IMPACT OF SUPPLEMENTARY *MORINGA OLEIFERA* LEAF EXTRACT ON RUMINAL NUTRIENT DEGRADATION AND MITIGATING METHANE FORMATION *IN VITRO*

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SUMMARY

Plant extracts may be highly effective as natural dietary supplementation options to alternate the dietary antibiotics as growth promotors in ruminant diets. The current study was conducted to evaluate the dose response effects of the moringa *(Moringa oleifera*) leaf extract (MLE) as a natural alternative to monensin in sheep diets, on ruminal methane production (CH_4), gas production (GP), nutrient degradability and fermentation parameters. The *in vitro* semi-automatic system of GP was used. The treatments were MLE added to a basal diet (consisted of 50 concentrate: 50 forage) at 0 (control), 50 (MLE_{low}) and 500 (MLE_{high}) mg/ kg dry matter, and the ionophore antibiotic monensin was added at 40 mg/kg dry matter. Abundant quantities of essential amino acids, monosaccharides, glycosides and benzene derivatives phytochemicals components were detected by the GC–MS analysis of MLE. The most effective treatments to decrease ($P < 0.05$) CH_4 were monensin and MLE_{high}, while only MLE_{high} enhanced ($P < 0.05$) the overall mean of total volatile fatty acids (VFAs) concentrations compared to the other treatments and the molar proportion of acetate compared to monensin. A decline ($P < 0.05$) in protozoal count was observed by monensin, while such effect did not appear at other treatments. No significant differences were observed among the experimental treatments in the ruminal degradability, ammonia concentrations or GP. This study demonstrated efficiency of MLE as an effective natural intervention to monensin in sheep diets.

Keywords: *Methanogenesis, monensin, ruminal fermentation and moringa leaf.*

INTRODUCTION

Methane (CH_4) emission from ruminants represents a considerable loss of dietary energy which could potentially be redirected towards the meat or milk production (Patra and Yu, 2015). This is especially the case in most areas of the developing countries, due to the low feed efficiency of the animals that lead to high cost in terms of CH_4 produced per unit of animal product (Soltan *et al*., 2012).

Antibiotic ionophores are widely used in ruminant industry to improve energy and protein utilization and decrease CH_4 emission (Russell and Strobel, 1989). However, there is a controversy about the use of these additives due to the risk of transferring residues into final animal products (meat and/or milk). These concerns have promoted the search for alternative natural additives such the secondary metabolites which occurring naturally in many plant species.

Moringa trees *(Moringa oleifera*) belonging to the Moringaceae family, have been used as an antibiotic in traditional medicine dates back thousands of years in many developing countries (Soliva et al., 2005 and Soltan *et al*., 2018). Among moringa parts, the leaf was the most part rich in various phytochemicals with high potency as antimicrobial, anti-cancerous, antianthelmintic, antispasmodic, anti-inflammatory properties (Sholapur and Patil 2013; Wang *et al*., 2016 and Soltan *et al*., 2017a). Thus there are many studies confirmed moringa leaves as dietary feed additives in livestock production, however, most of these studies were done for the whole leaf, while eliminating its extractions. Moreover, most of studies with the leaf extracts eliminated their antimethanogenic activity. Parts other than the leaves of moringa were suggested to

be natural alternatives to monensin antibiotic to modulate CH_4 emission towards more volatile fatty acids. Recently, Soltan *et al*. (2018), found a similarity between moringa whole root bark and monensin in enhancing the growth performance of the growing lambs, while reduced CH_4 emission relative to body weight gain. Thus, this provided a suggestion that if the chemical characterization of secondary metabolites of moringa leaf extract (MLE) and their mechanism of action can be clarified, they may introduce an alternative to replace the dietary antibiotic additives for ruminants. The objective of the current study is to evaluate *in vitro* the effects of two levels of MLE on ruminal fermentation, degradability and CH_4 production compared to monensin.

MATERIALS AND METHODS

Moringa origin, processing and analysis of the MLE:

Fodder leaves of Moringa *(Moringa oleifera)* had been harvested in the first cutting. About 25 kg of fresh leaves were collected from a private farm located 45 km south of Alexandria (30°50′56″N 29°36′42″E), Egypt. The leaves were collected from 50 trees, pooled, dried at 40°C for 72 h and milled through 1 mm screen.

Ten grams of moringa leaves were ground to a fine powder and mixed with 100 ml ethanol (700 ml/l). The mixture was then ultrasonically for 30 min. The ethanol extract solution was subsequently filtered and kept at −5 °C overnight and was filtered again. The supernatant was transferred to the rotary evaporator (RE301/601/801**,** Yamato Scientific America Inc., USA) and treated at 42 °C for 30 min in order to remove the ethanol. The concentrated extract recovered in the volumetric flask was lyophilized for 3 days to get the experimental MLE that was used for the chemical analysis and the *in vitro* assay. The MLE was subjected to an in-depth compositional analysis using gas chromatography/mass spectrometry (Thermo Scientific TRACE- 1300 series GC) as described in details by Soltan *et al*. (2018).

Basal diet, treatments and inocula preparation:

The control total mixed ration was consisted of (g/kg DM): 500 g clover (*Trifolium alexandrinum*) hay, 200 g ground maize, 27.5 g soybean meal, 114.5 g wheat bran, 125 g cotton seed meal, 20 g limestone, 10 g sodium chloride and 3 g mineral premix. The ration was chemically analyzed based on DM (g/kg) according to AOAC (1995) as: OM= 896.7and CP= 141.6 (as 6.25×N). The neutral detergent fiber (NDF) =505.9, acid detergent fiber (ADF) = 252.6, and lignin= 41 were measured sequentially using ANKOM Technology Corporation, Macedon, NY, USA, and expressed exclusive of residual ash as described by Goering and Van Soest (1970) and Van Soest (1973). The diet was formulated to meet NRC (2007) nutrient requirements recommended for growing sheep.

Four experimental treatments were evaluated as follow: control (the basal diet without supplementations, monensin [(the basal diet supplemented with the manufacturer's recommendation dose (40 mg/kg DM) of ionophore sodium monensin (Rumensin®, Elanco, Itapira, Brazil)], MLE was supplemented to the basal diet at two doses 50 (MLE_{low}) or 500 (MLE_{high}) mg/ kg DM, respectively. The ionophore antibiotic monensin was selected because it is among the most common additives used to decrease CH_4 emission and modulate ruminal fermentation characters (Soltan *et al*., 2018).

Four adult rumen-cannulated Barki sheep (58± 2.5 kg body weight) were used as inoculum donors. The donner animals were fed *ad libitum* berseem clover hay and a concentrate feed mixture (0.7 kg/100 kg body weight, and containing 145 g/kg DM crude protein), and had free access to fresh water. Each treatment was incubated in four inocula, with each inoculum, four bottles per treatment were prepared, two for truly degraded organic matter (TDOM) determination and the other two for estimating the fermentation parameters. The same procedure was applied for the blanks (bottles containing the ruminal inoculum and the buffer solution without samples) to be able to correct the GP from the inoculum, and for an internal standard (bottles containing clover hay, ruminal inoculum and the buffer solution) to correct for sensitivity changes induced by the inoculum (Soltan *et al*., 2012).

***In vitro* assay:**

A semi-automatic system of GP (Bueno *et al*., 2005) using a pressure transducer and a data logger (GN200, Sao Paulo, Brazil) with some modifications according to Soltan *et al*. (2018) was used.

For CH_4 determination, 2 ml of the head space gas was sampled by a syringe (med Dawliaico, Assiut, Egypt) at each measuring event and stored in a 10 ml vacutainer tubes (BD Vacutainer® Tubes, NJ, USA). Methane concentration was determined using a gas chromatograph (Model 7890, Agilent Technologies, Inc, Colorado 80537, USA), the separation conditions in details were described by Soltan *et al*. (2018). The test of linearity and calibration were accomplished using a standard gas curve in the range of probable concentrations of the samples using pure CH_4 (Abu Qir Petroleum Co., Alexandria, Egypt; 939 ml/l purity). The amounts of CH_4 produced were calculated according to Longo *et al*. (2006).

After termination of the incubation, all bottles were placed in ice to inhibit fermentation. Two bottles were assigned to the determination of the truly degraded dry matter and organic matter (TDDM and TDOM, respectively) following Blümmel and Becker (1997) method. The partitioning factor (PF: an indicator of ruminal microbial syntheses) was calculated as the ratio of TDOM (mg) and gas volume (ml) (Blümmel *et al*., 1997). The incubation liquid of the other two bottles was used for determining fermentation parameters and protozoal counts. The ammonia concentrations were evaluated calorimetrically by spectrophotometer (Alpha-1101 model; Labnics Equipment, California, USA) using commercial lab test (Konitzer and Voigt, 1963). The VFAs were determined following the method of Palmquist and Conrad (1971) using a gas chromatograph (Thermo fisher scientific, Inc., TRACE1300, Rodano, Milan, Italy) with some modifications described in details by Soltan *et al*. (2018). Protozoal abundance was counted by microscopy following the procedure of Dehority *et al*. (1983).

Statistical analysis:

Data were subjected to analysis of variance (ANOVA), using the PROC MIXED of SAS software package (2002). The four inocula were considered as the true statistical replicates. Each treatment was incubated in duplicate (analytical replicates) to achieve highly accurate estimate of a true replicate. The analytical replicates were averaged prior to statistical analysis with each inoculum being the statistical replicate, thus the statistical number of replications of treatments (n = 4) are the true statistical replications. The significant differences between individual means were considered significant at $P < 0.05$, whereas $0.05 < P < 0.10$ were considered as a tendency by using Tukey test.

RESULTS AND DISCUSSION

GC–MS analysis of MLE:

Under the current GC–MS separation conditions, the most abundant compounds identified for MLE were branched chain amino acids (BCAA) since L-valine, L-alanine, L-leucine and L-isoleucine were 4.14, 3.90, 2.72 and 2.65%, respectively. Other amino acids like L-threonine (1.38%) was detected, components with benzene ring were detected in high concentration, glycosides were found (7.3%), also 3-caffeoylquinic acid was 3.95% and butanedioic acid was 4.92% (Table 1). These combinations of moringa active components are found to be nutritionally and biologically active, e.g. its bioactive benzene fraction and glycosides are known to possess antibacterial, antifungal, and antioxidant properties (Alptüzün *et al*., 200 and Shah *et al*., 2016). Moreover, MLE can be considered as a good source of amino acids, because it contains considerable amounts of BCAA. These results are in accordance with Gopalakrishnan *et al*. (2016) who suggested that moringa leaves can be considered as a protein supplement due to the high content of essential amino acids (440 mg/kg DM). Although MLE have high content of various active components, little information is available about the effects of these components on ruminal fermentation or methanogenesis.

***Ruminal CH_4, fermentation parameters and degradability**:*

The results presented in Table (2) showed that no differences were observed for the gas production (GP), truly degraded dry matter (TDDM), truly degraded organic matter (TDOM) and partitioning factor (PF) among all the experimental treatments. The most efficient treatments to decrease ($P < 0.05$) CH_4 were monensin and MLE $_{high}$**,** where their proportional CH_4 reduction was 18.1 and 15.5%, respectively compared to the control. Currently, MLE $_{high}$ seemed to act against ruminal methanogenesis, thus likely adversely affecting Archaea as monensin did, and this finding suggest that MLE can be an alternative to the critical antibiotics feed additives in ruminant diets without adverse effects on GP or ruminal degradability. Previous studies also confirmed the antimethanogenic activity of moringa leaves studies, e.g. Dey *et al*. (2014) found an achievement of CH_4 inhibition combined with enhancement of the total GP and organic matter

degradability by wheat straw supplemented with moringa leaves in buffalo diets. Soltan *et al*. (2014) reported that extracts of moringa leaves and root barks could be used as effective natural alternatives to monensin in sheep diets, not only to decrease CH_4 emission, but also to increase the ruminal nutrient degradability. Similarly, Soliva *et al*. (2005) found that CH_4 production was inhibited significantly by 17% with moringa leaves based diet as compared to the diets containing rapeseed meal or soybean meal, without adverse effects on the ruminal fermentation or nutrient degradability, the authors suggested that such effects might relate to existence of bioactive components in moringa leaves, however no specific bioactive components were assigned to confirm such suggestion.

Table (1): Individuality of constituents in the moringa leaf extract (MLE) determined by gas chromatography/mass spectrometry.

Peak	Name	% Area	RT
1	Benzene, 1,1'-[4-(3-phenylpropyl)-1, 7-heptanediyl]bis- (CAS)	8.17	2.03
2	L-alanine, N-(trimethylsilyl)-, trimethylsilyl ester	3.90	7.295
3	L-valine, N-(trimethylsilyl)-, trimethylsilyl ester	4.14	10.090
4	L-leucine, N-(trimethylsilyl)-, trimethylsilyl ester	2.72	11.519
5	L-isoleucine, N-(trimethylsilyl)-, trimethylsilyl ester	2.65	12.085
6	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	2.88	12.147
7	Propanoic acid, 2,3-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	0.77	13.097
8	Carotene, 3,4-didehydro-1,1',2,2'-tetr ahydro-1'-hydroxy-1-methoxy-	3.03	13.842
9	L-threonine, N,O-bis(trimethylsilyl)-, trimethylsilyl ester	1.38	14.533
10	Butanedioic acid, [(trimethylsilyl)oxy]-, bis(trimethylsilyl) ester	4.92	17.032
11	Nd	2.40	17.763
12	Trimethylsilyl 2,3,4-tris[(trimethylsilyl)oxy]butanoate	2.54	18.896
13	Glucofuranoside, methyl 2,3,5,6-tetrakis-o-(trimethylsilyl)-, .alpha.-D-	1.61	24.118
14	D-fructose, 1,3,4,5,6-pentakis-o-(trimethylsilyl)-	6.02	24.335
15	D-fructose, 1,3,4,5,6-pentakis-o-(trimethylsilyl)-	4.73	24.494
16	Mannofuranoside, methyl 2,3,5,6-tetrakis-o-(trimethylsilyl)-, .alpha.-D-	3.57	25.105
17	no-2,4-dimethyl-1H-pyrrol- 3-yl)-2-methyl-4H-pyran-3 -carboxylic acid ethyl ester	1.95	25.464
18	Beta.-D-galactofuranose, 1,2,3,5,6-pentakis-o-(trimethylsilyl)-	6.90	27.940
19	Beta.-D-galactofuranose, 1,2,3,5,6-pentakis-o-(trimethylsilyl)-	2.69	28.229
20	4-(Pentadeuterio)phenylazulene	1.85	29.849
21	Colchiceine	1.86	34.958
22	Benzene, 2-(1-decyl-1-undecenyl)-1, 4-dimethyl- (CAS)	9.15	37.203
23	2,3-Bis(3'-Methoxy-2'-nitro phenylimino)-2H-indole	2.40	37.547
24	1,3,4,6-tetrakis-o-(trimethylsilyl)hex-2-ulofuranosyl 2,3,4,6-tetrakis-o-(trimethylsilyl)hexopyranoside	7.30	38.176
25	Alpha.-D-glucopyranoside, 1,3,4,6-tetrakis-o-(trimethylsilyl)-.beta.-d fructofuranosyl 2,3,4,6-tetrakis-o-(trimethylsilyl)-	5.96	38.973
26	2(3,4bis[(trimethylsilyl)oxy]phenyl)-3,5,7 tris[(trimethylsilyl)oxy]-4h-chromen-4-one	0.56	45.744
27	Hexatrimethylsilyl-trans-3-o-caffeoyl-d-quinic acid	3.95	46.101

Nd: not detected

Table (2): Effect of monensin, and moringa leaf extract (MLE) on ruminal gas production (GP), degradability and partitioning factor (PF).

Item	Treatments				SEM	*P* value
	Control	Monensin	MLE_{Low}	MLE_{High}		
GP (mL/g DM)	150.4	143.3	160.4	159.6	22.71	0.4241
CH_4 (mL/ g TDOM)	34.80^{a}	28.49^{b}	32.95^{A}	29.39^{b}	7.861	0.0199
TDDM (g/kg)	620.5	611.3	603.4	610.8	41.189	0.1214
TDOM (g/kg)	597.06	595.8	562.7	610.9	38.344	0.1993
Partitioning factor (PF)	2.2010	1.9396	1.939	2.431	0.4148	0.216

GP: net gas production; CH_4: methane; TDDM: truly degraded dry matter; TDOM: truly degraded organic matter; SEM: Standard error of the mean.

^{a,b}: Means within a row without a common superscript letter differ significantly ($P < 0.05$).

Most common methanogen inhibitors negatively affect the ruminal fermentation or/ and organic matter degradability at doses that achieve desirable methane reduction (Patra and Yu, 2015). Currently, although

the reasons for methane inhibition caused by MLE remain to be explored, it seems that MLE affected the methanogenesis directly, since the ruminal degradability and the protozoal counts remained unchanged. Thus combinations between antibacterial and antioxidant bioactive components in MLE might play a key role in that concern (Alptüzün *et al*., 2009, Soltan *et al*., 2018).

Table (3) presented the *in vitro* effects of MLE and monensin on ruminal pH, ammonia concentrations, protozoal count and VFAs. No significant differences were observed among the experimental treatments in the ruminal pH or ammonia concentrations, while a decline ($P < 0.05$) in protozoal count was observed by monensin, but such effect did not appear at other treatments. Methanogen inhibitors can reduce CH_4 production directly or indirectly ways through the inhibition of numbers or activity of methanogens and antiprotozoal properties, respectively (Cieslak *et al*., 2013). Monensin was found to alternate the ruminal hydrogen-sink products directly towards less CH_4 production through a shift in hydrogen usage from methanogenesis and /or formate to propionate or succinate production by the inhibition of gram-positive bacteria (Russell and Strobel, 1989 and Schären *et al*., 2017). Moreover, the antiprotozoal effect of monensin might partially help to explain the indirect reduction in CH_4 emission. Thus currently, the tendency ($P = 0.08$) in enhancement of propionate production and decreasing ($P < 0.05$) the acetate to propionate ratio caused by monensin without affecting the total VFAs production may support the above suggestion. On other hand, the reduction of CH_4 caused by MLE $_{high}$ was combined by an enhancement ($P < 0.05$) in the total VFAs production. This may be due to the presence of components with antioxidant activity (e.g. glycosides and 3-caffeoylquinic acid) in MLE. Recently, many studies confirmed that the presence of these components would lessen oxidative stress and promote better conditions for ruminal fermentation (Soltan *et al*., 2017b, 2018). Such enhancement in total VFAs production found by MLE $_{high}$ might partly confirm this hypothesis, since VFAs are the principal outcome of the ruminal fermentation (Calsamiglia *et al*., 2007). The reasons for the increases in VFAs by MLE are not clear, however the high content of essential amino acids (BCAA) found in MLE may parley explain such effect. Nouman et al. (2014) reported that the dietary supplementation of valine, leucine and isoleucine enhanced the production of total VFAs. Thus, the current results suggested that the fermentation pathways expended H_2 to produce VFAs than CH_4 and these increases of VFAs could be attributed to acetate enhancement, hence acetate are the major part of the total VFAs produced by ruminal microbes (Calsamiglia *et al*., 2007 and Soltan *et al*., 2017b). Reduction of CH_4 combined with enhancement ($P < 0.05$) of acetate caused by MLE $_{high}$ may suggest that MLE stimulates acetogenesis as an alternative to the ruminal methanogenesis. Ruminal methanogenesis pathway is the primary H_2 sink, while acetogens have a poorer affinity to H_2 than methanogens (Tan *et al*., 2011). Thus the current results may refer to a competition happened between methanogenesis and acetogenesis for H_2 binding. Recently, many studies have also suggested that acetogensis can serve as an alternative hydrogenotrophic pathway in the rumen (El-Zaiat *et al*., 2014 and Soltan *et al*., 2017b).

Table (3): Effect of monensin and moringa leaf extract (MLE) on some ruminal parameters.

Item	Treatments				SEM	*P* value
	Control	Monensin	MLE $_{Low}$	MLE $_{High}$		
pH	5.98	5.99	5.93	5.94	0.327	0.991
NH_3-N (mg/100 mL)	22.1	22.8	24.5	24.7	4.557	0.110
VFAs						
Total (mM)	46.9^{b}	46.7^{b}	48.1^{b}	57.9^{a}	1.267	0.001
Acetate, %	64.2^{ab}	61.4^{b}	63.7^{ab}	64.8^{a}	0.687	0.028
Propionate, %	16.2	18.5	16.9	16.5	0.358	0.082
Butyrate, %	14.1	15.39	14.0	15.3	0.467	0.164
Isobutyrate, %	1.07	1.18	1.09	1.18	0.120	0.880
Valerate, %	1.68	1.79	1.62	1.67	0.100	0.663
Isovalerate, %	1.56	1.80	1.57	1.78	0.246	0.889
C2:C3	3.74	3.38	3.82	3.99	0.091	0.084
Protozoa $\times 10^5$	4.943^{a}	3.937^{b}	5.381^{a}	5.212^{a}	0.7502	0.009

SEM: Standard error of the mean.
$^{a,b:}$ Means within a row without a common superscript letter differ significantly ($P < 0.05$).

The PF is an indicator of the efficiency of microbial protein synthesis (Blümmel *et al*., 1997), none of the experimental additives affected the PF (Table 3). The lack of change in PF values is consistent with the rather constant of ammonia concentrations suggested that the nitrogen use by microbes for their protein synthesis remained unchanged, and the VFAs probably enhanced by ruminal microbes which are not involved in the amino acids degradation.

No differences were detected between MLE $_{low}$ and the control treatments either in the ruminal degradability or the fermentation parameters. These findings suggest that MLE $_{low}$ was an inadequate dose to affect the ruminal microbial ecosystem, thus it is important to choose the effective dose of MLE to be applicable in the ruminant's diets. Generally, the effects of MLE either through reducing CH_4 or enhancing the production of VFAs may be nutritionally advantageous to ruminants, due to the increases in the energy supply to animals consequently enhance the whole animal productivity.

CONCLUSION

The current study suggested that MLE can be used as an effective additive for ruminants' diets in the field of smart agriculture. Both MLE and monensin exhibited a similar antimethanogenic activity without adverse effects on ruminal degradability however; they were different in their mode of action. Monensin reduced CH_4 through enhancing propionate production, while MLE enhanced the acetate production. These results also suggested that the consideration of MLE as a dietary supplementation to modify the ruminal fermentation was dose dependent. Further research should focus on the *in vivo* long-term effects of the dietary MLE to be applicable as one of the climate smart agriculture practices in the developing countries.

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تأثير إضافة مستخلص أوراق المورنجا علي هدم العناصر الغذائية بالكرش وخفض تكوين غاز الميثان معمليا

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تعد المستخلصات النباتية من أحد الوسائل الفعالة لاحلال المضادات الحيوية والتي تستخدم كاضافات علفية لعلائق المجترات. وقد إجريت هذه التجربة لدراسة تأثير مستخلص أوراق المورنجا كبديل طبيعي للمضاد الحيوي مونسن علي هدم الكرش للمكونات الغذائية, إنتاج غاز الميثان و تخمرات الكرش للاغنام معمليا بإستخدم نظام النصف اوتوماتيكي لانتاج الغاز معمليا خلال 24 ساعة. إستخدمت عليقة تتكون من 50% دريس برسيم و50% مركز كعليقة اساسية و كانت المعاملات كالتالي: العليقة الاساسية بدون اي إضافات (كنترول), العليقة الاساسية مضاف اليها مستخلص أوراق المورنجا بنسبة 50 (مستوي منخفض) و 500 ملليجرام لكل كيلوجرام مادة غذائية علي أساس جاف (مستوي مرتفع). و إستخدم المونسن بتركيز 40 ملليجرام لكل كيلوجرام ماده علفية علي أساس جاف. تم اكتشاف العديد من مركبات الاحماض الامينية الاساسية, السكريات الاحادية, الجلوكوسيدات و مركبات مشتقات البنزين كمكونات كبري لمستخلص أوراق المورنجا. أظهرت كل من مجموعة المستوي المرتفع من مستخلص أوراق المورنجا و المونسن إنخفاض معنويا ($P < 0.05$) في انتاج غاز الميثان , لكن أظهر المستوي المرتفع من مستخلص أوراق المورنجا زيادة معنويه ($P < 0.05$) في الانتاج الكلي من الاحماض الدهنية الطيارة مقارنه بكل المعاملات الاخري وزيادة ($P < 0.05$) لتركز الاسيتات مقارنة بمجموعة المونسن. مجموعة المونسن أظهرت خفضا بمعنوية ($P < 0.05$) في أعداد البروتوزوا بينما لم يظهر هذا التأثير في باقي المعاملات. لا توجد اختلافات معنوية في باقي خصائص هدم المكونات الغذائية بالكرش, انتاج الامونيا , و إنتاج الغاز.خلاصة نتائج هذه التجربة تشير الي إمكانية إحلال مستخلص أوراق المورنجا محل المونسن في علائق الاغنام لخفض إنتاج الميثان.