Scientific Paper

# Global warming indicator from rumen fermentation of feedstuffs with different energy and protein levels▲

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## Abstract

**Objective**: To evaluate the in vitro gas production technique as method to estimate in vivo a global warming indicator, in diets with different levels of metabolizable energy and crude protein.

**Materials and Methods**: The *in vitro* gas production technique was used to obtain a global warming indicator from $CO_2$ and $CH_4$ production, the potential emission of fermentation gases and digestibility of five diets: three balanced diets at low BB): 2,7:12,8; medium (MM): 2,7:15,3 and high (AA) levels: 3,0:17,6; and two unbalanced diets: BA: 2,6:18,5 and AB: 2,9:14,0 in metabolizable energy (Mcal $kg^{-1}$) and crude protein (%), with which 30 lambs (26,5 ± 3,7 kg LW) were fed in individual pens. Fattening lasted 60 days (three periods of 20 days). In each period samples of the offered and rejected feedstuff, and feces were taken per animal, which were fermented with the GPT during 24 h. The design was complete randomized with 5 x 3 factorial arrangement (diet x period).

**Results**: The *in vitro* dry matter digestibility and *in vitro* ruminal fermentation were 6 and 13 % higher for the balanced diets compared with the unbalanced ones, respectively. Regarding $CH_4$ production and the global warming potential indicator, higher production was observed in the third period of fattening with regards to the first 40 days. In addition, the diets high in metabolizable energy were the ones with higher environmental impact on global warming ($p < 0,05$).

**Conclusions:** The global warming potential indicator obtained with the gas production technique is useful to estimate the environmental impact of the diets for ruminants; and the balanced diets, independently from the energy and protein level, they had higher digestibility and fermentation.

**Keywords**: lamb, fermentation, environmental impact

## Introduction

Ruminants contribute to global warming by generating products of the carbohydrate anaerobic fermentation, such as heat, carbon dioxide ($CO_2$) and methane ($CH_4$), according to Castelán Ortega *et al*. (2014). The last one represents a 10-% loss of the energy contained in the feedstuff (Carmona *et al*., 2005), and is the second most significant contributor to greenhouse effect, capturing twenty times more heat than carbon dioxide (Yan *et al*., 2010). In addition, it is calculated that approximately one fourth of all the anthropogenic $CH_4$ is produced by enteric fermentation of ruminants (Beauchemin *et al*., 2008; Ramin and Huhtanen, 2013).

The estimation of greenhouse gas emissions, mainly $CH_4$, by *in vivo* methods is considered more accurate, and among them the breathing chamber, SF6 tracer and micrometeorological techniques stand out (Rodríguez *et al*., 2019). Nevertheless, these techniques are costly and laborious; and, to a lower or higher extent, affect the animal behavior. On the other hand, *in vitro* techniques are low-cost (Danielsson *et al*., 2017), little contaminant practices, and can be used to estimate the parameters of the gas production kinetics and predict methane production (Ramin and Huhtanen, 2012).

The *in vitro* gas production technique (GPT) generates gas directly and mainly, but not exclusively, from the ruminal fermentation of the carbohydrates in the feedstuff; thus, gas can be an indicator of the energy content (Menke and Steingass, 1988; Posada and Noguera, 2005). Some research works have proven the feasibility of applying the GPT in *in vivo* studies, in order

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to determine the effect of the diet on potential gas emission (Miranda Romero *et al*., 2018). Due to the above-explained facts, there is interest in evaluating the potential methane production related to the energy and protein content of the feedstuff; for which the objective of the research was to evaluate the in vitro gas production technique as method to estimate in vivo a global warming indicator, in diets with different levels of metabolizable energy and crude protein.

## Materials and Methods

*Study location*. The study was conducted in two stages: the first one consisted in fattening 30 lambs during 60 days and the sampling of the offered feedstuff; and took place at the Postgraduate and Research Unit of the Cuautitlán School of Higher Studies of the National Autonomous University of Mexico –located in Cuautitlán Izcalli, Mexico State–.The area is located at a latitude of 19,695826 North and longitude of 99,190613 West; the climate is temperate. Mean annual rainfall is 653 mm and average temperature is 15,7 ºC (INAFED). The second stage was carried out in the ruminant nutrition laboratory of the Animal Science Department of the Chapingo Autonomous University, located on the kilometer 38,5 of the Mexico-Texcoco road, Chapingo, Mexico State; and consisted in the fermentation in duplicate of the 90 samples of offered feed, through the *in vitro* gas production technique at 24 h of incubation.

*Treatment and experimental design*. With a complete randomized design with five treatments and six repetitions, five diets were formulated with different levels of metabolizable energy (Mcal/kg) and crude protein . For the control group (MM) the levels of metabolizable energy (2,8 Mcal/kg) and crude protein (16 %) recommended by the NRC (2007) for growing and finishing sheep, were used. For the experimental groups two metabolizable energy levels were used, a higher (3,0 Mcal/kg) and a lower one (2,7 Mcal/kg) with regards to the recommendations made by NRC (2007). Likewise, two crude protein levels were utilized: higher (18 % CP) and lower (14 % CP). With the combinations the following treatments were obtained: (Mcal/kg:% CP): AA (3:18), AB (3:14), BA (2,7:18) and BB (2,7:14). The four combinations are shown in figure 1.

*Animals and management*. Thirty male lambs (26,5 ± 3,7 kg LW), installed in 1-$m^2$ individual pens, were fed with the diets, which were randomly allocated. The lambs had an adaptation period of 10 days. The fattening lasted 60 days and was divided into three periods of 20 days each. In each period every third day samples of the offered feedstuff were taken, with which composite samples per animal and per period were made. The 90 samples were dried, ground and fermented by the modified gas production technique (Getachew *et al*., 1998),

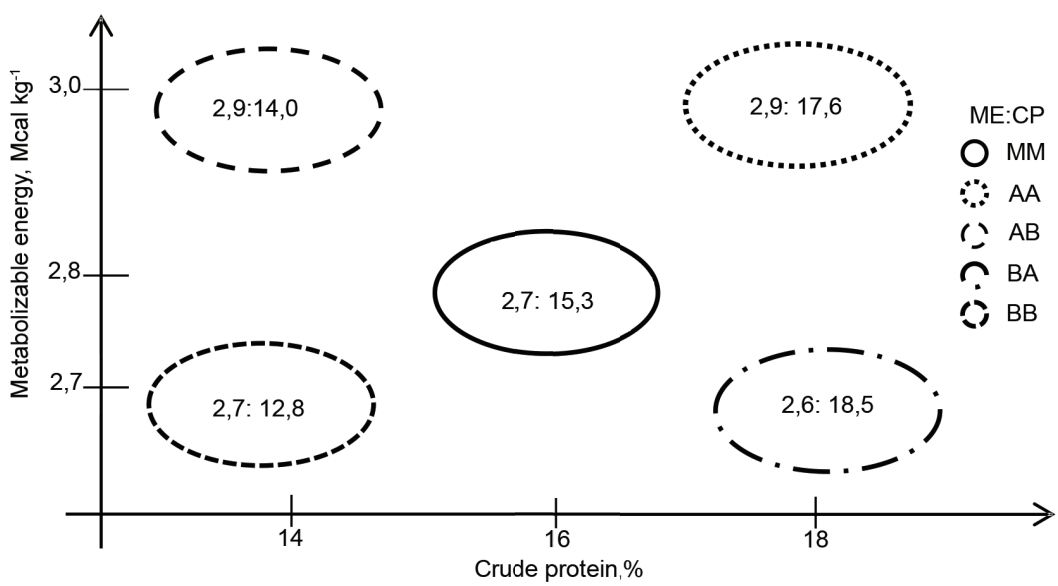


Figure 1. Metabolizable energy (ME) and crude protein (CP) in diets with high (A), medium (M) or low level (B) . MM: reference diet recommended by the NRC (2007) for sheep that gain 250 g/days. Values within the ovals correspond to the ME and CP levels for each diet.

to measure total gas production total (mL $g^{-1}$) and to estimate the production of $CO_2$, $CH_4$ plus minor gases ($CH_4$+MG) and *in vitro* digestibility (IVDMD) at 24 h of incubation.

The total gas production was referred as index of potential fermentation gas emission (IPFGE, mL $g^{-1}$). The $CH_4$+ GM values were adjusted as theoretical methane ($CH_4$) multiplying each value by 0,77 (Zhong *et al*., 2016). With the $CO_2$ and $CH_4$ values the potential global warming indicator (PGWI) was obtained, considering an equivalent value per methane of 23 (Berra *et al*., 2009) and the following equation:

$$PGWI\,(mL\,CO_2\,eq\,g^{-1}\,DM) = CO_2\,(mL\,g^{-1}) + CH_4\,(mL\,g^{-1})\,x\,23$$

The statistical analysis was carried out with the GLM procedure and Tukey's multiple mean comparison test (SAS 9.4).

**Results and Discussion**

According to the IPFGE, the unbalanced diet AB was les fermented than the balanced diets (AA, BB and MM) in the first two periods of fattening, and tended to be one of the least fermentable in the third period along with the balanced diet AA (table 1). This result is congruent with the IVDMD, because it was observed that the balanced diets at low, medium or high ME and CP level (BB, MM and AA) had higher digestibility ($p < 0,05$) than the unbalanced ones (AB y BA). The average digestibility was high in the first two periods. However, as they were adult animals there was no difference ($p > 0,05$) in digestibility, which coincides with the description made by Bastida *et al*. (2011).

Average methane production of the first three periods was lower than 16 %, which is in correspondence with concentrate-high diets; in turn, the value was lower than in forage-high diets (> 20 %), according to Dumortier *et al*. (2017).

It is notorious that the methane production was higher for the last fattening period (41-60 days). On the other hand, the diets with low energy (BB and BA) were observed to produce less methane ($p < 0,05$); while the diet with high energy and protein (AA) produced a higher quantity ($p < 0,05$) of methane (table 1).

On the other hand, *in vitro* ruminal fermentation of the diets showed that, with a higher ME

Table 1. *In vitro* fermentation gas production and potential global warming indicator of diets with different energy and protein levels.

| Period, days | Treatment, ME:CP | $CH_4$, % | $IPFGE_{24h}$, mL $g^{-1}$ | PGWI, mL $CO_2$ eq $g^{-1}$ $lamb^{-1}$ | IVDMD, % |
|---|---|---|---|---|---|
| 0-20 | BB | $0,1^{b}$ | $206,9^{a}$ | $219,9^{c}$ | $71,6^{ab}$ |
| | MM | $5,6^{a}$ | $195,7^{a}$ | $400,1^{b}$ | $74,1^{a}$ |
| | AA | $9,4^{a}$ | $182,0^{a}$ | $552,7^{a}$ | $75,0^{a}$ |
| | BA | $7,8^{a}$ | $131,4^{b}$ | $344,3^{bc}$ | $62,1^{b}$ |
| | AB | $9,9^{a}$ | $195,0^{a}$ | $610,0^{a}$ | $68,4^{ab}$ |
| 21-40 | BB | $4,2^{ab}$ | $171,3^{a}$ | $330,0^{a}$ | $73,4^{ab}$ |
| | MM | $1,7^{b}$ | $172,1^{a}$ | $239,5^{ab}$ | $72,6^{ab}$ |
| | AA | $4,7^{a}$ | $193,9^{a}$ | $390,4^{a}$ | $80,3^{a}$ |
| | BA | $2,3^{ab}$ | $91,6^{b}$ | $138,1^{b}$ | $68,0^{b}$ |
| | AB | $4,0^{ab}$ | $198,0^{a}$ | $369,2^{a}$ | $77,5^{ab}$ |
| 41-60 | BB | $5,4^{c}$ | $194,2^{ab}$ | $421,2^{c}$ | 75,3 |
| | MM | $16,4^{ab}$ | $206,5^{a}$ | $941,9^{a}$ | 77,1 |
| | AA | $16,6^{a}$ | $183,0^{b}$ | $842,1^{ab}$ | 74,1 |
| | BA | $12,4^{b}$ | $190,6^{ab}$ | $695,1^{b}$ | 75,3 |
| | AB | $15,6^{ab}$ | $189,0^{b}$ | $825,3^{ab}$ | 73,8 |

ME: metabolizable energy (Mcal $kg^{-1}$), CP: crude protein (% $kg^{-1}$), $CH_4$: methane, $IPFGE_{24}$: index of potential fermentation gas emission, PGWI: potential global warming index, IVDMD: *in vitro* digestibility

a, b, c: different letters within columns are different for $p < 0,05$

content (AA and AB), the PGWI had a higher value ($p < 0,05$) and, thus, they were the ones with higher environmental impact with regards to global warming, mainly in the first two periods of fattening. In the last period, the diet with medium ME and CP levels (MM) also had a high PGWI ($p < 0,05$). In contrast, the PGWI was lower ($p < 0,05$) with the fermentation of the diets with low ME level (table 1).

These results indicate that, independently from the ME and CP levels, ruminal fermentation of the feedstuff is optimized when these nutrients are balanced (BB, MM and AA); which, consequently, would increase the VFA production and available energy for the ruminant (Popa *et al*., 2016).

On the other hand, when the diet is unbalanced in ME and CP, the ruminal fermentation of the feedstuff is lower; and, subsequently, the VFA production is also reduced, particularly when there is excess of ME (AB). The results also indicated that the ME level in the diet is more determinant than the CP level in the environmental impact; because the diets AA and AB showed higher PGWIs and, in average of the three periods, had 31 % more of PGWI than the BB, MM and BA diets.

## Conclusion

The potential global warming indicator obtained by the *in vitro* gas production technique is useful to estimate the environmental impact by the fermentation of feedstuffs for ruminants. In this research it was proven that theP GWI was determined by the ME level in the diet. On the other hand, it was confirmed that the balanced diets, independently from their ME and CP level, were better fermented than the unbalanced ones; and, subsequently, higher energy availability from VFA for the ruminant is expected. This has implications for lamb production, because low-resource farmers should consider, before including energy or protein ingredients, that it is better to balance the diet at low ME and CP levels to improve ruminal fermentation and, probably, the productive performance of lambs.

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## Authors' contribution

- Brayan Eduardo Martínez-Hernández. Conceptualization, data curation, formal analysis, funding acquisition, research, project administration, visualization, writing of the original draft, revision and edition.
- Omar Salvador-Flores. Conceptualization, funding acquisition, methodology, resources, project administration, writing, revision and edition.
- Luis Alberto Miranda-Romero. Conceptualization, data curation, formal analysis, funding acquisition, methodology, project administration, resources, supervision, visualization, writing of the original draft, revision and edition.

## Conflicts of interests

The authors declare that there are no conflicts of interests.

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