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EFFECT OF ADDING DIFFERENT LEVELS OF BISR DATE POWDER IN TOTAL GAS AND METHANE PRODUCTION AND *in vitro* DIGESTIBILITY

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Abstract

This experiment was conducted in the nutrition laboratory, department of animal production /college of Agricultural Engineering science/University of Baghdad /Iraq. from the period 14/10/2018 to 17/2/2019. This experiment study the effect of adding four levels of bisr dates powder (2.7, 5.4, 8.1 and 10.8 %) to mixed ration (40% roughage + 60 % concentrate) on *in vitro* total gas and methane production, some rumen liquor characteristics and *in vitro* dry matter and organic matter digestibility after 24,48,72 and 96 hr. of incubation periods, The results showed a significant decreased ($p<0.01$) in total gas and methane production by adding bisr dates powder at different levels and different incubation periods compared to control treatment, bisr dates powder at low and medium levels (second and third treatments) led to a significant increased ($p<0.01$) *in vitro* dry matter and organic matter digestibility and metabolizable energy, a significantly increased ($p<0.01$) in volatile fatty acids concentration after different incubation periods, this value decreased significantly ($p<0.01$) in high levels (10.8 %) of bisr dates powder, ammonia nitrogen concentration decreased significantly ($p<0.01$) in all treatments after different incubation periods compared with control treatment, We conclude that addition of bisr dates powder at low and medium levels reduce total gas and methane production and increase *in vitro* of dry matter and organic matter digestibility and increased volatile fatty acids concentration.

Key words : *In viro* gas production, bisr dates, methane, *in vitro* digestibility, volatile fatty acids.

Introduction

Methane represents 9 - 4% of greenhouse gases (Bhatta, 2012), about 39% of methane is produced from the aerobic fermentation process in the ruminant digestive system (Gerber *et al.,* 2013), 2-15% of the total energy of feed converted into methane during fermentation in the rumen which reduces the utilizing of feed energy (Kennedy æ Charmley, 2012), therefore, it is important to find solutions to reduce methane production without harming the animal by controlling the rumen ecosystem through various additives that may be nutritional the most notable of which adding vegetable oils (Hassan and Irhaeem. 2016), or essential oils such as castor oil and flax (Kuttar *et al.*, 2017, Kuttar *et al.*, 2018), the a additives may be non-food such as nitrate and urea (Hassan and Hussan, 2018, 2020), cranberry leaf flavonoids (Al-Bayati and Hassan, 2018), in addition to the possibility of adding some plants containing tannin compounds that have a high potential to inhibit methane production with minimal negative effect of fermentation of nutrients in the rumen (Bhatta *et al.,* 2013), These compounds improvement rumen fermentation if used at a low concentration (Jayanegara *et al.,* 2012), its effect may be beneficial or harmful depending on the type of tannin consumed, chemical composition, molecular weight and the intake (Puchala *et al.,* 2012), tannins are a complex group of phenolic compounds that has been classified into hydrolyzed tannin, condensed and false tannins. Bhatta *et al.*, (2012) found that condensed tannin extracted from chestnut wood (*Castanea sativa*) reduced methane gas by 5.5%, and the reduction was linear as the amount of tannin increased in plants consumed by animals by its effect on microorganisms in rumen, especially that produces methane (Huang *et al.*, 2010), The aims of this experiment to investigate the effect of adding different levels of bisr dates powder (one of the sources of tannin) on *in vitro* methane production which

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is one methods to estimating the nutritional value of feed as well as its effect on the characteristics of rumen liquor and estimation *in vitro* dry matter and organic matter digestibility.

Materials and Methods

This experiment was conducted in the nutrition laboratory of department of animal production / college of agricultural engineering sciences / university of Baghdad / Iraq. To study the effect of adding different levels of bisr dates powder (one stage of immature dates) to mixed ration in some rumen liquor characteristics, total *in vitro* methane and gas production, *in vitro* dry matter and organic matter digestibility and metabolizable energy.

Experimental rations

The experiments included the use of mixed ration (40% roughage + 60% concentrate). The roughage included 23% Alfalfa hay and 17% barley straw. Khistawi bisr date (the third stage of the maturity dates), yellow color dates, sweet taste beginning and low levels of tannin, was dried at 60 ° C then at 105 ° C in order to maintain its content of tannin and other nutrients, Grinding and stored in plastic boxes until use. The powder was added to the mixed diet at levels 2.7, 5.4, 8.1 and 10.8% of the dry matter, The addition levels of powder was based on the percentage of condensed tannin (7.4%) with levels of tannin 0.2, 0.4, 0.6 and 0.8%, table 1 and 2 show the components, chemical composition and metabolic energy of mixed rations.

Estimation total gas and methane production

In vitro total gas and methane production was estimated by taking 6 replicates per sample according to Menke and Steingass (1988), Weighing 200 mg of experimental diets, 20 ml of artificial saliva and 10 ml of filtered rumen liquor taken from rumen of newly slaughtered lamb were added, Samples were placed in 100 ml glass syringes, and then added one time carbon dioxide to each syringe immediately before incubation. In order to prevent the descent of the liquor from the syringe, the syringe needle was closed with a plastic stopper. In a 39 ° C water bathe for periods 24, 48, 72 and 96 hours with blank for each stage of incubation periods, stirring of injection twice daily (similar to the rumen work), Injections were withdrawn to account total gas production and then 4 ml of 4% NaOH concentration was added to only 3 samples to calculate methane production by Fievez *et al.*, (2005).

Calculate metabolized energy, net energy for lactation, *in vitro* organic matter digestibility and total short-chain fatty acids

This indicators were calculated using total volume of gas production after 24 hr. of incubation periods using the following equations:

ME (MJ/kgDM) = 1.06 + 0.157 GV + 0.084 CP + 0.22 CF “ 0.081A (Ash)

IVOMD (%) = 14.88 +0.889 GV + 0.45 CP + 0.651 × A (ASH)

SCFA (m mol) = 0.0239 GV – 0.061

according to Menke and Steingas(1988)

NEL (MJ/Kg DM) = 0.096 × GV +0.0038 × CP+0.000173 × EE2+0.54

according to Getachew *et al.,* (1999)

Fermentation characteristics after each incubation period

Include PH, ammonia nitrogen concentration, total volatile fatty acids and protozoa number.

Determination of *in vitro* dry matter and organic matter digestibility

Digestibility was carried out in two stage in order to contain concentrate and roughage feed (microbial and enzymatic digestion) according to Tilley and Terry (1963).

Chemical analysis

Experimental diets were analyzed to determine dry matter, ash nitrogen and Ether extract according to the A.O.A.C. (2010).

Table 1: Chemical composition (% of dry matter) and ingredients of mixed ratios a

Ingredients	%	Nutrients %								
		DM	OM	CP	CF	EE	ASH	NFE	CT	ME*
Wheat bran	39	90.12	84.55	14.02	11.00	4.53	5.56	64.89	-	12.72
Yellow corn	11	89.10	82.67	8.85	3.21	4.37	6.43	77.14	-	13.38
Alfalfa hay	23	87.97	80.62	12.88	16.57	1.93	7.35	61.28	-	11.55
Barley straw	17	91.37	85.10	3.37	35.17	1.32	6.27	53.88	-	10.11
Soybean Mea	8	90.48	85.47	43.77	5.49	3.57	5.01	42.17	-	12.54
Salt	2	-	-	-	-	-	-	-	-	-
Bisr dates	-	75.60	73.09	3.90	2.30	1.20	2.01	90.59	33.80	13.64

*ME (MJ/Kg DM)= 0.012 × CP + 0.03 × EE +0.005× CF +0.014 ×NFE (MAFF, 1975) CT: Condense tannin

Table 2: The chemical composition of mixed ration %.

Rations	%							ME
	DM	OM	CP	CF	EE	ASH	NFE	
T_1:0 %	92.95	82.45	19.25	26.78	2.31	10.51	41.17	10.13
T_2:2.7 %	93.53	82.03	19.25	27.51	1.83	11.53	39.94	9.84
T_3: 5.4%	92.18	80.54	19.42	27.80	1.95	11.64	39.24	9.80
T_4 :8.1 %	91.59	80.29	19.48	28.33	1.82	11.30	39.09	9.79
T_5 :10.8%	93.42	81.42	19.55	28.52	2.01	11.53	38.43	9.77

T_1: control, T_2: added 2.7 % bisr date powder,T_3: added 5.4 % bisr date powder, T_4: added 8.1bisr date powder, T_5: added 10.8 % bisr date powder.

Statistical analysis

The data were analyzed statically complete randomized design, treatments means were separated using Duncan (1955) multiple range test, level of probability using the SAS, (2012) program.

Results and Discussion

In vitro total and methane gas production (ml / 200 mg dry matter)

In the current study, after 24 hr. of incubation periods control ration produced the highest (p<0.01) cumulative gas, while the lowest volume (p <0.01) of gas in fifth treatment (10.8% bisr dates powder) table 3, regard to methane production, the results showed a high significant differences (p <0.01) between rations, the volume of methane gas ranges between 14.67 - 5.33 ml / 200 mg dry matter, The highest methane volume in the control ration, the fifth ration (10.8% bisr dates powder) has lowest methane production, as shown in Table 3 data showed a significantly decreased (p <0.01) in total gas volume after 48 hr. The gas volume decreased in all rations compared to control ration, reaching 38.33 ml / 200 mg dry matter. The decrease in total gas production was recorded in the fourth and fifth rations at 24.67 and 22.67 ml / 200 mg dry matter, respectively. With regard to methane production, after 48 hr. of incubation, Table 3 indicated significant differences (p <0.01) between rations, the volume of methane ranged from 16.67 to 7.33 ml / 200 mg dry matter, the highest volume of methane in the control ration and the lowest volume in the fifth rations, In the same context, the total volume of gas formed after 72 hr. of incubation was the highest volume in the control ration as it reached 40.67 ml / 200 mg dry matter, It is widely accepted that feedstuffs which have higher gas production tend to have higher CH_4 production per gram DM incubated (Durmic *et al.,* 2010), The lowest volume of gas production was in the fourth and fifth rations (26.67 and 24.67 ml / 200 mg dry matter, respectively). With regard to methane production after 72 hr., there was a significant decrease (p <0.01) in all rations compared to the control ration, The volume of methane gas ranged between 17.33 - 10.67 ml / 200 mg dry matter, The highest methane production record in control ration, As for in fourth and fifth rations there was a significant (p <0.01) reduction in methane volume (12.67 and 10.67 ml / 200 mg dry matter, respectively), The results of the statistical analysis in Table 3 showed that the total volume of gas and methane formed after 96 hr. of incubation recorded highest volume in the control rations, reaching 43.33 and 20.67 ml / 200 mg dry matter, respectively. The lowest volume in the fifth rations was 27.33 and 12.67 ml / 200 mg dry matter, respectively, It is worth mentioning that the reduction of gases formed as a result of fermentation of different nutrients in the rumen depends on a number of factors, including the impact of additives directly affecting the microorganisms, especially methanogenesis microorganisms which are more sensitive to presence of tannin in the rumen liquor, and reduces the numbers or inhibits their activity (Lemine *et al.,* 2014), as well as reducing the production of hydrogen available in the rumen, which is an important element in methane production. It is clear from the data

Table 3: Effect of adding different levels of bisr dates powder on invitro total gas and methane production (ml / 200 mg dry matter) after different incubation periods.

Rations	24		48		72		96	
	Total gas	Methane	Total gas	Methane	Total gas	Methane	Total gas	Methane
T_1 control	36.67+ 0.67a	14.67+0.67a	38.33+0.33a	16.67+0.66a	40.67+0.67a	17.33+0.66a	43.33+ 0.67a	20.67+ 0.66a
T_2: 2.7%	32.67+ 0.67b	12.67+ 0.67a	33.33+ 0.67b	14.66+ 0.67b	36.67+ 0.66b	16.66+ 0.66b	38.67+ 0.01b	18.03+ 0.01b
T_3: 5.4%	26.67+ 0.67c	10.67+0.66b	32.67+ 0.67b	12.63+ 0.33c	32.67+ 0.67c	14.67+ 0.01c	35.33+ 0.67c	16.67+ 0.66c
T_4: 8.1%	22.76+ 0.67d	7.33+ 0.67c	24.67+ 0.67c	10.67+ 0.66c	26.67+ 0.67d	12.67+ 0.66d	30.67+ 0.67d	15.33+ 0.67c
T_5: 10.8%	19.76+0.67e	5.33+0.66c	22.67+ 0.67c	7.33+ 0.66d	24.67+ 0.66d	10.67+0.67d	27.33+0.67e	12.67+0.33d
Significance level	**	**	**	**	**	**	**	**

T_1: control , T_2:added 2.7 % bisr date powder,T_3: added 5.4 % bisr date powder, T_4: added 8.1bisr date powder, T_5: added 10.8 % bisr date powder. ** means that there are significant differences at the probability level (P <0.01).

Table 4: Effect of adding bisr dates powder on metabolized energy(MJ/Kg DM), net energy for lactation (MJ/Kg DM),invitro organic matter digestibility (%) and total short-chain fatty acids(ml /100ml).

Rations	Metabolized energy	Invitro Organic matter digestibility	Short-chain fatty acids	Net energy for lactation
T_1 control	13.38±0.01a	62.68±0.29 a	0.83±0.01a	4.08±0.01a
T_2: 2.7%	12.92±0.01 b	60.06±0.57 b	0.73±0.01b	3.71±0.02b
T_3: 5.4%	12.05±0.10 c	54.57±0.26 c	0.60±0.01c	3.14±0.03c
T_4: 8.1%	11.48±0.01 d	50.79±0.23 d	0.48±0.01d	2.78±0.02d
T_5: 10.8%	11.08±0.10 e	48.18±0.14e	0.41±0.01e	2.51±0.01e
Significance level	**	**	**	**

T_1: control, T_2:added 2.7 % bisr date powder,T_3: added 5.4 % bisr date powder, T_4: added 8.1bisr date powder, T_5: added 10.8 % bisr date powder.
** means that there are significant differences at the probability level (P <0.01).

Table 5: Effect of bisr dates powder on invitro digestibility of dry matter, organic matter (%) and metabolizable energy (MJ / kg dry matter).

Rations	IVDMD (%)	IVOMD (%)	(ME) MJ/Kg DM
T_1 control	68.19±0.14 c	70.61±0.54 c	10.76±0.01c
T_2: 2.7%	80.80±0.14 a	82.33±0.21 a	12.51±0.01 a
T_3: 5.4%	75.83±0.24 b	78.03±0.17 b	11.38±0.04 b
T_4: 8.1%	63.05±0.19 d	65.17±0.19 d	9.94±0.04 d
T_5: 10.8%	57.96±0.34 e	60.00±0.10 e	8.93±0.09e
Significance level	**	**	**

T_1: control, T_2: added 2.7 % bisr date powder,T_3: added 5.4 % bisr date powder, T_4: added 8.1bisr date powder, T_5: added 10.8 % bisr date powder.
** means that there are significant differences at the probability level (P <0.01).

in table 3 that the trend of decrease cumulative gas production and methane was with the increase of level of bisr date powder which affects the increase of tannin amount in all rations and in different incubation time, the presence of plant secondary metabolites (e.g. condensed tannins and saponins) may account for this,. Tannins, common in biser date palm, have been shown to reduce CH4 production by reducing fibre digestion. binding with proteins thus reducing degradation of the plant protein in the rumen and through the direct inhibition of the growth of methanogens (Tavendale *et al.,* 2005). The effect of tannin in suppression of methane production has also been reported by Hess *et al.*, (2004) who observed *in vitro* that the inclusion of the tropical legume (270 g of condensed (270 g of condensed tannin/kg DM) in grass-based diet suppressed methane production relative to DM degraded by over 30 %.

Estimate metabolized energy, net energy for lactation, invitro organic matter digestibility and total short-chain fatty acids

(Table 4) shows the results of statistical analysis of the effect of biser date powder addition on the metabolizable energy, invitro organic matter digestibility, short chain fatty acids and net energy for lactation, estimated from total gas volume after 24 hr. of incubation period. The values ranged between 11.08 in control ration and 13.38 MJ/ Kg DM in T5 (10.8% biser date powder) for ME as a result of increase levels of tannin in biser date powder, Similar trend was observed for invitro organic matter digestibility (range 48.18-62.68%) and short-chain fatty acids (0.41-0.83 ml / 100ml), and the value of net energy for lactation range 2.51- 4.08 MJ/ Kg DM. Being seeds they are high in crude protein and fibre as energy sources for microorganism.

Statistical analysis of invitro digestibility of dry matter, organic matter and metabolizable energy are given in Table 5, showed a significant increased (p <0.01) in second ration compared to the others, The values were 80.80, 82.33% and 12.51 MJ / kg dry matter, respectively, The lowest values of the studied traits were recorded in the fifth treatment (10.8% bisr dates powder) reaching 57.96, 60.00% and 8.93 MJ / kg dry matter, respectively. The second ration (2.7% bisr dates powder) is the best response between the different rations in value of invitro digestibility followed by third treatment (5.4% bisr dates powder). bisr dates powder, it is an important source of energy, providing 87% of digestable energy (Nunes, 1994), as well as high total digestable nutrients (Al- Dobaib, 2009). The low and medium levels of bisr dates powder resulted in the improvement of invitro digestibility of dry and organic matter due to increased utilization of nutrients, especially crude protein. This is consistent with Al-Homidy *et al.*, (2011), who stated that the use of the bisr dates in diets increased the digestion coefficient, *In vitro* digestibility of dry matter, organic matter and metabolic energy decreased with the addition of bisr dates powder due to the increase of tannin level in the rumen liquor causing a decrease in number of protozoa digestive cellulose and protein (Table 8).

Some characteristics of rumen fermentation after each incubation period

1. Ammonia nitrogen concentration (mg/100 ml)

(Table 6) shows the effect of adding different levels of bisr dates powder on ammonia nitrogen concentration after different invitro incubation periods. We note that ammonia nitrogen concentration increased significantly (p <0.01) in control ration, Values 34.79, 32.53,30.13 and 26.53 mg / 100 ml after 24, 48, 72 and 96 hr. of

Table 6: Effect of adding different levels of bisr date powder on concentration of rumen ammonia nitrogen (mg /100 ml) after different incubation periods.

Incubation periods				Rations
96 hr.	**72 hr.**	**48 hr.**	**24 hr.**	
26.53 ±0.34 a	30.13 ±0.30 a	32.53 ±0.24 a	34.79 ±0.23A	T_1 control
18.16±0.13 b	20.11±0.22 b	24.13 ±0.18 b	26.69±0.22 b	T_2 2.7 %
13.67±0.22 c	16.12±0.18 c	18.64±0.24 c	22.83±0.11 c	T_3 5.4 %
11.02±0.44 d	13.11±0.18 d	15.04 ±0.39 d	17.86±0.16 d	T_4 8.1 %
10.51±0.14 e	11.87±0.22 e	12.10±0.18 e	13.80±0.12 e	T_5 10.8 %
**	**	**	**	Significance level

T_1: control, T_2: added 2.7 % bisr date powder,T_3: added 5.4 % bisr date powder, T_4: added 8.1bisr date powder, T_5: added 10.8 % bisr date powder.

** means that there are significant differences at the probability level (P <0.01).

Table 7: Effect of adding different levels of bisr date powder on concentration of volatile fatty acids (mmol/100 ml) after different incubation periods.

Incubation periods				Rations
96 hr.	**72 hr.**	**48 hr.**	**24 hr.**	
59.83 ±0.11 c	63.02 ±0.29 c	70.94 ±0.10 c	80.87 ±0.08b	T_1 Control
61.67±0.22 a	69.75±0.23a	74.69±0.33 a	82.10±0.36 a	T_2 2.7 %
60.92±0.44 ab	65.14±0.19b	72.80±0.27b	81.17±0.41ab	T_3 5.4 %
60.09±0.39cb	62.81±0.46 c	71.15±0.61c	79.43±0.45c	T_4 8.1 %
58.22±0.33d	61.85±0.21 d	70.58±0.18 c	75.22±0.34d	T_5 10.8 %
**	**	**	**	Significance level

T_1: control, T_2: added 2.7 % bisr date powder,T_3: added 5.4 % bisr date powder, T_4: added 8.1 % bisr date powder, T_5: added 10.8 % bisr date powder.

** means that there are significant differences at the probability level (P <0.01).

incubation periods respectively, The lowest concentration was in the fifth treatment (10.8% bisr date powder) as it was 13.80, 12.10, 11.87 and 10.51 mg / 100 ml, respectively. This may be due to the effect of increasing the percentage of tannin present in the bisr date powder to the low concentration of ammonia nitrogen by its effect on the formation of complexes with protein, which reduces digestion and reduce the removing amine root from protein to form ammonia nitrogen in rumen liquor, This is agree with the study of Hassan Zadeh and Dayani (2012) reported that the addition of different levels of bisr date reduced ammonia nitrogen concentration in the rumen liquor, Numerous studies have also shown that use of bisr date increases utilization of nitrogen in the rumen by increasing the efficiency of microbial protein production. The low concentration of ammonia (10.51 mg / 100 ml) in the present study are the optimum limits for best microbial growth and microbial protein senesces (Khezri and Daneshmesgaran, 2010, Javidan and Khezri, 2013 and Bayati Zadeh *et al.,* 2013).

Volatile fatty acid concentration (mmol / 100 ml)

As can be seen from table 7 there were significant differences (p<0.01) among rations in terms of volatile fatty acids concentration, noting that second ration (2.7% bisr date powder) recorded a high significant (p <0.01) after deferent incubation hours (24, 48, 72 and 96 hours) were 82.10, 74.69, 69.75 and 61.67 mmol /100 ml, respectively. followed by the third ration (5.4% bisr date powder), wile the fifth ration recorded the lowest significant decrease (p <0.01) among the rations, The significant increase in the volatile fatty acids concentration as a result of the addition of bisr date powder, especially at low and moderate concentrations may be due to contain bisr date powder large quantities of soluble carbohydrates as well as degradable fibers, which increases the efficiency of volatile fatty acids production. Because of moderate levels of tannin, volatile fatty acids will be available for longer incubation periods due to the complexity of tannin with carbohydrates, which coincides with the release of nitrogen, increasing the efficiency of microbial protein senesces (Krueger *et al.,* 2010; Siddiq *et al.,* 2013 and Eoin, 2016). However, in the high levels of bisr date powder as in the fourth and fifth rations, the high tannin content in this additive inhibits the fibrolytic bacterial as well as the deposition of various nutrients, which reduces the digestibility and thus low volatile fatty acids concentration of in rumen liquor. This relationship is suggested to be a reflection of feed digestibility (Demeyer and Van Nevel, 1975; Holtshausen *et al.,* 2009).

pH

(Table 8) shows the effect of adding different levels of bisr date powder on the pH value of the rumen liquor, and the differences were highly significant (p <0.01), The pH value increased after 24 hours of incubation period in the third, fourth and fifth rations (6.7, 6.7 and 6.7, respectively) compared to the control and the second rations (6.5 and 6.5 respectively), Table 8 also shows that the pH value after 48 hr. of incubation period a significant decreased (p <0.01) in the third ration (5.4% bisr date powder) at 6.6, as well pH value after 72 hr. of incubation period recorded the highest significant value (p <0.01) in the control ration (7.3), however, The lowest values in second and Third rations (6.7 and 6.7,

Table 8: Effect of adding levels of bisr date powder on pH and protozoa (cell ×10^5/ ml) after different incubation periods.

Protozoa cells×10^5/ml	PH				Rations
	Incubation period				
	96 hr.	72 hr.	48 hr.	24 hr.	
3.83 ±0.01A	7.5 ±0.06 a	7.3± 0.07 A	6.9 ±0.06 A	6.5 ±0.03B	T_1 Control
3.73± 0.01 b	7.0± 0.03ab	6.7± 0.03 D	6.8 ±0.01B	6.5± 0.03B	T_2 2.7 %
3.61± 0.01C	6.8 ±0.03d	6.7± 0.03 D	6.6± 0.03 C	6.7± 0.03 A	T3 5.4 %
3.34± 0.02d	7.1± 0.03b	7.0± 0.03B	6.8± 0.03 B	6.7 ±0.03 a	T_4 8.1 %
3.14± 0.02e	6.9± 0.03c	6.8± 0.03C	6.8± 0.03 B	6.7 ±0.03A	T_5 10.8 %
**	**	**	**	**	Significance level

T_1: control, T_2: added 2.7 % bisr date powder,T_3: added 5.4 % bisr date powder, T_4: added 8.1bisr date powder, T_5: added 10.8 % bisr date powder.

** means that there are significant differences at the probability level (P <0.01).

respectively), Regarding the pH value after 96 hr. of incubation period, the highest value (p <0.01) was recorded in the control ration (7.5). The results show that although the pH value varies with different levels of bisr date powder to diets in different incubation period, the pH falls within the normal range of rumen liquor (6.5-7.0) (Cardozo *et al.,* 2002). This agree with Griswold *et al.,* (2003) who observed that increasing levels of bisr date powder in rations affects pH value of the rumen liquor.

Number of protozoa (cell × 10^5 / ml)

The results in Table 8 show that the addition of bisr date powder had a significant effect (p <0.01) in reducing the protozoa numbers in the rumen liquor, it is ranging from 3.14 to 3.83 cell × 105 / ml. This decrease in protozoa numbers can be explained by the fact that the tannin found in bisr date powder acts as an inhibitor of microorganism groups, especially methanogeneses organisms, which include protozoa without affecting the other groups digestive different nutrients. Contains numerous mineral elements and vitamins that promote the growth of tannin-resistant microorganisms (Al- Harrasi *et al.,* 2014).

General conclusion

The production of large quantities of dates reflected on large quantities production of biser dates of all kinds, which can be used as additives in ruminant animals rations, In the current study, levels of bisr date powder were able to favorably modulate rumen fermentation to produce methane concentrations lower than expected when considering their cumulative gas production, This is turn, is negatively correlated with gas production, We can be added biser dates, whether dry or wet at low or medium levels, to ruminant diets (2-6% dry or 3-8% wet) to be used as a product not suitable for human consumption. Moderate percentage of tannin that improved digestibility of nutrients due to increased tilization, especially crude protein, which is positively reflected on weight gain or milk production and other productive traits, The tannin compound in biser dates reduces methane gas formation in the rumen as a result of fermentation, This feed keep lost energy in the form of methane, and the energy goes for production purposes. It also reduces the proportion of methane in the atmosphere, which contributes to improving the environment and reducing global warming.

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