EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

MICROBIOLOGY

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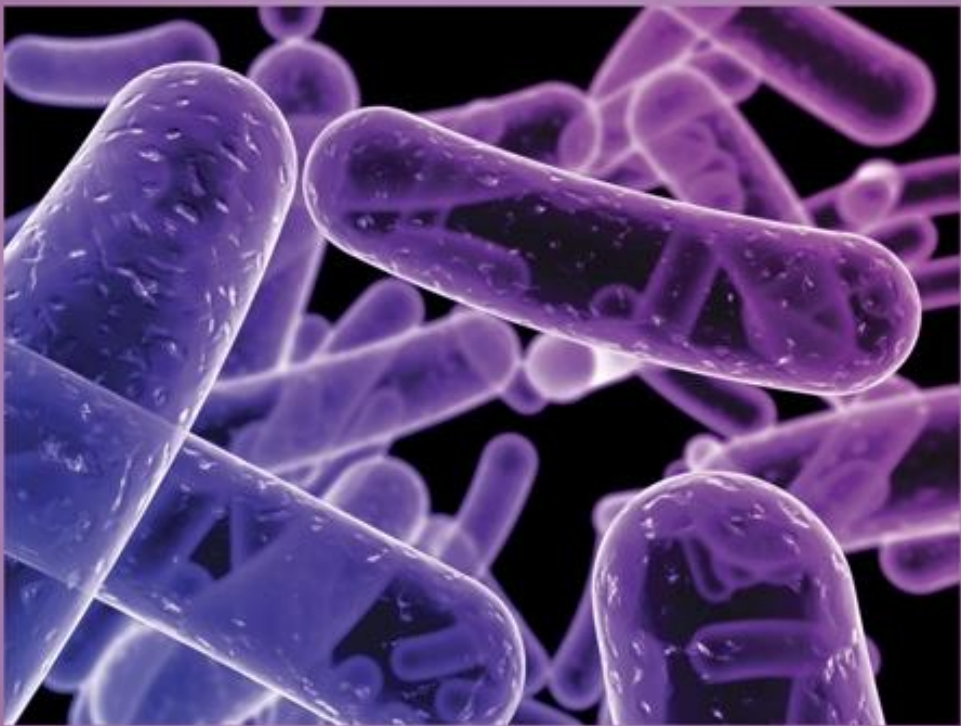

ISSN
2090-0872

WWW.EAJBS.EG.NET

Vol. 15 No. 1 (2023)

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.14 (2) pp.117- 128 (2022)*

DOI: 10.21608/EAJBSG.2023.294274

Egypt. Acad. J. Biolog. Sci., 15(1):117-128(2023)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg

Protein and Energy Levels at *in vitro* Microbial Fermentation of Diets for Fattening Sheep

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ARTICLE INFO

Article History
Received:27/2/2023
Accepted:7/4/2023
Available:11/4/2023

***Keywords*:**
Microbial fermentation, global warming, gas production, sheep fattening.

ABSTRACT

The utilization efficiency of the feed depends on the proportion of energy and protein it contains. In order to determine, *in vitro*, the effect of protein level (CP) and energy (ME) of the feed on the extent of digestibility (IVDMD), the volume (mV), rate (R) and Lag phase (L) of gas production; the fast (FFF), medium (MFF) and slow (LFF) fermentation fractions; methane production, global warming potential indicator (GWPI) and environmental impact index (EII), five diets were formulated with different ratios of metabolizable energy and crude protein (ME: CP) 2.8:16 (MM), 3:17.2 (AA), 2.8:14.5 (AB), 2.6:17.2 (BA) and 2.6:13.8 (BB). The R was higher ($p<0.05$) in the diets with the highest energy content (AA, AB), and the mV was lower ($p<0.05$) for the diet low in ME and high in CP (BA). The AA diet had the highest ($p<0.05$) values of FFF and MFF, while the highest proportion of LFF ($p<0.05$) and lowest IVDMD ($p<0.05$) was observed for the BA and BB diets. In these same diets, the highest proportion ($p<0.05$) of CH_4 was found and the BB diet caused the highest ($p<0.05$) GWPI and EII. It is concluded that diets with a higher content of non-structural carbohydrates have better fermentative kinetics and digestibility, and generate lower proportions of CH_4. The GWPI may be a good estimator of the environmental impact since it considers the amounts of CO_2 and CH_4 produced in the rumen.

INTRODUCTION

In sheep-intensive production systems there is a growing trend to increase the use of concentrates in rations for intensive fattening of sheep (González-Garduño *et al.*, 2013); however, the production costs associated with feeding are higher due to the high price of grains such as corn and sorghum, which increases year after year (Pérez et al. 2010). The problem is exacerbated when commercial and balanced diets are deficiently used by ruminants (Salinas-Chavira *et al.*, 2011; Martínez et al., 2007). The energy-protein ratio in the diet is a determinant in the productive response of the animal (Ebrahimi *et al.*, 2007) and the efficiency of feed utilization by ruminants (Sileshi *et al.*, 2021; Azevedo *et al.*, 2021; Huerta 2008), which is related to rumen microbial fermentation.

As The rumen is a complex ecosystem where microorganisms such as bacteria, protozoa, and fungi digest the consumed nutrients anaerobically. The main end products of fermentation are volatile fatty acids (VFAs) and microbial biomass, which are used by the host ruminant. The interaction between microorganisms and the host animal results in a symbiotic relationship that allows ruminants to digest diets rich in fiber and low in protein, it is important to determine the effect of the energy-protein ratio in the diets formulated for intensive fattening of sheep on the ruminal microbial fermentation. The ruminal microorganisms have the ability to produce the enzymes necessary for fermentation processes that allow ruminants to obtain the energy contained in forages efficiently (Burns 2008). On the other hand, the *in vitro* gas production technique (GPT) is a practical procedure that allows indirect estimation of the extent and kinetics of diet fermentation (Aragadvay-Yungán *et al*., 2022). The process of ruminal fermentation is not completely efficient as it produces some final products such as methane gas (Kingston-Smith *et al.* 2012) and excess ammonia (Russell and Mantovani 2002). The gas derived from *in vitro* fermentation is mainly, but not exclusively, related to the level of non-structural (soluble sugars and starch) and structural (cellulose, hemicellulose and pectin) carbohydrates in the diet (Villalba *et al.,* 2021; Tirado- González et al., 2016; Murillo *et al.,* 2012), the ruminal gas production (Huertas-Molina *et al.*, 2020), the energy content of the feed and the synthesis of microbial biomass (Castillo-López & Domínguez-Ordóñez, 2019, Opatpatanakit et al 1994, Schofield *et al.*, 1994). *In vitro* gas production has been widely used to estimate the nutritive quality of different classes of forages (Njidda, 2010). Miranda-Romero *et al.,* 2020, proposed the application of the *in vitro* gas production technique (GPT) to estimate not only the extent and kinetics of diet fermentation but also the fractions of fast, medium, slow and total fermentation, related to soluble carbohydrates, starch and cellulose, respectively. Similarly, this technique has been used to estimate environmental impact indicators such as methane production, GWPI and EII through the chemical capture of carbon dioxide (Martínez-Hernández *et al.,* 2019).

Therefore, the objective of this research was to determine, *in vitro*, the effect of the crude protein and metabolizable energy levels of the diet for sheep intensive fattening, on digestibility, fermentation kinetics, fermentable fractions and environmental impact indicators.

MATERIALS AND METHODS

Place and Treatments:

This research was carried out in the Microbiology Lab, Faculty of agriculture Menoufia University. The treatments consisted of five diets according to the content of metabolizable energy (ME, Mcal kg^{-1} DM) and crude protein (CP, %); three balanced at low levels (BB; 2.6:13.6), medium (MM; 2.8:16) and high (AA; 3.0:17.2); and two unbalanced (AB, 2.8:14.5; BA, 2.6:17.2) in ME: CP (NRC, 2001). Crude protein (CP), ash, ethereal extract (EE) and dry matter (DM) content in were determined these diets (AOAC, 1990); as well as neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al. 1991). The composition of the diets is presented in Table 1.

Table 1: Composition of diets for intensive fattening of sheep.

	*Diets				
Ingredients (%)	MM	AA	AB	BA	BB
Corn grains	25	28	26	15	20
Sorghum	28	24	31	22.6	30.8
Corn straw	15	13	15	27	25
Soybean meal	11.1	15.3	7.1	13.5	7.3
Soybean hulls	7	2	7	7	5
Corn gluten	4	4	4	6	3
Molasses	5	5	5	5	5
Animal fat	1	4.8	1	0	0
Mineral Salt	1.5	1.5	1.5	1.5	1.5
$CaCO_3$	1.4	1.4	1.4	1.4	1.4
common salt	0.5	0.5	0.5	0.5	0.5
Urea	0.5	0.5	0.5	0.5	0.5
**Relation F:C	22:78	15:85	22:78	34:66	30:70
Nutrients (%)					
DM	87.6	88.1	87.5	86.6	86.5
RNDP	6.4	6.8	5.9	6.7	5.5
RDCP	9.6	10.4	8.6	10.5	8.3
EE	3.7	7.3	3.7	2.3	2.5
CF	10.1	7.7	10	14.2	12.6
NDF	24.2	20.1	24.2	31.9	29.1
ADF	13.7	10.8	13.6	18.8	16.7
CP	16	17.2	14.5	17.2	13.8
ME, (Mcal kg^{-1}D M)	2.8	3	2.8	2.6	2.6

CP, crude protein; ME, metabolizable energy; DM, dry matter; RNDP, rumen non-degradable crude protein; RDCP, rumen degradable crude protein; EE, ethereal extract; CF, crude fiber; NDF, neutral detergent fiber; ADF, acid detergent fiber.
*Treatments with different metabolizable energy content (ME, Mcal kg-1 DM) and crude protein (CP, %); MM (2.8:16), AA (3.0:17.2), AB (2.8:14.5), BA (2.6:17.2) and BB (2.6:13.8).
** F:C, Forage: concentrate ratio in diets.

In vitro Determination of Fermentation Gas Production and Dry Matter Degradation:

The fermentation of the diets was measured indirectly by the gas production technique GPT (Menke & Steingass, 1988; Theodorou *et al.*, 1994), for which 500 mg of DM of each diet were placed in amber glass flasks of 125 mL capacity, 90 mL of ruminal inoculum and, simultaneously, a continuous flow of CO_2. The vials were firmly covered with a rubber stopper and an aluminum ring, and incubated in a water bath at 39 ºC.

Subsequently, the fermentation gas pressure was measured with a 0.0 to 1.0 kg cm^{-2} capacity manometer (METRON®, 51100), at 0, 2, 4, 6, 8, 10, 14, 18, 24, 30, 36, 42, 48, 60 and 72 h of incubation. Pressure values were transformed into gas volume (V; mL g^{-1} DM) according to the regression model V (mL) = [P (kg cm^{-2}) + 0.0495]/0.0185, which was obtained by relating the pressure (p) generated by known volumes of air injected into the bottles maintained under the same handling conditions. At the end of the incubation period, the contents of the flasks were filtered through previously weighed filter paper and, subsequently, dried at 65 °C for 48 h in a forced-air oven and weighed to obtain the residual dry matter of the diet by the difference of weight. The TGP procedure was repeated twice over time. The rumen inoculum was obtained from steers cannulated in the rumen and adapted to a maintenance diet (70:30% of forage: concentrate). Prior to sampling the rumen fluid, the steers fasted for 12 h and the obtained rumen inoculum was filtered through four layers of gauze and mixed with a reduced mineral solution in a 1:9 (v/v) ratio. Each liter of mineral solution contained

K_2HPO_4 (0.45 g), KH_2PO_4 (0.45 g), $(NH_4)_2SO_4$ (0.45 g), NaCl (0.90 g), $MgSO_4$ (0.18 g), $CaCl_2$ (0.12 g), Na_2Co_3 (4g), which was reduced with 20 mL L^{-1} of a solution composed of Na_2S (0.2 g) and L-cysteine (0.2 g), dissolved in one liter of NaOH solution (0.8 ml L^{-1}). As an indicator of the reduction, two drops of rezarzurin 0.1 % were added., (Cobos & Yokoyama, 1995; Miranda-Romero *et al.*, 2020)

The accumulated gas volume as a function of incubation time was used to estimate the kinetic parameters: maximum volume (mV; mL g^{-1} DM), rate (R; mL h^{-1}) and lag phase (L; h) of gas production, the logistic model Vo = mV / (1+$e^{(2-4*S*(t-l))}$) (Pitt et al. 1999) and the NLIN procedure (SAS, 2015) On the other hand, the accumulated gas volume was also used to calculate the fermentable fractions according to the following procedure: the fractional volumes (fV) of accumulated gas were obtained for the intervals from 0 to 8 (fV_{0-8h}), 8 to 24 (fV_{8-24h}) and from 24 to 72 (fV_{24-72h}) hours of incubation (Miranda-Romero et al., 2015). With these fractional volumes of gas, the percentage of the fast (% FFF), medium (%M FF), and slow (%L FF) fermentation fractions were obtained in relation to the total volume of gas at 72 h of incubation.

The *in vitro* digestibility of the DM at 72 h (IVDMD72h) was calculated with the initial DM added to each bottle and the residual DM obtained by filtration, using the following formula:

$$\text{IVDMD72h} = \frac{\text{initial DM} - \text{residual DM}}{\text{initial DM}} * 100$$

Environmental Impact Indicators:

Another sample of DM from each diet was fermented *in vitro*, following the same procedure described above, with the following modifications: incubation was carried out at 39 °C for 24 hours in a water bath, gas volume (V) was measured at 0, 6, 12, 18 and 24 h of incubation with a 50 mL graduated glass syringe. In each measurement, the gas trapped in the syringe was carefully transferred to another 60-mL flask firmly closed with a rubber stopper and an aluminum ring, containing 45 mL of a KOH solution (1 M) for the capture of carbon dioxide (CO_2) from the gas mixture. The volume of gas that returned to the syringe corresponded to the amount of methane plus minor gases (V_{CH4+GM}). The V_{CH4+MG} values were adjusted to theoretical methane (V_{CH4}) with a factor of 0.77, according to the proportion of methane determined in the gas sample generated in the rumen (Zhong et al. 2016). The difference of V minus V_{CH4+GM} corresponded to the volume of CO_2. (V_{CO2}). This procedure was repeated twice in time and the values of V, V_{CH4+MG}, V_{CO2} and V_{CH4} were expressed in % and mL g^{-1} DM in 24 hours of incubation. The V_{CO2} and V_{CH4} values were used to calculate the global warming potential indicator GWPI (mL CO_2 eq g^{-1} DM) according to the following equation: GWPI = [CO_2 (mL g^{-1} DM) + CH_4 (mL g^{-1} DM) * 23]; where the constant 23 corresponds to the CO_2 equivalents for methane (CH_4) (Berra *et al.*, 2009). The GWPI and V values were used to calculate the Environmental Impact Index (EII; CO_2 eq) according to the following relationship: EII = GWPI/TV.

Statistical Analysis:

A generalized block experimental design was used with five treatments and six treatment repetitions in each block. The block corresponded to the repetition in time and the treatments to the diets. The GLM procedure and Tukey's multiple comparison tests of means (SAS, 2015) were used.

$$Y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \epsilon_{ijk}$$

Y_{ijk}= variable response corresponding to treatment i, block j and repetition k.

μ = general mean

τ_i= effect of the i-th treatment, i=1, 2, … 5,

β_j= effect of the j-th block, j=1 and 2.

$(\tau\beta)_{ij}$= block interaction effect by treatment

ϵ_{ijk}= random error associated with the k-th observation of the response variable in treatment I and block j. It is assumed ϵ_{ijk}~ NIID (0, $\sigma^2\varepsilon$).

RESULTS AND DISCUSSION

The ME and CP varied from 2.6 to 3.0 Mcal kg $^{-1}$ DM and from 13.6 to 17.2 % (Table 1) in the diets, these levels of ME and

CP are used in diets for the intensive fattening of developing sheep with the objective of daily weight gain between 325 and 400 g day^{-1} (NRC, 2007), so the identification as AA, MM and BB referred to balanced diets at high, medium and low energy and protein levels, and as AB and BA to unbalanced diets, but within the range for intensive fattening of sheep. The F: C ratios for these diets were 22:78 (MM), 15:85 (AA), 30:70 (BB), 22:78 (AB), and 34:66 (BA). The MM and AB diets had the same F: C ratio because they contained the same energy level (2.8 Mcal kg^{-1} DM). On the other hand, the NDF percentage for the treatments was 24.2% for MM, 20.1% for AA, 24.2% for AB, 31.9% for BA and 29.1% for BB (Table 1).

The parameters of the gas production kinetics: mV, R, and L are presented in Table 2. The L phase was not affected ($p>0.05$) neither for the balanced diets (AA, MM and BB) nor for the unbalanced ones (AB, BA) and had an average value of 4.2 h. The R of gas production was higher ($p<0.05$) in the balanced diets with medium or high levels of ME and CP (MM, AA), compared to the low-energy diets (BA, BB). The mV of gas, which indicates the extent of fermentation, was lower ($P<0.05$) in the unbalanced diet with excess protein and energy deficiency (BA), with respect to the other diets (Table 2). The mV and L were the same ($P>0.05$) for BB, MM and AA diets, which indicated that the length of fermentation and the time it takes to start fermentation, for this type of diet, are not affected by the level of ME and CP, as long as they are balanced. Although the imbalance of ME and CP decreased R and mV, it was observed that an excess of CP and a deficiency of ME (BA diet) affect such parameters more (Table 2), among other reasons, because protein fermentation produces little amount of gas (Makkar, 2004) and suggests that energy is more determinant than protein in ruminal fermentation.

Table 2. Parameters of in vitro gas production kinetics for fattening sheep diets.

Diet			Parameters		
	ME	CP	L	R	mV
	Mcal kg	%	h	mL h^{-1}	mL g^{-1}
MM	2.8	16.0	4.24^{a}	0.038^{a}	488.7^{a}
AA	3.0	17.2	4.24^{a}	0.038^{a}	477.5^{ab}
AB	2.8	14.5	4.24^{a}	0.036^{ab}	491.8^{a}
BA	2.6	17.2	4.24^{a}	0.034^{b}	462.1^{b}
BB	2.6	13.8	4.24^{a}	0.035^{b}	493.2^{a}

^{abc}Means with different letters in the same column are different ($p<0.05$).
L, lag phase; R, fermentation rate; mV, maximum gas volume, CP, crude protein, ME, metabolizable energy.
MM, AA, AB, BA, BB; Diets with high or low levels of ME or CP.

Cui *et al.* (2019) also demonstrated with *in vivo* studies that energy is determinant in productive variables (weight gain, feed consumption, feed conversion). Beckett *et al.* (2021), in their studies, observed that the energy content increases the proportion of volatile fatty acids and consequently gas production (Karabulut *et al.,* 2007). The mV for grains and concentrates is higher than that produced by green forages and stubbles (127-238 mL g^{-1} MO; Calabro *et al.*, 2005) and, the mV of gas for the diets formulated for the intensive fattening of sheep, was similar to that found for grains or diets high in concentrates (Sánchez *et al.*, 2019; Bueno *et al.*, 2005). Table 3, shows the values of the fermentable fractions. The FFR and FFM in the balanced diet at a high level of ME and CP (AA) were higher ($p<0.05$) compared to the balanced diet at a low level of ME: CP (BB) and to the unbalanced diets (AB and BA), because the AA diet contains a greater amount of concentrate (85 %) than BA and BB (66 and 70 %) (Table 1). These fractions (FFF and MFF) are associated with soluble, non-structural and reserve carbohydrates

(Ramírez-Díaz *et al.,* 2020 Miranda-Romero *et al.,* 2020; Ruiz *et al.,* 2018) and their increase in a diet induces greater gas production (Miranda-Romero *et al.,* 2020). When comparing the unbalanced diets, it was observed that the RFF is the same ($p>0.05$) despite the fact that the AB diet has a high concentrate level than the BA diet (78 vs 66%); while the MFF is higher ($p<0.05$ for the AB diet compared to BA, as expected due to its concentrate content. On the other hand, the comparison of the AB and AA diets showed that the first one contains less RFF and MFF, despite the fact that both are high in concentrate (85 vs 78 %), which showed that the ME: CP imbalance caused a deficient fermentation of the feed, particularly when the diet contains an excess of ME and the CP is poor. In contrast, the LFF associated with structural carbohydrates such as cellulose (Miranda-Romero et al., 2020), was higher ($p<0.05$) in those diets BA and BB which have a higher proportion of forage (34 and 30 %) and lower ME (2.6 Mcal kg-1 DM), compared to the diets with a lower proportion of forage and higher energy content (MM and AA). The unbalanced diet with an excess of ME and a low amount of CP (AB) had a higher LFF; despite the fact that it is poor in forage and rich in concentrate (85% and 15%), which could be due to the fact that a late fermentation of non-structural carbohydrates causes a low microbial biomass, due to its low content of CP.

The IVDMD is shown in Table 3. The balanced diet with a medium level of ME and CP (MM) had the highest ($p<0.05$) IVDMD, while the diets low in ME (BA and BB) had the lowest ($P< 0.05$) IVDMD. This was attributed to the fact that these last diets were formulated with higher forage content (30 and 34 %) and fiber (31.9 and 29.1 %) compared to the MM diet (Table 1). The same was observed by Gurrola *et al.* (2014). However, this hypothesis is not fulfilled when comparing the IVDMD of the MM diet with that of AA and AB, since the latter had lower IVDMD ($p<0.05$; Table 3), which could be due to the high-fat content of the AA diet, (4.8 %), which led to a higher percentage of EE (7.3 %), which could affect IVDMD (Harahap *et al.,* 2022). On the other hand, the lower IVDMD of the AB diet could be due to CP deficiency (Bastida Garcia *et al.,* 2011). This finding shows that diet can have high digestibility but lower fermentability and vice versa; sometimes due to the imbalance in ME and CP, and in others due to the content of anti-nutritional secondary compounds contained in the food (Jiménez-Santiago *et al.,* 2019).

Table 3. Fermentable fractions and *in vitro* dry matter digestibility (IVDMD) of diets for fattening sheep, at 72 h.

Treatment			Fermentable fractions			IVDMD
	ME	**CP**	**FFF**	**MFF**	**LFF**	**%**
	Mcal kg	%	.--------- % -----------.			
MM	2.8	16.0	20.8^{b}	51.8^{a}	27.4^{b}	77.4^{a}
AA	3.0	17.2	21.9^{a}	52.3^{a}	25.8^{c}	75.3^{b}
AB	2.8	14.5	20.4^{b}	50.4^{b}	29.2^{a}	74.5^{b}
BA	2.6	17.2	20.6^{b}	49.0^{c}	30.3^{a}	69.4^{c}
BB	2.6	13.8	19.9^{b}	49.8^{bc}	30.3^{a}	70.9^{c}

^{abc}Means with different letters in the same column are different ($p<0.05$
FFF, MFF, LFF, fast, medium and slow fermentation fractions

The environmental impact indicators are shown in Table 4. The sum of CH_4 and CO_2 was an average of 96%, the remaining 4% corresponded to minor gases such as H_2, N_2, etc. The increase in CH_4 decreased the proportion of CO_2 (Culma et al., 2017). The proportion of CH_4 varied ($p<0.05$) between treatments from 10.5 to 13.5 %, which is similar to that found for diets high in grains by Martínez-Hernández et al. (2019). Low ME diets (BB and BA) produced the highest CH_4 ($p<0.05$) compared to medium or high ME diets (MM, AA and AB; Table 4), which could be influenced by the highest proportion of forage and NDF in the first diets with respect to the MM, AA, and AB diets (Table 1), this is due to the fact that the fibrinolytic bacteria of the rumen are the main producers of H2 and CO2, previous molecules for the synthesis of CH_4 (Vélez- Terranova et al., 2014). On the other hand, the AA diet produced a higher V_{24h} ($p<0.05$) compared to the BA and BB diets (Table 4), which was attributed to the higher concentrate content. The diet low in ME and high in CP (BA) had the lowest V_{24h} ($P<0.05$), even than the low-level balanced (BB) and unbalanced (AB) diets. This is indicative that an imbalance in which ME is deficient and CP in excess causes poor feed use in the first 24 h of incubation. This may be due to the fact that the microorganisms have to ferment the protein when the amount of carbohydrates is insufficient and, consequently, the gas production is lower (Rodríguez et al., 2007). Regarding the GWPI (Table 4), the BB diet causes the highest ($p<0.05$) GWPI than the diets (AB and MM), which was attributed to its higher forage and NDF content (Table 1) which induced greater CH_4 (Table 4) used to estimate the GWI. There is no data in the literature that refers to the GWPI to compare them with those found in this research; however, a similar indicator known as emission factor (EF) has been calculated by various authors, which is calculated based on gross energy and the percentage of gross energy in the feed converted into methane (IPCC, 2006), In these studies, it was also found that EF increases in animals consuming diets low in energy and high in fiber, in contrast to those diets high in energy and low in fiber which reduce the emission factor (Lombardi *et al.*, 2021). The GWPI is a more realistic value of environmental impact than the measurement of CH_4 only since the two greenhouse gases produced in the rumen (CO_2 and CH_4) are considered for its calculation, and the calorific equivalence (Sandoval - Pelcastre *et al.*, 2020). The CH_4 value estimated by other methods is lower than the GWPI and is useful to compare one treatment with another, but not the environmental impact, which is only assumed to be higher than the methane production and the GWPI is an indicator of the warming potential caused by the ruminal fermentation of one gram of DM in 24 h of incubation.

The environmental impact index (EII) (Table 4) is a comparison value to estimate how much more one diet impacts the environment with respect to another, and this value is an indicator of the CH_4 amount produced. For this, it was assumed that the volume (V_{24h}) is fully equivalent to CO_2 eq. The EII for the diets varied between 3.3 and 3.9 (Table 4), the low-energy diets (BB and BA) have the highest EII ($p<0.05$) compared to diets with a medium and high ME content (MM and AB; Table 4), due to the same reasons previously stated for methane and GWPI, based on forage content and NDF. The EII values obtained are typical for diets high in concentrate and ME, such as those used in this research, since when forages or diets high in forage are used, their value can reach 10 (unpublished data).

Table 4 Environmental impact Indicators and *in vitro* gas production after 24 h of incubation at 39 °C of fattening sheep diets.

Treatment			Environmental impact indicators				
	ME	CP	*V_{24h}	CO_2	CH_4	GWPI	EII
	Mcal kg	%	mL g^{-1} DM	%	%	mL CO_2 eq g^{-1}DM	CO_2 eq
MM	2.8	16.0	211.8^{ab}	86.4^{a}	10.5^{c}	692.0^{b}	3.3^{c}
AA	3.0	17.2	218.2^{a}	85.3^{ab}	11.4^{bc}	754.9^{ab}	3.5^{bc}
AB	2.8	14.5	215.2^{a}	85.8^{a}	10.9^{c}	722.2^{b}	3.4^{c}
BA	2.6	17.2	198.1^{c}	83.1^{bc}	13.0^{ab}	757.3^{ab}	3.8^{ab}
BB	2.6	13.8	207.0^{b}	82.5^{c}	13.5^{a}	809.8^{a}	3.9^{a}

V_{24h}, gas volume at 24 h; GWPI global warming potential indicator; EII, Environmental impact indicators. [Abc] Means with different letters in the same column are different ($p<0.05$)

CONCLUSION

Ruminal fermentation is the result of the metabolism of bacteria, anaerobic fungi, and protozoa that are present in this environment. The rumen microorganisms play a vital and major role In proper animal nutrition, so it is important to maintain creative knowledge in the study of rumen fermentation and microbial ecosystems to improve ruminant feeding. In intensive sheep production systems, where feeds high in ME and CP are required, the balance of these nutrients is essential to make rumen fermentation parameters more efficient and to environmental impact related to global warming. However, an imbalance caused by an excess of protein can unfavorably modify the fermentative kinetics of the food, the fermentable fractions and environmental impact indicators. On the other hand, the use of energetic ingredients such as fat, with the purpose of reaching high energy levels can cause a high digestibility, but a lower fermentability.

Based on the proposed objectives, it is concluded that diets with a higher content of non-structural carbohydrates have better fermentative kinetics and digestibility, in the same way, that they generate lower proportions of CH_4. On the other hand, the GWPI can be a better estimator of the environmental impact since it considers the amounts of CO_2 and CH_4 produced in the rumen.

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