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In vitro Gas Production, Methane Emission and Rumen Fermentation Characteristics with Increasing Roughage to Concentrate Ratios

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Abstract. Effects of roughage to concentrate ratio on gas production (GP) and nutrient degradability were evaluated *in vitro*. Rations I: 0R:100C, II: 20R:80C, III: 40R:60C, IV: 60R:40C, V: 80R:20C and VI: 100R:0C were incubated for 24h. Concentrate (0R:100C) produced greater GP/gDM, TDOM and MCP. TDDM was higher for rations I to IV than V and VI. Roughage (100R:0C) had lower PF and higher pH and protozoal count with no VFA effect. Ration III and IV containing 60 and 40% concentrate, respectively exhibited balanced distribution of soluble substrate between microbial biomass production and fermentation waste products and are optimal for sheep nutrition.

Keywords: Gas production, Methane, Roughage, Concentrate, Ammonia.

1. Introduction

Forage production in arid and semi-arid regions is low where producers in these parts of the world are raising their animals on cereals and/or concentrate mixtures, thus rations offered to animals mostly are unbalanced. Previous studies revealed that supplementation with diets high in concentrate: roughage (C:R) ratio may decrease the digestibility of nutrients (Castrillo *et al.*, 1995). However, supplementation with high fibre diets with easily digestible carbohydrate and protein can increase the nutrient digestibility (Swanson *et al.*, 2000 and Yang *et al.*, 2000). It is well established that feeding concentrates promotes rapid growth of animals (McDonald *et al.*, 1996), reduce ruminal methane emission, thereby lowering undesirable losses of energy leading to higher overall efficiency of utilization of dietary energy for body weight gain (Mandebvu and Galbraith, 1999). However, diets high in cereals reduce ruminal pH (Franzolin and Dehority, 1996), which may induce acidosis thereby causing reduced feed intake and nutrient absorption as well as retarded animal performance (Owens *et al.*, 1998). Shem *et al.* (2003) and Tessema and Baars, (2004) reported that concentrate supplements improved the utilization of poor feeds by ruminants by improving digestibilities of dry matter, organic matter and crude protein by stimulating rumen fermentation. Therefore, there may be an optimal concentrate supplementation level for a given kind of roughage, which allows the animal to use the nutrients in the roughage most efficiently (Liu *et al.*, 2005). Optimum roughage to concentrate ratio is crucial for proper rumen fermentation and availability of nutrients. Therefore, the objective of this work was to evaluate the effect of different ratios of roughage to concentrate intended for feeding sheep in arid regions on gas production (GP), methane emission, and nutrient degradability *in vitro*.

2. Materials and Methods

2.1 Feeds and Experimental Rations

Alfalfa hay was used as the roughage (R) and a wheat-based commercial concentrate mixture manufactured by the Saudi Grains Organization (SAGO), Jeddah, KSA was used as concentrate (C) to formulate the following the six experimental rations: I (concentrate, 0R:100C), II (20R:80C), III (40R:60C), IV (60R:40C), V (80R:20C) and VI (roughage, 100R:0C).

2.2 Proximate Chemical Analysis

The dried samples were ground to pass a 1mm screen using Wiley mill. Feed analyses were performed according to AOAC (2006). Dry matter contents of feeds were determined by drying at 135℃ for 2h. Organic matter was determined as the weight loss during ashing at 550^{o}C for 2h. Contents of nitrogen (N) were determined by the kjeldahl method, and crude protein (CP) was calculated as 6.25×N content. Ether extract was determined according to AOAC (2006). The neutral detergent fibers (NDF) and acid detergent fiber (ADF) were determined using the procedures of Van Soest *et al*. (1991). No sodium sulfite or α- amylase was used in the procedure for NDF determination. Both NDF and ADF are expressed without residual ash (Table 1).

2.3 Inoculums Donors and Preparations

Three rumen-cannulated adult rams of body weight 49.0±2.3 kg were used as inoculum donors. Both solid and liquid rumen contents were collected separately before morning feeding through the cannula using a stainless steel probe (2.5 mm screen) attached to a large capacity syringe. Liquids and solids were placed in prewarmed (39°C) insulated flasks and transported under anaerobic conditions to the laboratory. Pooled rumen contents (50:50 v/v) were squeezed through four layers of cheese-cloth and kept in a water bath at 39°C saturated with CO_2 until inoculation took place.

2.4 In vitro Gas Production and Methane Production

The *in vitro* gas production (GP) assay was carried out as described by Theodorou *et al*. (1994) but was adapted to the semi-automatic system using a pressure transducer and data logger (GN200, Sao Paulo, Brazil) in 120ml serum bottles incubated at 39°C for 24 h. Ground samples (0.3g as-fed) were incubated in 120ml serum bottles along with 15ml mixed rumen fluid and 30ml of MB9 incubation medium. The composition of MB9 was NaCl (2.8g), $CaCl_2$ (0.1g), $MgSO_4.7H_2O$ (0.1g), KH_2PO_4 (2.0g) and Na_2HPO_4 (6.0g) per 1.0 litre of distilled water. Then the pH was adjusted to 6.8 and CO_2 was flushed for 30 min (Onodera and Henderson, 1980). After filling, bottles were closed with rubber stoppers, shaken and placed in the incubator at 39°C. The bottles were shaken manually after recording the gas headspace pressure (GP) at 3, 6, 9, 12 and 24h incubation using a pressure transducer. Gas production was calculated by the following equation: $V = 7.365 \times p$ ($n = 500$; $r2 = 0.99$; unpublished data) where: V is gas volume (ml); p is measured pressure (psi). Four runs of GP were used for each assay. Measurements of GP were performed in quadruplicate. Each run included four bottles containing buffered rumen fluid without substrate (blank), four bottles containing substrate without additive (control), and four bottles containing substrate for each treatment.

The gas values were expressed as ml per g of incubated DM.

2.5 Methane Emission Analyses

Representative gas samples were collected from the bottles by a syringe (2ml) each time and accumulated in vacutainer tubes (10ml) five times at 3, 6, 9, 12, 24 h incubation. The methane was determined by gas chromatography (Model 7890, Agilent Technologies, Inc., Colorado 80537, USA) with three-valve system using 1/8 inch packed columns having early back flush of the C6 components and equipped with a thermal conductivity detector. Separation was achieved using micro packed column with helium as carrier gas and a flow rate of 28.0ml/min. The detector and column temperatures were 250°C and 60°C, respectively. The test of linearity and calibration were accomplished using a standard gas curve in the range of probable concentrations of the samples. The methane production at the end of incubation was calculated as described by Tavendale *et al*. (2005): CH_4, ml = (total gas volume + headspace) × CH_4 concentration. Both GP and CH_4 were expressed as ml/g DM and calculated by correcting the values of total gas production and incubated or truly degraded organic matter for the corresponding blank.

2.6 Rumen Degradability and Fermentation Characteristics

After termination of the incubation (24h), the contents of two bottles were used to determine the true digestibility of dry matter (DM) and OM (TDDM, TDOM) with 50ml neutral detergent solution and refluxed for 3hrs at 105°C. The bottles content were filtered in pre-weighed crucible, washed with hot water then with acetone and the residual DM and ash were determined according to Blümmel and Becker (1997). The partitioning factor (PF) was calculated as the ratio of TDOM (mg) and gas volume (ml in 24h) (Blümmel *et al.*, 1997). The content of each bottle were transferred to a centrifuge tube and centrifuged at 3000 rpm for 15min. Five millilitres from the supernatant were transferred into 10ml glass bottle and stored at -20°C until analysed for NH3-N and short chain fatty acids (SCFA). Ammonia concentration was calorimetrically analysed using a commercial Kit (Bio diagnostic Company, Egypt). Protozoa were counted microscopically following the procedure described by Kamra *et al*. (1991).

2.7 Statistical Analyses

The experimental design used in this study was the completely randomized design (CRD). Statistical analyses of the *in vitro* data were analysed by the generalized linear model procedure (SAS, 2002). The following model was assumed: $Yij= \mu+ Ti + eij$ where: μ is the overall mean, Ti is the treatment, eij is the random error term. Proper contrasts comparisons were used to determine the linear, quadratic and cubic variables response to increasing levels of the concentrate in the diet. Differences between treatments were declared significant at 0.05 level using the revised LSD for multiple comparisons.

3. Results

Mean values of the proximate analysis rations I (concentrate, 0R:100C), II (20R:80C), III (40R:60C), IV (60R:40C), V (80R:20C) and VI (roughage-alfalfa hay, 100R:0C) on dry matter basis, are presented in Table 1. Roughage (100R:0C) as a good quality alfalfa hay had higher crude protein (20.19%) and ash (10.74%) content compared to concentrate (17.93 and 6.61%, respectively). Similarly, was the crude protein and ash contents in rations II to V following similar trend with the increase in roughage percentage replacing concentrate in the rations. Higher values of nutrients detergent fiber (NDF), acid detergent fiber (ADF), acid lignin fiber (ADL) and cellulose (48.56, 32.56, 6.91 and 23.85%, respectively) were observed in the roughage compared to the concentrate (32.65, 8.76, 2.23 and 6.53%, respectively).

3.1 In vitro Rumen Degradability and Fermentation Characteristics

The effects of tested rations on cumulative *in vitro* gas production (IVGP), TDDM, TDOM and partitioning factor (PF) after incubation for 24 h. *in vitro* are presented in Table 2. The addition of roughage was found to be accompanied with decreasing gas production (ml/gDM) as compared to that of concentrate ration. No significant (*P*=0.16 and *P*=0.15) differences were observed among rations in their GP per g TDDM and TDOM, respectively. Truly digestible dry matter (TDDM) was significantly (*P*<0.01) high for ration I, concentrate (0R:100C) and remained high even with 60% roughage replacing concentrate in ration IV (60R:40C) then started to decrease in ration V (80R:20C) containing 80% hay and ration VI (roughage alone). Inclusion of roughage at 60% in the rations improved TDDM in a linear (*P*<0.01)) then quadratic (P= 0.01) fashion. However, inclusion of roughage at any tested ratio (20, 40, 60 and 80%) decreased the truly digestible organic matter (TDOM) in a linear (*P*<0.01) then quadratic (*P*=0.01) fashions where the concentrate (ration I) recording the highest TDOM and the roughage (ration VI) recording the lowest. All rations including concentrate (ration I) and rations containing increasing ratios of concentrate (rations II, III, IV and V) had higher (*P*<0.01) partitioning factor than alfalfa hay alone (ration VI, roughage alone). The change in PF after inclusions of (20, 40, 60, and 80%) ratios of concentrate was quadratic (*P*<0.01).

3.2 In vitro Rumen Methane Emission

The effects of tested rations on cumulative methane production, ammonia, microbial protein production and pH after incubation for 24h *in vitro* are presented in Table 3. No significant differences were observed among all rations from I to VI in their methane gas emission per g DM, TDDM,TDOM and ammonia N (CH_3-N, mg/100ml) except ration VI (0C:100R) that contains 100% roughage produced slightly less methane (29.6 m/g DM) (Table 3). In contrast to methane and ammonia production, increasing the ratio of roughage in the rations decreased (*P*<0.01) MCP (g/kg DOM) linearly (*P*<0.01) and quadraticaly (*P*<0.01) compared to concentrate. Rations II, III and IV containing 20, 40 and 60% roughage, respectively produced significantly less MCP than ration I (concentrate, 0R:100C) and significantly higher than rations V and VI that contained 80 and 100% roughage. The inclusion of roughage increased significantly (*P*<0.01) the pH values in ration II (5.5), III (5.60), IV (5.70), V (5.80) and VI (5.80) compared to (5.48) for the concentrate (ration I). The increased pH values followed a linear (*P*<0.01) then quadratic (*P*<0.01) patterns with the inclusion of roughage in rations.

3.3 Total Volatile Fatty Acids (VFA) and Protozoal Growth

In vitro production of total volatile fatty acids (VFA) and protozoal count are presented in Fig. 1. No differences in the concentrations of total volatile fatty acids (meq/100ml) were found after fermentation of the six experimental rations *in vitro* (Fig. 1). However, significantly (*P*<0.05) lowest protozoal growth was recorded with concentrate ration, whereas protozoal growth increased significantly as percentages of roughage increased in the ration.

4. Discussion

4.1 In vitro Rumen Methane Emission Characteristics

Gas production is the result of fermentation of carbohydrates to volatile fatty acids *i.e*., acetate, propionate and butyrate, and GP from protein fermentation is relatively lesser compared to carbohydrates (Makkar *et al.,* 1995). The higher GP observed in ration I (0R:100C) might be due to increased production of propionate as CO_2 is produced when propionate is made by ruminal bacteria via the succinate: propionate pathway (Seshaiah *et al.,* 2014). Also increased GP after incubation of ration I (concentrate, 0R:100C) can be attributed to increased numbers of starch fermenting bacteria due to abundance of soluble carbohydrates (starch). Decreased GP from rations II to VI containing 20, 40, 60, 80 and 100% roughage might be considered a positive effect as the inclusion of roughage content in the rations could increase utilization of rations nutrients, decrease GP, and presumably reduce the risk of bloat *in vivo*. The pH value (5.48) was lower when inoculum was incubated with concentrate and was gradually increased with the gradual increase in roughage ratio replacing concentrate in the inoculum. This could be due to that roughage (alfalfa hay) contained more NDF, ADF and cellulose than concentrate (maximum concentrate, 100%) which could explains the higher gas production when roughage fraction was minimal (0%). The pH value measurements can be used as a tool to evaluate the fermentation process in the rumen (Kumar *et al.,* 2013). The pH value decreased as the amount of concentrate mix in the ration was maximal (100%) which was similar to findings reported by Kumar *et al*. (2013). In our study, pH values ranged from 5.48 to 5.8, was relatively stable, and were greater than 5.0 to 5.5 range suggested by Hoover (1986) at which ruminal microbial bioactivity was negatively affected.

The truly digestible dry matter (TDDM) and truly digestible organic matter (TDOM) were reduced significantly (*P*<0.05) by increasing roughage level (Table 3). The decrease in degradability in roughage content can be due to more lignin content in roughage compared to concentrate (Table 2). It is generally agreed that lignin content of forages is negatively correlated with extent of digestion (Jung, 1997). The lignin supressing effect is probably resulting from a reduction in attachment of ruminal microbes to feed particles and inhibition of microbial growth and microbial enzyme activity (McSweeny, 2001). Others (Reddy *et al.,* 2016) reported that *in vitro* OMD and TDOM increased linearly with the increase of concentrate proportion in the diet. They attributed such increase to gradual decrease in cellulose and lignin in their diets, which act as a limiting factor to lowering digestibility (Al-Masri, 2009 and Kumari *et al.,* 2012). The increase of *in-vitro* OMD in rations I, II, III and IV containing 100, 80, 60 and 40% concentrate, respectively (Table 3) might be due to the increase of readily available energy and protein contents of these rations which might have improved microbial growth and fermentation (Chatterjee *et al.,* 2006). Therefore, ration IV (60R:40C) improved TDDM similar to ration I (concentrate, 0R:100C) and its effect on OMD was intermediate.

More importantly, rations III (60% concentrate) and IV (40% concentrate) are considered the optimal rations that minimized energy wastage in the form of GP with DM and OM values remaining similar to that of ration II with higher concentrate (20R:80C). The levels of NH3-N concentrations ranged from 18.55 to 20.53 mg/dl which was similar to that by Wanapat and Pimpa (1999) (15-30.0 mg/dl) in the rumen and Polyorach *et al*. (2014) *in vitro*. An increase in the nitrogenous concentrate supply generally increases NH3-N content in rumen juice, however no significant difference was found between concentrate mix and hay regarding NH3-N content but concentrate mix had higher microbial CP than hay alone. In the meantime, increased concentrate ratio was associated with more MCP production. In case of rations high in concentrate, energy availability was probably higher than rations with less concentrate. Microbes in rations high in concentrate would require more NH3-N to sustain their growth, which would decrease NH3-N to levels similar to rations low in concentrate, which was probably the case in the current study. Microbial CP concentration as a function of microbial biomass explains the sustained higher growth rate of microbes in high concentrate rations in the present study. During incubation, the liberated ammonia will be incorporated into microbial protein synthesis, but this incorporation depends on synchronization between availability of nitrogen and energy (Thirumalesh and Krishnamoorthy, 2013). Therefore, partitioning factor (PF) provides a meaningful information for prediction of microbial biomass and available CP in the rumen (Thirumalesh and Krishnamoorthy, 2013). They also, reported a positive correlation between microbial biomass and PF of total mixed rations. PF is an index of the distribution of truly degraded substrate between microbial biomass and fermentation waste products, as reported by Thirumalesh and Krishnamoorthy (2009). This mean that higher PF indicate that more of truly degraded substrate was converted to microbial protein synthesize. Therefore, all ration except roughage alone had elevated PF and the later (roughage alone) had the lowest MCP. More importantly, ration III (60%C) and IV (40%C) had balanced distribution of soluble substrate between microbial biomass production and fermentation waste products.

The total VFA was not significantly affected by increased levels of concentrate in the rations. However, others (Suharti *et al.*, 2011 and Kumar *et al.,* 2013) reported that total VFA was significantly affected by forage to concentrate ratio in the diet. Total protozoa count in ruminal fluid generally increases with the addition of concentrate to forage in the diet (Franzolin and Dehority, 1996 and Saini *et al.*, 2012). Further, feeding high-grain diets can result in reduced protozoa numbers or even defaunation (Hristov *et al.,* 2001). Ramos *et al*. (2009) reported that increasing concentrate from 30 to 70% in the diet with alfalfa hay as a forage increased protozoa numbers by 48%, but this effect was not observed when grass hay was used as a forage. In our study, total protozoal count was lowest in 100% concentrate (ration I) and protozoal count increased gradually with inclusions of more roughage in the rations with highest count recorded in ration VI (Roughage, 100R:0C). In agreement with the findings reported by Franzolin and Dehority (1996) and Hristov *et al.* (2001) where high-grain diets may lower the pH values and greater amounts of starch in high concentrate rations may produce greater lactic acid concentrations (Slyter, 1976) and hence lower pH measurements concurrent with the pH values observed in the current study negatively affecting protozoal growth.

Concentrate feeding has been shown to reduce methane (CH_4) output by reducing the protozoal population (Van Soest, 1982 and Iqbal *et al.*, 2008). Nevertheless, no difference in CH_4 production among the incubated rations with different roughage to concentrate ratios in the current study.

5. Conclusions

Considerable attention should be paid during formulation of sheep rations in order to produce rations with optimum roughage to concentrate ratio for efficient nutrient utilization. Based on the results obtained in the present study, ration III and IV containing 60 and 40% concentrate, respectively exhibited balanced distribution of soluble substrate between microbial biomass production and fermentation waste products and can be considered optimal rations for sheep raised under harsh nutritional and climatic conditions.

Table 1. Proximate analyses of the tested rations on dry matter basis.

Items	Rations					
	I	II	III	IV	V	VI
Organic matter, %	93.39	92.65	91.80	91.10	90.15	89.26
Ash,%	6.61	7.35	8.20	8.90	9.85	10.74
Crude protein,%	17.93	19.25	17.71	20.56	20.68	20.19
Ether extract,%	5.39	5.64	5.02	4.40	5.75	4.31
Neutral detergent fiber,%	32.65	34.75	38.64	41.55	44.63	48.56
Acid detergent fiber,%	8.76	13.43	19.51	23.41	28.20	30.77
Acid detergent lignin,%	2.23	3.23	4.31	5.30	7.14	6.91
Hemicellulose,%	23.89	21.32	19.13	18.14	16.43	17.80
Cellulose,%	6.53	10.19	15.20	18.11	21.06	23.85

Roughage: alfalfa hay C: Concentrate mix manufactured by Saudi Grains Organization (SAGO), heat-based.

Table 2. *In vitro* gas production (IVGP) per dry matter (DM), truly digestible dry matter (TDDM), truly digestible organic matter (TDOM) and partitioning factor (PF) of the tested rations.

Rations	IVGP (ml/g)			TDDM (g/100g)	TDOM (g/100g)	PF (mg/ml)
	DM	TDDM	TDOM			
I, (0R:100C)	134.4[a]	145.3	157.7	88.85[a]	82.23[a]	3.90[a]
II, (20R:80C)	113.4[b]	134.0	140.8	86.20[a]	79.10[b]	3.38[a]
III, (40R:60C)	102.2[b]	120.8	131.7	84.90[a]	77.65[b]	3.72[a]
IV, (60R:40C)	100.4[b]	120.2	131.0	85.20[a]	78.13[b]	3.61[a]
V, (80R:20C)	101.6[b]	132.9	147.0	76.90[b]	68.85[c]	3.70[a]
VI, (100R:0C)	110.3[b]	137.5	150.7	75.03[b]	66.63[d]	2.70[b]
SEM	3.17	4.27	4.83	1.30	1.20	0.11
P values	<0.01	0.16	0.15	<0.01	<0.01	<0.01
Linear	0.76	0.22	0.08	0.01	<0.01	0.07
Quadratic	0.67	0.54	0.74	0.01	0.01	<0.01
Cubic	0.09	0.11	0.15	0.59	0.09	0.11

Means in the same column bearing different letters differ significantly. SEM = standard error of the mean.

Table 3. *In vitro* methane emission per dry matter, (DM), truly digestible dry matter (TDDM), truly digestible organic matter (TDOM), CH3-N (ammonia nitrogen), microbial protein production and pH of the tested rations.

Rations	Methane, ml/g			CH_3-N (mg/100 ml)	MCP (g/kg DOM)	pH
	DM	TDDM	TDOM			
I, (0R:100C)	36.4	40.8	44.3	19.80	158.73a	5.48[d]
II, (20R:80C)	36.1	36.4	39.7	18.55	152.68[b]	5.50c
III, (40R:60C)	31.4	36.9	40.4	19.83	149.88[b]	5.60[b]
IV, (60R:40C)	33.7	39.4	43.1	19.28	150.78[b]	5.70[b]
V, (80R:20C)	30.9	40.0	45.2	20.53	132.88[c]	5.80[a]
VI, (100R:0C)	29.6	39.4	44.7	19.98	128.58[d]	5.80[a]
SEM	1.15	1.3	1.6	0.44	2.3	0.03
P values	0.28	0.90	0.89	0.89	<0.01	<0.01
Linear	0.15	0.35	0.24	0.34	<0.01	<0.01
Quadratic	0.54	0.51	0.72	0.70	<0.01	<0.01
Cubic	0.25	0.78	0.80	0.99	0.08	<0.01

Means in the same column bearing different letters differ significantly. SEM = standard error of the mean.

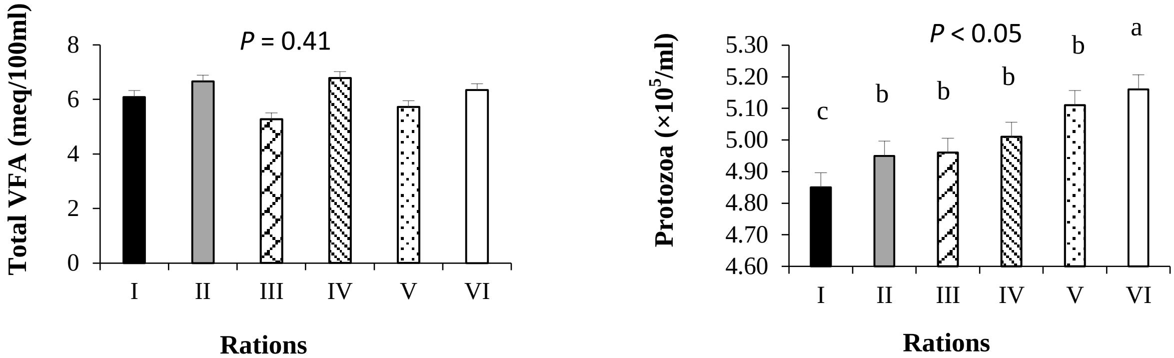

Fig. 1. ***In vitro*** **production of total volatile fatty acids (VFA, meq/dl) and protozoa concentrations (105/ml) in rations I (concentrate, 0R:100C), II (20R:80C), III (40R:60C), IV (60R:40C), V (80R:20C) and VI (roughage, 100R: 0C) after 24 hrs of incubation *in vitro*.**

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إنتاج الغاز التراكمي والميثان وخصائص تخمر الكرش معمليًا بإضافة نسب متزايدة من المادة المالئة

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المستخلص. أجريت هذه الدراسة لتقدير تأثير البرسيم الحجازي (ماليء) والمادة المركزة ونسب متباينة من الماليء: المركز على إنتاج الغاز التراكمي وتحرر الميثان ومعاملات هضم المواد الغذائية معمليًا. تم تكوين ستة علائق متباينة في نسب الماليء: المركز كما يلي: المركز (٠: ١٠٠) و (٢٠: ٨٠) و(٤٠: ٦٠) و(٦٠: ٤٠) و(٨٠: ٢٠) والماليء (١٠٠: ٠) ماليء: مركز ٪ على التوالي. بهدف التعرف على أنسب نسبة ماليء: مركز لاستخدامها في تغذية الأغنام. تم اختبار العلائق معملياً لمدة ٢٤ ساعة بواسطة نظام نصف آلي لإنتاج الغاز. وقد أظهرت النتائج أن المادة المركزة أنتجت غازًا مرتفعًا معنويًا (مل/جم مادة جافة) وأعلى معامل هضم حقيقي للمادة العضوية وأعلى معامل هضم حقيقي للمادة الجافة. العليقة المالئة حققت أقل معامل تجزئ (PF) وأقل معدل إنتاج بروتين ميكروبي وأعلى قيم لدرجة الحموضة pH وأعداد البروتوزوا مقارنة بجميع العلائق، ولم يلاحظ تغير معنوي في معدل إنتاج الأحماض الدهنية الطيارة ومعدل تحرر غاز المثان، وكذا إنتاج الأمونيا. مما سبق نتبين أن العلائق ٣ و٤ المحتوية على ٦٠ و٤٠٪ مادة مالئة على التوالي وفرت أفضل توازن للمواد الذائبة ما بين إنتاج الكتلة الميكروبية الحيوية ونواتج التخمر المفقودة، وتعتبر هذه العلائق الأنسب لتغذية الأغنام المرباة تحت الظروف البيئية والغذائية القاسية بهدف تحسين أداءها الإنتاجي.

كلمات مفتاحية: إنتاج الغاز، الميثان، علف ماليء، علف مركز، الأمونيا.