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Addition of garlic powder to the ration mixture and its effect on digestion coefficient of dry and organic matter, total gas and methane in vitro production

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***Abstract*---**The aim of the experiment is to find out the effect of garlic powder on the digestion coefficient and the rumen environment in vitro by adding four levels (0.0, 1.0, 2.0 and 3.0) g/100 g of dry matter added to the mixture feed concentrate: roughage (40:60). Total gas was calculated in 24 hours .The digestibility was determined after 96 hours incubation . Results showed differences ($p < 0.01$) in the total gas and methane production, the lowest value for the treatment was 1.0 g/100g DM , the dry matter and organic matter digestibility significantly increased ($p<0.01$) T3 (2.0%) garlic powder with (61.28 and 79.87) respectively compared with control. Significant difference($p<0.01$) between all treatments in total count of bacteria. Addition garlic powder to mix ration 60: 40 roughages : concentrate at the level 2.0 g/100 g DM improve dry and organic matter digestibility , increase total gas and decrease methane production.

***Keywords*---**garlic powder, gas production, dry and organic digestibility, total count bacteria.

Introduction

Rumen fermentation and methane production increase the greenhouse effect, so some feed additives have been used to reduce their production (Patra, and Saxena 2010) . There are many addition that have been used, such as yeast, ionophores, probiotics and Aspergillus oryzae have been introduced to the ration ruminants (Karakurt, et .al 2012).These microbes increase volatile fatty acid reducer

protozoa numbers, and lower methane production(Iqbal ,et .al 2008). Currently, natural products that have been introduced into the mitigation of methane it is often cheap and inexpensive(Kamra, et . al 2012). For example, garlic , thyme and laurel are plants that have an effect on the process of methanogens (The study searches Bodas, et. al 2012) for the best addition level of garlic base, feed mixture, digestion factor, rumen characteristics, total gas and methane gas production in the in vitro.

Material and Methods

Use of the feed mixture (60:40) Concentrate: hay to determine the basic components of garlic powder (0, 1.0, 2.0, 3.0%) / 100 gm in the laboratories of digestion, rumen fermentation and gas production in the laboratory. The chemical composition of the ration Table (1).

***In Vitro* total gas production**

The artificial saliva was prepared for gas measurement in a ratio of 1:2 (10 rumen liquid + 20 artificial saliva) and incubated in glass syringes with a capacity of 100 ml four replicates for each treatment with the addition of carbon dioxide gas and put in a water bath at 39 ° C for 24 hours and then was added 4% hydroxide to measure methane production according to(Menke and Steingass 1988). At the end of the incubation period, a sample was taken for each treatment to determine the NH3-N and VFA by(Warner, 1964) . After the incubation period, the contents of each syringe were transferred, washed with hot water, and dried at 105°C (Van Soest and Robertson 1985). After the crucibles were dried and burn at 550 °C for about 6 hours and weighed after cooling in a dryer to determine the TOMD.

Table 1. Proximate chemical composition of the diet

Ingredient	Barley	yellow corn	soybean meal	wheat bran	vitamin-mineral premix
%	46	10	10	32	2

Table 2.The chemical composition of the components of the concentrated ration and (%) roughage alfalfa hay 40:60

Dry matter	Organic matter	ASH	Ether extract	Crud Fiber	Crud protein	NFE	Metabolizable *energy MJ/Kg DM
91.77	85.19	6.58	3.49	13.05	14.79	53.86	11.04

* Metabolizable energy MJ/Kg DM by(MAFF, 1975)

***In vitro* dry matter and organic matter digestibility**

In vitro digestion coefficient of dry and organic matter was estimated according to the method of (Tilly and Terry, 1963) . Different levels of garlic powder (0, 1.0,

2.0 and 3.0%)/100 g DM were added to 1g sample of mixture consisting of concentrated: Roughage 40:60 chemical composition of the components of the concentrate ration (%) table 2 according to (AOAC,2005) and it includes two stages: The first stage is microbial digestion of the feed material. Using artificial saliva and rumen liquid of a slaughtered animal for 48 hours, followed by the second stage: enzymatic digestion for 48 hours. This is similar to intragastric digestion of HCL dissolved in an acidic medium of pepsin with the addition of the fourth enzyme rennet. The experiment was conducted in the Animal Nutrition Laboratory / College of Agricultural Engineering Sciences

Total count of bacteria

Using pour plate method for counting the number of bacteria according to (Kass and Norden,1968) .After the solidification of the agar the plate is incubated of $37C^0$ for 24-48 hours

Statyical analysis

Data were analyzed by SAS (2012). The design included one factors, was the levels of garlic powder (0, 1.0,2.0 and 3.0%)/100g DM. Data were tested according to Duncan's multiple range test (Duncan, 1955) following. $Yij=\mu+ Ai + eij$; where Yij, μ is the common mean, Ai is the effect of garlic powder levels (0.0, 1.0, 2.0, and 3.0%) and eij is the randomly normal distributed experimental error associated with each observation

Result and Discussion

Table 3 revealed that adding four levels of garlic (0. 0,1.0,2.0 and 3.0) % The results showed increased ($p < 0.01$) in the total gas production for T2,T3 andT4 compared with T1 amounting to 53.00, 52.66, 60.33 and 43.00, respectively. Whereas, significant decrease ($p < 0.01$) In the production of methane gas, withT2 (23.00) and highest value for the T4 (57.33) when adding garlic powder and there were no significant differences in the production of methane gas (ml) and the organic matter digestibility factor (%) and a significant decrease ($p<0.05$) in the production of metabolic energy MJ/kg dry matter for treatments T1, T2 and T3 compared to treatment T4 and it amounted to 12.68, 11.03 , 11.87 and 13.75 MJ/kg dry matter. The high percentage of total gas when adding garlic powder is a clear indication of the level of the fermentation showed by the addition increased with an increase in the percentage, as it was observed a decrease in methane production with the second treatment compared to the other treatments, and this indicates a decrease in the food energy lost in the form of methane gas when adding 10% garlic powder (Kongmun et.al 2010) who observed a decrease in total gas with adding garlic powder .On the other hand Anassori ,et.al.(2012) reported that a decrease in gas production with raw and oil garlic this result agree with(Kekana, et.al.2021). The production of methane gas is an indicator of the effectiveness of anaerobic fermentation of bacteria (Calsamiglia,et.al 2008) .

Complex sugars are converted into simple sugars and converted into volatile fatty acids. Bacteria benefit from the production of CH_3 and CO_2 from fermentations in the production of methane(Morgavi, et.al 2010) this agrees with (Danielsson,et.al

2017) Which showed that volatile fatty acids have an effect on the production of methane and hydrogen gas and their use as an energy source (Panthee,et.al 2017) the use of garlic leaves led to a decrease in the production of methane gas, while the addition of garlic powder by 64 mg increased the production of gas (Sahli ,et.al 2018).

Table3. Effect of adding different levels (0, 1.0, 2.0 and 3.0%) /100gDM of commercial garlic powder to the ration in metabolizable energy (MJ/kg DM), organic matter digestibility (%)by gas (ML/200mg DM)total gas and methane *in vitro*

Parameters	T1 0	T2 1.0	T3 2.0	T4 3.0	Significant
TG ml/200mg DM	2.64 b±43.00	3.51 a ±53.00	2.90a±52.66	1.20a±60.33	**
CH3 ml/200mg DM	38.00±2.00 b	3.51 C ±23.00	4.05a±48.66	2.66a±57.33	**
CH3/ML	1.00±5.00	0.57 ±3.00	1.15±4.00	1.52±3.00	NS
ME MJ/kg DM	0.51ab±12.68	0.41b±11.03	0.86b±11.87	0.18a±13.75	*
OMD %	7.22± 68.62	3.46 ± 75.89	2.58±72.26	2.63±80.85	NS

abc Means with different superscript on the same raw are differ significantly ($p<0.05$)*($p<0.01$)**T1,0 garlic powder /100 g DM,T2:0. garlic powder /100 g DM,0.3 garlic powder /100gDM

Table 4 showed that significantly increased ($p<0.01$) the dry matter digestibility by (61.28) with T3 (2.0%) garlic powder compared with control by (44.66), whereas organic matter digestibility significantly increased ($p<0.05$) by (79.87) with T3 (2.0%) garlic powder compared with control by (51.62). Garlic powder may have improved the rumen environment, which led to increase *in vitro* digestion. These results were similar with Kongmun et.al (2010) who reported that the addition of 16 mg of garlic powder showed a significant increase in the digestion coefficient . On the other studies observed that no changes in DM digestibility when adding garlic powder to ration(Wanapat, et.al 2008 and Kongmun et.al 2010) . Zhong, et.al (2019) showed that the lambs were fed 50 g/kg the digestibility of DM and CP increased by garlic Powder supplementation. Whereas , Kekana, et.al.(2021) on their studies observed that no changes in DM digestibility when adding garlic powder to ration(Wanapat et.al 2008; Kongmun et.al 2010 and Zhong,et.al 2019) the lambs were fed diet without or with 50 g/kg increased digestibility of DM and CP by garlic Powder . Whereas , Kekana, et. al (2021) pointed that powder and juice garlic resulted in differences in DM digestibility decreased with garlic juice (1ml) compared with control.

Table4 Effect of adding different levels (0, 1.0, 2.0 and 3.0%) /100g DM of commercial garlic powder to the ration *in vitro* dry matter and organic matter digestibility (%)

Treatments	DMD	OMD
T1	.72b ±544.66	0.71c±51.62
T2	0.27b±45.20	59.61±2.05b
T3	5.36a±61.28	0.23 a± 79.87
T4	3.23a±57.81	3.95bc±58.40
Significant	**	*

abc Means with different superscript on the same Colum are differ significantly ($p<0.05$)*($p<0.01$)**T1:0.0 garlic powder T1:1 .0 g garlic powder /100g DM,T2: 2.0g garlic powder /100gDM,T3.:3.0 g garlic powder /100 g DM

Table 5 showed that different levels of garlic powder supplementation in diets concentrate : roughage (40:60) significant difference ($p<0.01$) between all treatments. During the study, the number of total bacteria was observed with an increase in the percentage of garlic powder addition compared to the control treatment, which amounted to (8.00,5.66,13.33 and 29.00). cfu/ml . This result may be due to the active substances of garlic powder alliin and oil sulphur, which led increasing the activity of microorganisms and improving the rumen environment led to an increase in their numbers, as shown by some studies. Wanapat, et.al (2013) note that gram-positive bacteria are inhibited when a mixture of garlic powder and other plants is added Ikyume, et.al(2017) pointed that supplementing four levels (0, 0.5,1and 1.5%) /100 kg garlic powder fed diet at ratio 70:30 roughage :concentrate had inhibition on gram positive bacteria. The addition of some different garlic sources (garlic skin supplements) showed changes in the numbers of some types of bacteria, increased production of microbial protein, such as *Methanosphaera, Howardella, Bulleidia,* and *Prevotella, and reduced Fretibacterium* (*Zhu* et.al.2021).

Table 5.Effects of different levels(0.0, 1.0, 2.0 and 3.0%) /100gDM of commercial garlic powder to the ration in total count of bacteria^{11} $10_{\times}$ cfu /ml

Treatments	bacterial $)^{11}10$ x(numbers
T1	c8.00±0.57
T2	d5.66±0.33
T3	b13.33±0.88
T4	a29.00±0.57
Significant	**

abcd Means with different superscript on the same Colum are differ significantly ($p<0.01$)**T1:0.0 garlic powder T1:1.0 g garlic powder /100g DM,T2: 2.0g garlic powder /100gDM,T3.:3.0 g garlic powder /100 g DM

Outcome

In conclusion, Through this study, it can be concluded that adding garlic powder with levels 1.0 and 2.0 g / 100 g decreased methane gas and metabolizable energy compared to 3.0 g/ 100 g of and improvement dry and organic matter in the *in vitro* digestibility, significant differences in the number of bacteria observed with an increase in the levels of garlic .

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