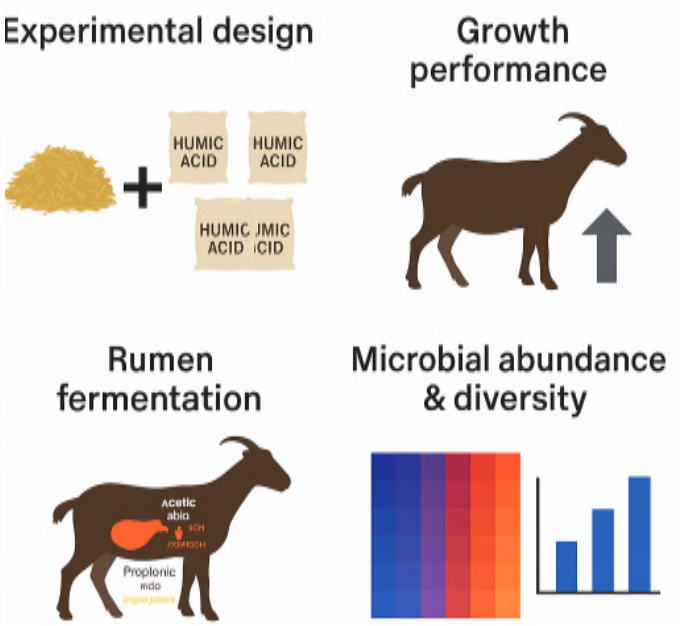

Figure 1: Various phases of the experimental system-starting with feeding treatments to rumen fermentation and microbial analysis.

Sampling and analysis

The amount of feed consumed on a daily basis was recorded; body weights were taken after every two weeks. The pH, volatile fatty acid (VFA), ammonia-N, and microbial DNA extraction were sampled using rumen fluid at day 90. qPCR was used to quantify the microbial DNA based on *Ruminococcus albus, Fibrobacter succinogenes, Prevotella ruminicola, Streptococcus bovis, Butyrivibrio fibrisolvens, Ruminococcus flavefaciens, Methanobrevibacter smithii and Methanobacterium formicicum.*

Table 1: Chemical composition and estimated metabolizable energy (ME) of feed ingredients and concentrate mixture used in the experimental diets (% on dry matter basis).

Nutrient	Alfalfa hay	Corn	Soybean meal	Wheat bran	Barley	Concentrate mixture
Dry matter (%)	91.11	92.40	91.70	90.42	90.32	90.24
Crude protein (%)	16.40	8.70	45.90	13.50	10.50	14.15
Ether extract (%)	1.28	4.50	7.21	4.61	2.12	2.38
Crude fiber (%)	32.28	7.31	2.51	10.71	6.11	17.47
Ash (%)	6.75	2.33	6.14	4.35	2.99	4.77
Nitrogen-free extract (%)	34.40	77.16	38.24	66.83	78.28	56.80
Metabolizable energy (MJ/kg DM)	8.95	13.20	12.60	11.70	12.80	12.10

Values are means of examined triplicates. The values of metabolizable energy (ME) were calculated based on the digestible nutrient content using NRC (2007).

Determination of volatile fatty acids (VFAs)

On day 90, the samples of rumen fluid (around 50 mL) were obtained through stomach tubing before the morning feeding. Samples were filtered on the spot using 4 layers of sterile cheesecloth, snap-frozen in liquid nitrogen, and stored at −80 °C until microbial DNA extraction. Aliquots of 1 mL of the total genomic DNA were lysed with the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer instructions and bead-beating (2 × 30 s at 6 ms^{-1}) to enhance Gram-positive bacterial lysis. The purity and concentration of the DNA were spectrophotometrically monitored (NanoDrop 2000, Thermo Fisher Scientific) and normalized to 20 ng μL^{-1}. A StepOnePlus Real Time PCR System (Applied Biosystems, USA) was used to perform a quantitative PCR (qPCR) using SYBR Green chemistry. In every 20 µL reaction, 10 µL SYBR Green Master Mix (2x), 0.5 µM primer, and 2 µL template DNA were used. The conditions of cycling were initial denaturation at 95 °C (5min); 40 cycles of 95 °C (15s), 60 °C (30s) and 72 °C (30s) each. The absolute quantification was conducted using standard curves which were created using serial dilution of 10-fold (102-108 copies 250-3002) of plasmids containing cloned fragments of 16S rRNA genes. The results were calculated as the log 1/0 copies that were subtracted by each target microorganism rumen content in g. These targeted taxa were six dominant bacterial species, namely: *Ruminococcus albus, Ruminococcus flavefaciens, Fibrobacter succinogenes, Prevotella ruminicola, Butyrivibrio fibrisolvens, and Streptococcus bovis as well as two dominant archaea namely: Methanobrevibacter smithii and Methanobacterium formicicum.* The microbial diversity was determined by using the universal 16S rRNA primers (341F/806R). Sequencing of amplicons was done on an Illumina MiSeq system (2 × 250 bp paired-end). Processing of sequences was done with QIIME 2 v2024.2 and DADA2 was used to denoise and SILVA 138 to taxonomically classify the sequences. Indices of diversity (Chao1, Shannon, Simpson) and relative abundances of the genera were determined.

Microbial quantification and diversity analysis

On the morning of day 90 of the experiment, rumen fluid samples (about 50 mL) were gathered using stomach tubing. Samples were filtered right away in four layers of sterile cheesecloth and transferred to sterile tubes, frozen in liquid nitrogen and kept in -80 °C before microbial DNA extraction. The 1 mL aliquots were processed to total genomic DNA using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) according to the manufacturer protocol including an extra bead-beating step (2 x 30 s at 6m s^{-1}) to enhance the lysis of Gram-positive bacteria. Purity/concentration of the DNA was viewed spectrophotometrically (NanoDrop 2000, Thermo Fisher Scientific) and normalized to 20 ng μL^{-1}. Quantitative PCR (qPCR) was done on a StepOnePlus Real-Time PCR System (Applied Biosystems, USA) using SYBR Green chemistry. The reaction volume in each of the 20 µL tubes was 10 µL of 2× SYBR Green Master Mix, 0.5 µM primer of each primer and 2 µL template DNA. The cycling conditions included: first denaturation at 95 °C, 40 cycles at 95 °C, 15 s, second cycle, then 60 °C, 30 s, and 72 °C, 30 s. Standard curves were prepared using serial 10-fold dilution (10 2108 copies µ L^{-1}) of plasmids carrying cloned fragments of the 16S rRNA genes. The standard curves were used to determine the absolute value. The results were presented as the number of log 10 copies of g^{-1} rumen content of each target microorganism. The selected taxa were six major bacterial species: *Ruminococcus albus, Ruminococcus flavefaciens, Fibrobacter succinogenes, Prevotella ruminicola, Butyrivibrio fibrisolvens and Streptococcus bovis and two predominant archaea: Methanobrevibacter smithii* and *Methanobacterium formicicum.* The microbial diversity was evaluated on the basis of universal 16S rRNA primers (341F/806R). The sequences of the amplicons were sequenced on an Illumina MiSeq (2 × 250 bpaired end). The processing of sequences was done in QIIME 2 v2024.2 in combination with DADA2 to denoise the sequences and SILVA 138 to classify the sequences taxonomically. The relative abundance, diversity indices (Chao1, Shannon, Simpson) were determined at the genus level.

Statistical analysis

One-way ANOVA and the multiple range test of Duncan were used in data analysis. Differences of any kind were regarded as significant at $p < 0.05$. The rumen content data of rumen microbial diversity analysis was founded on the log10 copies g^{-1}. The values were then transformed into absolute linear abundance and put into relative percentages and the indicators of microbial diversity were obtained to be statistically analyzed. Performing the calculation of α-diversity indices based on the Shannon and Simpson coefficients to obtain the values of these variables helped to establish the richness of microbes and homogeneity in the coefficients. The significance of the β-diversity was tested using PERMANOVA based on the number of microbial communities that can be different in each area using the Bray-Curtis distance. Both differences in the relative abundance of the major bacterial classes of the data were analyzed by One-way ANOVA, and the results were compared against the Tukey HSD test at the level of significance ($P \leq 0.05$). The diversity of microbial communities (alpha and beta diversity) was compared with the phyloseq (McMurdie and Holmes, 2013) and vegan (Oksanen *et al*., 2022) packages of R software (version 4.3.0).

RESULTS AND DISCUSSION

Growth performance

The data on growth is summed up in Table 2. Final body weight and daily gain of goats fed with humic acid-supported diets was much greater than control animals ($p < 0.05$) and ratio of feed to gain was improved. The improvement was higher at 5 kg HA/t DM DM. Weight of lambs fed on humic acid supplementation at 3 and 5 kg/t dry matter (28.9 and 29.6 kg) differed between the control group and the lambs fed humic acid. This rise in end weight is probably explained by an improved digestion and growth of more nutrients preconditioned by humic acid, which positively impacts the environment of the rumen and nutrient uptake (Terry *et al*., 2018). Mean daily gain also varied significantly ($P= 0.03$) with the second and third treatments having a higher rate (97 and 105 g/d, respectively) as compared to the control (78 g/d). This evidence discovered that this increased protein and energy conversion efficiency with the addition of humic acid positively affected the growth too (de Lourdes *et al*., 2022). Feed conversion ratio ($P= 0.02$) increased with increase in humic acid dosage and improved, as compared to (8.5) in the control group, (7.1) and (6.8) in the second and third groups, respectively. This reduction indicates that the use of humic acid as forage catalyst has become more efficient, which was expected in the view of the fact that humic acid as forage catalyst is confirmed to be necessary to maximize the output of production and minimize the loss of food content (Malyugina *et al*., 2024). The findings are in line with those of Kholif *et al*. (2021) that also reported comparable performance improvements in ruminants fed humic compounds.

Table 2: Growth performance of goats fed diets with different levels of humic acid.

Parameter	Control	3 kg HA/t DM	5 kg HA/t DM	SEM	p value
Initial weight (kg)	18.6	18.7	18.6	0.12	0.98
Final weight (kg)	26.8b	28.9a	29.6a	0.31	0.04
Average daily gain (g/day)	78b	97a	105a	2.6	0.03
Feed conversion ratio	8.5a	7.1b	6.8b	0.27	0.02

Values in the same row with different superscripts differ significantly ($p < 0.05$).

Rumen fermentation and microbial populations

Table 3 and Figure 2 indicate that the humic acid did not influence the pH of the rumen ($P = 0.21$) such that it was not within the range of (6.5–6.7), which is said to be the optimal microbiotic activity range in the rumen (6.27). These results lead to a conclusion that the introduction of acid did not play an important role in the balance of the rumen environment and pH, which means that the acid did not have a negative effect on the natural fermentation processes (Terry *et al*., 2018). The concentration of NH3-N was significantly high in the groups with the humic acid supplementation (17.6 and 18.1 mg/dL) compared to the control group (14.3 mg/dL). It was likely caused by such increase that there was an improved digestion of protein, and that the production of proteolytic products out of rumen was increased by the activation of microorganisms, which were primarily proteolytic bacteria and fibers. These outcomes were comparable to Yin *et al*. (2025). According to Malyugina *et al*. (2024), humic acids activate the development of microbial cells in the rumen and enhance the internal environment of rumen, the level of NH3-N needed by microorganisms to synthesize proteins will also be higher. There was a significant increment of VFA concentration with increasing humic acid ($P = 0.03$) and the values were 88.5, 93.8, and 96.1 mmol/L under normal conditions and 3 kg and 5 kg, respectively. This is an augmented microbial fermentation (in this instance microbial fermentation performance), and carbohydrate digestion, which in turn advances energy output by means of energy to support microbial growth (El-Zaiat *et al*., 2018). Regarding the estimated production of the methane, in the humic acid addition (25.1 and 23.9 ml/g dry matter), it was found to decrease statistically ($P = 0.01$) compared to the control group (28.4 ml/g). This decrease is associated with an alternative fermentative route between methane and propionate, as it considers the negative impact of certain acid elements on methane organisms, which adds to the minimization of energy losses out of methane. This

is in accordance with the results described in the report Kaevska *et al*. (2021) that humic acid has the ability to align microbial state to the extent that it was observed that the metabolic activity of methanogens was inhibited in the rumen. The findings revealed that addition of humic acid at the 3 and 5 kg/ t dry matter enhanced rumen fermentation thus resulting in the generation of volatile fatty acids, reduction of methane generation without any significant effect on rumen pH. It can be observed that there was an improved fermentation environment and improved nutrient use efficiency on the lambs fed the humic acid-fortified feeds.

Table 3: Rumen fermentation and microbial populations.

Parameter	Control	3 kg HA/t DM	5 kg HA/t DM	SEM	p value
Rumen pH	6.5	6.6	6.7	0.03	0.21
NH_3-N (mg/dL)	14.3b	17.6a	18.1a	0.42	0.04
Total VFA (mmol/L)	88.5b	93.8a	96.1a	1.8	0.03
Methane estimation (mL/g DM)	28.4a	25.1b	23.9b	0.74	0.01

Values in the same row with different superscripts differ significantly ($p < 0.05$).

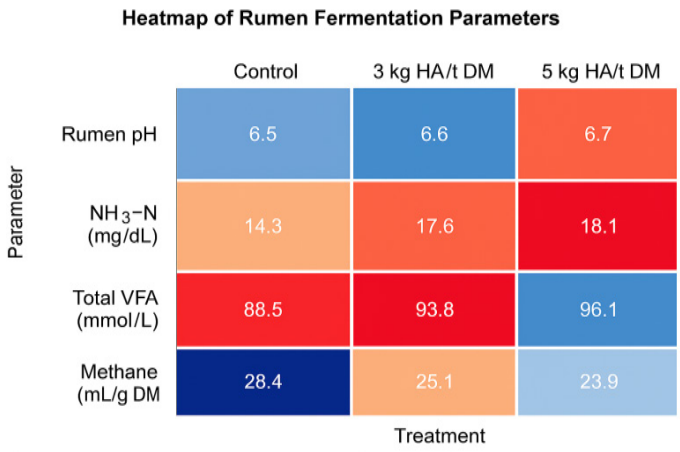

Figure 2: Heatmap of rumen fermentation parameters.

Volatile fatty acid profile

Supplementation of the rumen with humic acid changed rumen fermentation process, VFA profile was shifted towards greater proportion of propionate and lower proportion of acetate to propionate (Table 4). The concentrations of propionate and overall VFA were significantly greater ($p < 0.05$) in these HA-containing groups, and the proportions of acetate were lower than those of the control. Such elevation in the glucogenic fermentation profile also leads to elevation in the energy efficiency by using substrate to hepatic gluconeogenesis. It is possible that the positive influence of humic acid on *Prevotella ruminicola* and other amylolytic bacteria that have been reported to yield propionate selectively (Nur Atikah *et al*., 2017; Ren *et al*., 2019) could increase the content of propionate and, consequently, a higher concentration of propionate should be expected. The reduced ratio of acetate to propionate identified in this research is in line with high feed conversion efficiency and low methane generation since acetate is an intermediate energy source to release hydrogen and propionate as a hydrogen sink (Shinkai *et al*., 2024; del Prado *et al*., 2025). Butyrate concentrations did not significantly change, which means that humic acid selectively regulated the fermentation pathways and did not disorganize rumen metabolism. Overall, these findings show that humic acid does not only enhance the quantity of VFAs produced but also maximizes their ratio to be used in energy generation and reduce the production of enteric methane.

Table 4: Effect of dietary humic acid supplementation on rumen volatile fatty acid (VFA) concentrations (mmol/100 mmol total VFA).

Parameter	Control	3 kg HA t^{-1} DM	5 kg HA t^{-1} DM	SEM	p value
Acetate (C_2)	64.8^a	62.1^b	61.3^b	0.41	0.04
Propionate (C_3)	20.9^b	23.8^a	24.4^a	0.36	0.02
Butyrate (C_4)	11.6	11.8	12.0	0.19	0.37
Acetate: Propionate ratio	3.10^a	2.61^b	2.51^b	0.07	0.03
Total VFA (mmol/L)	88.5^b	93.8^a	96.1^a	1.8	0.03

Values in the same row with different superscripts differ significantly ($p < 0.05$).

Rumen microbial population dynamics

The rumen microbial community was significantly altered by the humic acid in diet (Table 5). Cellulolytic bacteria *Ruminococcus albus, Ruminococcus flavefaciens and Fibrobacter succinogenes* were more prevalent with both HA concentration, which is probably due to improved fiber degradation ability. This is in line with Zhijie *et al*. (2021) who found that more cellulolytic numbers are found when the humic acid is added to goat food. The enhanced population of *Prevotella ruminicola* suggests the augment of proteolytic, carbohydrate fermenting activity and consequently the augmented creation of volatile fatty acids that have been recorded above. On the other hand, predicted results showed a reduction in methane emission that was supported by the reduction in the profiles of methanogenic archaea (*Methanobrevibacter* smithii and *Methanobacterium formicicum*) after the addition of HA ($p < 0.05$). Humic substances contain phenolic and quinone groups, which may be used to inactivate the methanogen activity, or indirectly lower the growth with alterations in the hydrogen supply (del Prado *et al*., 2025). Together, these microbial alterations show, that humic acid helps to make the rumen more energy efficient and better health that helps ensure the prevailing type of fermentation: cellulolytic and

suppresses the growth of microorganisms that produce methane. The application of the humic acid supplement is observed to be beneficial in altering the microbial condition of the rumen of the local Iraqi black goats (Table 3). Significant increase ($p < 0.05$) in the number of cellulolytic bacteria, i.e. *Ruminococcus albus, Ruminococcus flavefaciens and Fibrobacter succinogenes* was found in the goats receiving HA compared to the control group. These microorganisms cannot be ignored to deconstruct the cell wall components of plants and its growth is a good indicator of better performance in degrading fibers. This impressive finding is supported in Zhijie *et al.* (2021) and Li *et al.* (2021) that also demonstrated such increases in the activity of cellulolytic activity following the supplementation of humic acid in the diet of goats and cattle. There was also a relative increase in the abundance of *Prevotella ruminicola* and *Butyrivibrio fibrisolvens* in overall abundance as an indication of a stimulation in proteolytic and butyrate-producing bacterial groups. This means that HA supplementation enhanced the overall fermentation efficiency of the rumen as it was in line with higher VFA levels previously recorded. Instead, the population of the methanogenic archaea (*Methanobrevibacter smithii* and *Methanobacterium formicicum*) significantly reduced ($p < 0.05$) in both specific conditions with HA. This reduction is equal to the reduction in estimation of methane and could be linked to the redox activity of humic compounds and could prevent methanogenic pathways or reduce the supply of hydrogen (Betancur-Murillo *et al.* 2022; del Prado *et al.*, 2025). In conclusion, it can be concluded that the humic acid promotes the cellulolytic and fermentative bacteria, which causes a good rumen microenvironment, and suppresses the production of methane-producing archaea. The best use of nutrients and growth pattern by goats fed with humic acid in the roughage is likely to be strengthened by this microbial modulation.

Table 5: Relative abundance (log_{10} copies g^{-1} rumen content) of major rumen microbial taxa in goats fed different levels of humic acid.

Microbial group	Control	3 kg HA t^{-1} DM	5 kg HA t^{-1} DM	SEM	p value
Ruminococcus albus	6.25b	6.84 a	7.03 a	0.09	0.01
Fibrobacter succinogenes	6.12b	6.73 a	6.88 a	0.08	0.02
Prevotella ruminicola	6.51b	6.95 a	7.02 a	0.07	0.03
Butyrivibrio fibrisolvens	5.83b	6.21 a	6.35 a	0.10	0.04
Streptococcus bovis	5.67a	5.52 a	5.48 a	0.11	0.42
Ruminococcus flavefaciens	5.94b	6.48 a	6.61 a	0.09	0.02
Methanobrevibacter smithii	5.31a	4.78 b	4.55 b	0.08	0.01
Methanobacterium formicicum	5.16a	4.83 b	4.70 b	0.07	0.03

Values in the same row with different superscripts differ significantly ($p < 0.05$).

Rumen microbial diversity indices

Humic acid supplementation has the potential to significantly improve rumen microbiota indices of alpha-diversity (Table 6). Rumen microbiota with more balanced and diverse microbiome structure was also found in goats treated with HA with much higher Chao1 richness, Shannon diversity, and Simpson evenness indices compared to controls. At 5 kg of HA/t DM the increase appeared to be more significant showing a dose dependent effect. Such an additional variety can be explained by humic acid that can provide the cell with an additional organic carbon and phenolic compound as either substrate or regulator of microbial growth. Increased microbial richness, in turn, results in the increase of metabolic capability because of the improved breakdown of complex vegetal polysaccharides and secondary compounds (Shinkai *et al.*, 2024). The findings correspond to earlier studies of Li *et al.* (2021) and Shinkai *et al.* (2024) that point to the idea that humic substances are more likely to stabilize the microbial population and eliminate the opportunistic species of the rumen. This implies that biodiversity and, therefore, ecological resilience is also increased, conservation of fermentation processes under varying dietary conditions. Together the diversity data will support the quantitative microbial data by indicating that humic acid has a non-random impact on abundance of relevant taxa but indeed enhances structural complexity of the global rumen microbiome, which will support the apparent gains in nutrient use and animal performance.

Table 6: Alpha-diversity indices of rumen microbiota in goats fed diets supplemented with humic acid.

Diversity index	Control	3 kg HA t^{-1} DM	5 kg HA t^{-1} DM	SEM	p value
Chao1 richness	612.4^{b}	648.7^{a}	662.1^{a}	8.3	0.03
Shannon index	4.21^{b}	4.58^{a}	4.67^{a}	0.07	0.02
Simpson index	0.89^{b}	0.92^{a}	0.94^{a}	0.01	0.04
Observed OTUs	589^{b}	632^{a}	645^{a}	6.9	0.02

Values in the same row with different superscripts differ significantly ($p < 0.05$).

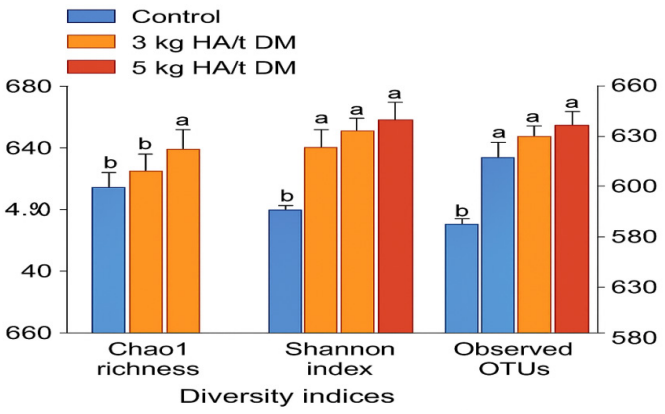

Figure 3: A bar chart showing alpha-diversity indices (Chao1, Shannon, Simpson, Observed OTUs).

Correlation between dominant microbial species and productive trait

Based on correlation analysis, the abundance of cellulolytic bacteria (*Ruminococcus albus, Fibrobacter succinogenes*) showed strong positive correlations with productive properties, including the average daily gain (r= 0.84 and 0.81, respectively) and total volatile fatty acids (Table 7). The methanogenic archaea (*Methanobrevibacter smithii, Methanobacterium formicicum)*, on the other hand, yielded negative relationships with growth and fermentation efficiency but a positively significant relationship with methane estimation (r = 0.88 and 0.83). These associations contribute that humic acid supplementation higher performance is mainly involved in cellulolytic activity and prevention of methanogenesis. It is a correlation between rumen microbial and methanogen activity that aligns with the prior observation of negative interaction between the methanogen populations and rumen energetic efficiency (Shinkai *et al*., 2024; del Prado *et al*., 2025). The results show that rumen microbial population balance, that is, cellulolytic population balance and methanogenic population balance are very sensitive to the impact of not only goat feed ratio but also goat environmental output when using humic acid-treated roughages.

Table 7: Pearson correlation coefficients (r) between major rumen microbial groups and performance or fermentation parameters in goats.

Microbial group	Average daily gain	Feed conversion ratio	Total VFA	Methane estimation
Ruminococcus albus	0.84	–0.79	0.82	–0.76
Fibrobacter succinogenes	0.81	–0.73	0.77	–0.70
Prevotella ruminicola	0.69	–0.65	0.74	–0.61
Butyrivibrio fibrisolvens	0.63	–0.60	0.69	–0.57
Methanobrevibacter smithii	–0.78	0.74	–0.71	0.88
Methanobacterium formicicum	–0.72	0.69	–0.68	0.83

All correlations are significant at $p < 0.05$.

Correlation of the dominant bacterial species with the volatile fatty acid profile

The correlation analysis indicated the linkages between the microbial taxa and the rumen fermentation profiles (Table 8). The presence of acetate and propionate concentrations and total VFA were positively correlated with the cellulolytic bacteria (*Ruminococcus albus, Fibrobacter succinogenes*), which was demonstrated to be implicated in the degradation of fiber and the production of energy. *Prevotella ruminicola* was most positively correlated with propionate (r= 0.83, $p < 0.001$) demonstrating its role in the succinate-propionate pathway and the induced glucogenic shift when fed on humic-acid. Methanogenic archaea (*Methanobrevibacter smithii* and *Methanobacterium formicicum*) on the other hand, showed a strong positive relationship with acetate and acetate: Propionate ratio but negative relationship with propionate and total VFA. This implies that methanogens will compete with propionate-formers in the competition of hydrogen, and therefore energy efficiency will be lowered. These correlations indicate that humic acid modulates the rumen fermentation by promoting cellulolytic and propionate fermentation and repressing the hydrogen-dependent fermentation of methanogenesis. The resultant fermentation profile facilitates propionate production and growth in the overall VFA production, which have led to the growth performance and feed ratio improvements. According to Zhijie *et al.* (2021) and Li *et al.* (2021), the functional role of microbial has also been confirmed through corresponding relations between community structure and VFA patterns.

Table 8: Pearson correlation coefficients (r) between key rumen microbial species and major volatile fatty acids in goats fed humic-acid diets.

Microbial species	Acetate (C_2)	Propionate (C_3)	Butyrate (C_4)	Total VFA	A:P ratio
Ruminococcus albus	0.74 **	0.81 ***	0.46 ns	0.79 ***	–0.68 *
Fibrobacter succinogenes	0.69 **	0.76 ***	0.40 ns	0.73 ***	–0.65 *
Prevotella ruminicola	–0.48 *	0.83 ***	0.57 *	0.77 ***	–0.70**
Butyrivibrio fibrisolvens	0.32 ns	0.58 *	0.79***	0.66 **	–0.45 *
Methanobrevibacter smithii	0.85***	–0.82***	–0.50 *	–0.79***	0.89***
Methanobacterium formicicum	0.79***	–0.74 **	–0.41ns	–0.73 **	0.81***

*Significance levels: $p < 0.05$ (), $p < 0.01$ (), $p < 0.001$ (); ns = not significant.

Correlation between microbial diversity indices and volatile fatty acids profile

The indices of microbial alpha-diversity (Chao1, Shannon, Simpson) were positively associated with the total VFA and the acetate level as well as propionate level and negatively correlated with the acetate: propionate ratio with acetate (Table 9). These relationships imply that the more diverse (richer and more balanced) rumen microbiota is, the more preferential it is to a fermentation that produces higher propionate and a more efficient energy production. The increase of cellulolytic and amylolytic species following soil supplementation with humic acid would also have been conducive to the substrate availability of succinate-propionate pathways and the inhibition of the methanogen would have reduced the hydrogen competition. In line

with this, increased diversity of microbial microorganisms promoted increased metabolic flexibility and redirected reducing equivalents to propionate synthesis with a potential of reduced methane production. The relationships between rumen diversity and fermentation yield are consistent with the prior study that the high microbial content is correlated with an enormous increase in the conversion of yield and energy losses in goats and cattle (Shinkai *et al*., 2024; Li *et al*., 2021). Humic acid is thus a microbial control and a biochemical control that stabilizes fermentation and enhances the metabolic efficiency of rumen systems.

Table 9: Pearson correlation coefficients (r) between microbial alpha-diversity indices and major rumen volatile fatty acids in goats fed humic-acid diets.

Parameter	Acetate (C_2)	Propionate (C_3)	Butyrate (C_4)	Total VFA	A:P ratio
Chao1 richness	−0.72 *	0.81 ***	0.44 ns	0.78***	−0.68*
Shannon index	−0.69 *	0.84 ***	0.51 *	0.80***	−0.71*
Simpson index	−0.63 *	0.77 ***	0.38 ns	0.74***	−0.66*

Significance levels: * p < 0.05 (), p < 0.01 (), p < 0.001 (); ns = not significant.

CONCLUSION

The roughage diet of the Iraqi local black goats supplemented with humic acid improved the growth performance, the feed economy and the rumen fermentation characteristics. In addition to its nutritional benefits, it also impacted rumen microbial balance positively (enriching cellulolytic bacteria and fermentative bacteria (*Ruminococcus albus, Fibrobacter succinogenes, Prevotella ruminicola*) and suppressing the growth of methanogenic bacteria (*Methanobrevibacter smithii, Methanobacterium formicicum*). Moreover, the presence of the fact that alpha-diversity indices (Chao1, Shannon, Simpson) have also risen also speaks in support of the fact that humic acid can facilitate the establishment of a more diverse and stable microbial community, help to more efficiently degrade fiber and reduce the possibility to produce methane. Such beneficial transformations of microbial abundance and diversity are why the nutrient capacity and animal production were greater in the humic acid-amended groups. Humic acid is a possible natural additive that can improve the quality of roughage and the ecology of the rumen microbial in goats. It has dual effects of increasing performance, and decreasing the emissions of enteric methane, points to a high possibility of effect on sustainable systems of small ruminant rearing in arid and semi-arid regions.

Practical implications

Humic acid content of 350-500 g of feed on goat diets increases feed efficiency and healthy, diverse rumen microbial composition. It can be used to enhance the use of low-quality roughages, which are usually in abundance in all places, enhance growth performance of goats, and suppress the emission of methane, thus it presents a low-cost and viable source of natural additives.

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NOVELTY STATEMENT

This work provides a novel contribution to small ruminant nutrition by demonstrating how humic acid can be used strategically to enhance the feeding value of roughages and improve the productive performance of Iraqi Local Black Goats.

AUTHOR'S CONTRIBUTIONS

JAAB: Writing and reviewing. HAJA-G: Writing and reviewing, field work. ANK: Field work, statistical analysis.

Ethics and data availability statement

The Animal Ethics Committee of the College of Agriculture, University of Basrah approved all the procedures (Approval No. AEC/2025/04). One can request data when there is a reasonable need to do so with the respective author (hanaa.jabar@uobasrah.edu.iq).

Generative AI and AI-assisted technology statement

The authors declare that generative AI tools were merely used to improve the language, grammar, and clarity of the manuscript. The scientific content, data analysis, interpretation of results, and conclusions were developed entirely by the authors.

Conflict of interest

The authors have declared no conflict of interest.

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