Effect of humic acid supplementation on rumen parameters in Arab male lambs

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Summary

This study was conducted in the animal field of the Department of Animal Production/College of Agriculture/ University of Basrah. The study lasted for 105 days from 1/11/ 2021 until 15/2/ 2022. In the experiment, 12 Arabi lambs purchased from local markets aged 5-4 months. The lambs were placed in similar cages 3 x 3 m for each treatment inside a semi-closed barn. The cages were provided with feeders and fountains for drinking water. The lambs were fed for 14 days on the basic ration. A preliminary period was counted for the purpose of adaptation, and then the lambs were weighed using an electronic scale. This was counted as the initial weight (26.5 kg), then the lambs were divided into three treatments according to their weight, four lambs each treatment. The three treatments were the control treatment, the addition of 6 gm humic acid/kg fodder, and the addition of 12 gm humic acid/kg fodder. pH measured after 3 hours of feeding at the beginning and at the end of the study of the addition treatment 6 gm humic (6.73, 6.79 respectively) exceeded over the control treatment (6.46, 6.10 respectively). Significant superiority in the total bacteria count after feeding at the end of the study for the addition treatments 6 and 12 gm humic (1.00×10^9 and 0.85×10^9) CFU/ml, compared to the control treatment (0.65×10^9) CFU/ml as well as the addition treatment of 6 gm humic was superior to the addition treatment of 12 gm humic. Significant superiority in the concentration of total volatile fatty acids for the addition treatment of 6gm Humic (86.77) mmol/L compared to the control treatment and the 12gm Humic supplementation treatment (76.41 and 83.33 mmol/L), respectively. There was also a significant superiority in the proportion of propionic acid for the addition treatment of 6 gm humic (28.75%) compared to the control treatment and the addition treatment of 12 gm humic (24.00% and 24.00%), respectively. Significant decrease in methane gas emission in the addition treatment of 6 gm humic (23.19) L/day compared to the control treatment and the addition treatment of 12 gm humic (26.66 and 26.40) L/day, respectively. The supplement with humic acid (6 gm/ Kg) improves rumen ecology and parameters.

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make better use of feed by providing appropriate conditions for the growth and development of microorganisms in the rumen, especially those that work on analyzing and digesting fibers with no negative impact on the animal or its accumulation in the rumen, tissue and its negative effects on the consumer, such as

Introduction

Improving rumen conditions is one of the most effective ways to improve the efficiency of animal production (Khattab et al., 2020).Therefore, nutritionists and microbiologists have been searching for various food additives from natural sources that improve the performance of animals and

lamb's mouth so that the lamb could not close its mouth on the suction tube and thus block or rupture the tube.The head of the lamb is raised to the top when the tube is inserted to ensure that the tube, which is 1 cm in diameter, does not enter the respiratory tract, and then lower the head of the lamb to the bottom in order to facilitate the descent of the rumen fluid.The first rumen fluid was drawn in the morning immediately before giving the diet, and the second draw was done three hours after giving the diet, and the rumen fluid was collected in the tubes, and then the required tests were conducted.

pH of rumen fluid

The pH of the rumen fluid was measured for each sample with a pH meter model PH9909 made in Germany. The measurement was made immediately after the rumen fluid was withdrawn. After the examination of each sample, the electrode was washed with distilled water, dried, and the reading was reset (calibration) to ensure accuracy.

Bacteria total count

The method of counting colonies in the cultured dishes was adopted, as the number of colonies growing in the cultured media represents the number of bacterial cells, and this number depends on the approved dilution ratio, as a series of dilutions are usually used in the dilution process. Nine ml of peptone water was added to 1 ml of rumen fluid and shaken well, then 1 ml of the new solution is taken and 9 ml of peptone water is added to it again, then this process is repeated until we get nine different dilutions, their dilution ratio ranges between (1/10 to 1/1000). ,000,000) and then 1 ml of the last dilution (the ninth dilution) was transferred to sterile Petri dishes using sterile pipettes for each dilution.Nutrient agar was added sterilized by autoclave at 120°C and cooled to 45-50°C for each of the mentioned dishes. The plates were moved gently clockwise and counterclockwise or moved in the form of a number 8 to mix the diluted suspension with the culture medium. Then the dishes were left until the culture medium hardened, then the dishes were incubated upside down for 24-48 hours at a temperature of 37 °C. Then, the what causes antibiotics (Shaaban et al., 2021). Among these additives are the humic substances that consist of 1- humus (humic), 2- humic acid, 3- fulvic acid and 4- minerals (Kocabağli et al. 2002).

Humic acid is an alkali-soluble, high molecular weight molecule capable of composting nutrients and then transferring them from the soil to living organisms. It has antimicrobial, detoxification, and element absorption properties, and promotes the reproduction of beneficial bacteria in the rumen (Islam et al., 2005) and increase immunity and reduce disease (Rahman et al., 2020).Humic is also considered as a natural and safe feed additive (Teter et al., 2021).Murbach et al., (2020) indicated the feasibility of further study to develop the product as a dietary supplement.

Therefore, the current study aimed to use different levels of humic acid to recognize the effect of humic acid on the rumen parameters, which include the pH, microorganisms, volatile fatty acids and the concentration of methane gas in the rumen.

Materials and Methods

Animals and feeding

The study included 12 Arabi male lambs purchased from the local market at the age of 4-5 months, with an average weight of 26.5 kg. The lambs were distributed equally and according to their average weights into three groups that included the control and the first treatment, adding 6 g/kg humic acid to the control diet, and the second treatment adding 12 g/kg of the control diet.The control diet consisted of 60% concentrate diet and 40% roughage fodder (alfalfa hay). The concentrate diet consisted of (50% barley, 35% wheat bran, 10% yellow corn, 4% soybeans, 1% vitamins and minerals) with a metabolic energy of 13 MJ/ kg dry matter and 14.67% crude protein. Lambs of each treatment were placed in 3x3m boxes.

Rumen parameters

Withdrawal of the rumen fluid through a 50 ml syringe connected to a tube manufactured locally for this purpose, a transparent plastic tube 1.5 meters long and 1 cm in diameter.A plastic tube of 25 centimeters in length and 5 centimeters in diameter was placed in the

(default):CH4= 0.45 C2 – 0.275 C3 + 0.40 C4, Where, C2 = acetate, C3 = propionate, C4 = butyrate.

Results and Discussion

pH

Table (1) shows that there were no significant differences in the pH of the rumen fluid between treatments when measuring the pH before feeding at the beginning and at the end of the study. However, there was a significant difference ($p < 0.05$) in the pH when measured after feeding (after 3 hours) in the beginning of the study for the addition treatment of (6 gm humic), which scored (6.73), over the control treatment(6.46). Significant superiority in pH when measured after feeding at the end of the study for the addition treatment (6 gm humic), which scored (6.79), over the control treatment, which scored (6.10).

This result is consistent with the findings of El-Zaiat et al., (2018) where it was found that the addition of humic acid to the diet of Barki goats at a rate of 2 g / day / goats led to an increase in pH in the rumen of the addition treatment compared to the control treatment.It agrees with the result of Kholif et al., (2021) who noticed that sodium humate added to the diet of dwarf goats led to a significant decrease in acidity in the rumen for the two addition treatments equally compared to the control treatment, and the reason behind that was attributed to the decrease in ammonia and the increase in the concentration of VFA. On the other hand, the result of this study did not agree with the results of (McMurphy et al., 2009 & 2011, Tunç et al., 2012, Terry et al., 2018 a&b and Marcin et al., 2020 Bujňák et al., (2020) and Hassan et al., 2020) who found no significant effect in pH when adding humic acid to diets.

number of bacterial cells was calculated using the following equation: number of bacterial cells/ ml= number of colonies x inverted sample dilution.

Cellulolytic bacteria

Nine ml of peptone water is added to 1 ml of rumen fluid and shake well, then 1 ml of the new solution is taken and 9 ml of peptone water is added to it again, then this process is repeated until we get seven different dilutions whose dilution ratio ranges between (1/10 to 1/10,000,000). Then 1 ml of the last dilution (the seventh dilution) was transferred to sterile Petri dishes using sterile pipettes for each dilution.Mast Diagnostica sterilized culture medium was added by autoclave at 120°C and cooled to 45-50°C for each of the mentioned dishes. The plates are gently moved clockwise and counterclockwise, or they are moved in the form of a number 8 to mix the diluted suspension with the culture medium.As this medium contained plant tissue juice as a source of cellulose for the growth of cellulose digesters, then the dishes were left until the culture medium hardened, then the dishes were incubated upside down for 24-48 hours at a temperature of 37 °C. Then, the number of bacterial cells was calculated using the following equation: number of bacterial cells/ ml= number of colonies x inverted sample dilution.

Volatile fatty acids

The volatile fatty acids were estimated in the laboratories of the College of Pharmacy / University of Basra by GCmas device manufactured by the Japanese company HLMADZU.

Methane gas

Methane emission concentration was calculated as described by Moss *et al.* (2000), considering the hydrogenrecovery of 90%

Table (1) Effect of adding humic acid at different levels on the pH of the rumen fluid of Arabi lambs (mean ± standard deviation)

Treatments	Initial pH before feeding	Initial pH after feeding	Final pH before feeding	Final pH after feeding
Control (0 humic acid)	6.73±0.21	6.46±0.08 b	6.58±0.25	6.10±0.14 b
T2 (6 gm humic acid/kg feed	6.97±0.03	6.73±0.07 a	6.35±0.07	6.79±0.08 a

T3 (12 gm humic acid/ Kg feed)	**6.93±0.01**	**6.57±0.07 ab**	**6.58±0.10**	**6.46±0.14 ab**
P Value	**NS**	**0.05**	**NS**	**0.05**

***NS= not significant**

observed on the rumen microbiology when humic was added to the diet of rams.It agrees also with Tunç et al., (2012), when adding humic acid to the sheep ration and Ikyume et al., (2020) when sodium humate was added to the goat diet.

Total and Cellulolytic bacteria counts

Table (2) shows that there were no significant differences in the total bacteria count and cellulolyticbacteria among the treatments at the beginning of the study.The result of this study agrees with the result of Galip et al., (2010), where no significant effect was

Table (2) Effect of adding humic acid at different levels on the total and cellulolytic bacteria count (CFU/ml of rumen fluid) at the initial of the study

Treatment	**Total Bacteria count X 10^9 before feeding**	**Total Bacteria count X 10^9 after feeding**	**Cellulolytic Bacteria count X 10^7 before feeding**	**Cellulolytic Bacteria count X 10^7 after feeding**
Control (0 gm humic acid)	**0.65±0.05**	**0.75±0.02**	**3.81±0.80**	**4.48±0.13**
T2 (6 gm humic acid/kg feed)	**0.69±0.03**	**0.81±0.03**	**3.80±0.90**	**4.59±0.12**
T3 (12 gm humic acid/ Kg feed)	**0.67±0.04**	**0.79±0.07**	**3.35±0.20**	**4.17±0.13**
P Value	**NS**	**NS**	**NS**	**NS**

***NS= not significant**

countsafter feeding at the end of the experiment for the addition of 6 gm humic(6.26×10^7) CFU/ml compared to the control treatment (5.77×10^7) CFU/ml.

This result is consistent with the result of El-Zaiat et al., (2018) who found that the addition of humic acid to the diet of Barki goats at a rate of 2 g / day / goats led to a decrease in the number of protozoa and a significant increase in rumen bacteria. The reason behind the increase in bacteria might attributed to the decrease in the number of protozoa in the rumen. It agrees with Kholif et al., (2021), the addition of humic substances to the cow diet reduces the numbers of protozoa and increases the number of bacteria significantly, the increase of microbes count in the rumen either to the protein resulting from the decomposition of humates in the rumen or the rumen microflora has the ability to use humate as receptors for electrons, which leads to the growth and proliferation of rumen organisms.

Table (3) indicated a significant superiority ($p < 0.05$) of the total bacteria count and cellulolytic bacteria before feeding at the end of the study for the addition of 6 gmand 12 gm humic(0.67×10^9 and 0.62×10^9) CFU/ml respectively compared to the control treatment(0.53×10^9 CFU/ml) and the addition of 6g humic over the addition of 12gm humic.The same table showed a significant superiority ($p<0.05$) of total bacteria count after feeding at the end of the study for the addition of 6 and12gm humic (1.00×10^9 and 0.85×10^9) CFU/ml, respectively. The control treatment recorded (0.65×10^9) CFU/ml.The addition of 6 g humic was superior to the addition of 12 g humic.

Also, a significant superiority ($p<0.05$) was found in the number of cellulolytic bacteria before feeding at the end of the experiment for the addition of 6 gm humic (5.50×10^7) CFU/ml compared to the control treatment (4.90×10^7) CFU/ml.In addition, a significant superiority ($p<0.05$) of cellulolytic bacteria

Table (3) Effect of adding humic acid at different levels on the preparation of total bacteria and cellulolytic bacteria (CFU/ml of rumen fluid) at the end of the experiment

Treatments	Total bacteria count x 10^9 before feeding	Total bacteria count x 10^9 after feeding	cellulolytic bacteria count x 10^9 before feeding	cellulolytic bacteria count x 10^9 after feeding
Control (0 gm humic acid)	0.53±0.07 c	0.65±0.19 c	4.90±0.02 b	5.77±0.03 b
T2 (6 gm humic acid/kg feed)	0.67±0.08 a	1.00±0.08 a	5.50±0.02 a	6.25±0.08 a
T3 (12 gm humic acid/ Kg feed)	0.62±0.02 b	0.85±0.16 b	5.20±0.01 ab	5.48±0.04 ab
P Value	0.05	0.05	0.05	0.05

treatment and the addition of 12 g humic (9.75% and 10.50% respectively).

These results are in agreement with the findings of Sheng et al., (2017 & 2019), where it was found that humic substances led to a decrease in the molar ratio of acetic ($p < 0.05$) at 12 hours of incubation.It partially agrees with the results of El-Zaiat et al., (2018) where it was found that the addition of humic acid to the diets of Barki goats led to a significant increase in acetic acid and propionic acid in the rumen, the reason for the increase in volatile fatty acids is attributed to the increase in humic substances that improve digestion.The results agree with Hassan et al., (2020), that CD-HS supplementation did not affect the proportion of butyric acid, but it led to a quadratic increase in the total volatile fatty acids and the proportions of acetic and propionic acid.

Volatile fatty acids and methane in the rumen

Table (4) shows a significant superiority in the total volatile fatty acids for the addition of 6 g humic(86.77 mmol/L) compared to the control treatment and the addition of 12 g humic, which recorded (76.41) and (83.33) mmol/L, respectively. The addition of 12 g humic exceeded the control treatment. The same table indicates a significant ($P<0.05$) decrease in acetic acid percent for the addition of 6 g humic(62.00%) compared to the control treatment and the addition of 12 g humic(65.25%and 64.00% respectively). A significant superiority ($P<0.05$) in propionic acid percent for the addition of 6 g humic (28.75%) compared to the control treatment and the addition 12 g humic(24.00% for both).In addition, there was a significant decrease in butyric acid for the addition of 6 g humic (8.00%) compared to the control

Table (4) Effect of adding different levels of humic acid on the production of volatile fatty acids and methane gas emission in the rumen of Arabi lambs (mean ± standard deviation)

Treatments	Total volatile fatty acid (mmol/L	Acetic%	Propionic%	Butyric%	Methane (L/day)
Control (0 gm humic acid)	**76.41±1.30 c**	**65.25±1.50 a**	**24.00±0.81 b**	**9.75±0.95 a**	**26.66±0.61 a**
T2 (6 gm humic acid/kg feed)	**86.77±1.36 a**	**62.00±0.81 b**	**28.75±1.50 a**	**8.00±1.41 b**	**23.19±1.16 b**
T3 (12 gm humic acid/ Kg feed)	**83.33±4.33 b**	**64.00±0.81 a**	**24.00±0.81 b**	**10.50±0.57 a**	**26.40±0.63 a**
P Value	**0.05**	**0.05**	**0.05**	**0.05**	**0.05**

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The results of this study disagreed with the findings of McMurphy et al., (2011), Tunç et al., (2012), Terry et al., (2018), Bujňák et al., (2020), Marcin et al., (2020) and Ikyume et al., (2020), where they found no effect of adding humic acids on the percentage of total volatile fatty acids or the percentage of acetic acid, propionic or butyric acid.Kholif et al., (2021) found an increase in the concentration of total volatile acids, acetic acid and propionic in the treatment with (40) g humic acid/day and a decrease in butyric in the two addition treatments compared to the control.

The same table shows a significant decrease of methane emission in the addition of 6 g humic(23.19 L/day) compared to the control treatment and the addition of 12 g humic(26.66 and (26.40 L/day, respectively).This result is consistent with the findings of Sheng et al., (2017 & 2019) that the addition of humic materials leads to a reduction ($P < 0.05$) in methane production after 48 hours of incubation.The addition of humic materials, according to Terry et al. (2018 a & b), had no impact on the rumen's ability to produce methane.

Conclusion

The supplement with humic acid (6 gm/ Kg) improves rumen pH, volatile fatty acids, total and cellulolytic bacteria and reduce CH_4 emission.

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