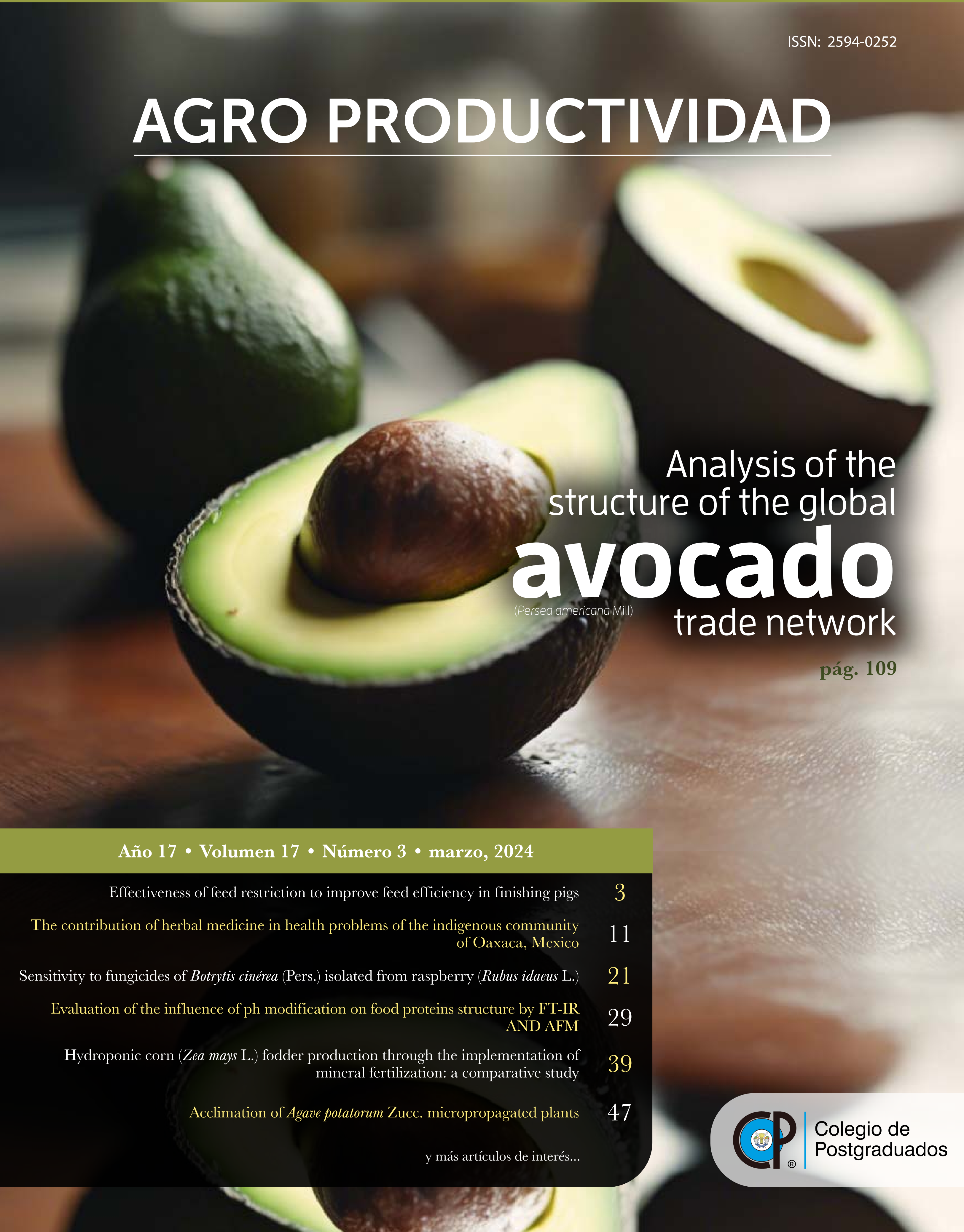
ISSN: 2594-0252

AGRO PRODUCTIVIDAD



Analysis of the structure of the global avocado trade network

(Persea americana Mill)

pág. 109

Año 17 • Volumen 17 • Número 3 • marzo, 2024

Colegio de Postgraduados

AGRO PRODUCTIVIDAD

Colegio de
Postgraduados

In vitro nutritional assessment and estimation of methane emissions from Kikuyu grass pastures overseeded with rye

Marín-Santana, María N.[1]; **López-González, Felipe**[1]; **Morales-Almaraz, Ernesto**[2]; **Arriaga-Jordán, Carlos M.**[1*]

[1] Universidad Autónoma del Estado de México, Campus UAEM, Instituto de Ciencias Agropecuarias y Rurales. El Cerrillo. El Cerrillo Piedras Blancas, Toluca, Estado de México, México. C. P. 50090.
[2] Universidad Autónoma del Estado de México, Campus UAEM El Cerrillo. Facultad de Medicina Veterinaria y Zootecnia. El Cerrillo Piedras Blancas, Toluca, Estado de México, México. C. P. 50090.
* Correspondence: cmarriagaj@uaemex.mx

ABSTRACT

Objective: To determine the *in vitro* digestibility and gas production, and to estimate the methane emissions from Kikuyu grass pastures, and Kikuyu grass overseeded with rye.

Design/methodology/approach: Two pastures were assessed. One was the subtropical grass Kikuyu (*Cenchrus clandestinus*) (KY), and the other was Kikuyu grass plus overseeding with rye (*Secale cereale*) (KYCEN), both associated with white clover (*Trifolium repens* cv. Ladino). Sample collection was in June and July 2021. *In vitro* digestibility of dry matter (MS), organic matter (MO), and Neutral Detergent Fibre (FDN), as well as the methane emissions were estimated. The *in vitro* digestibility and gas production variables were analysed with a split-plot experimental design, and the methane emission variables were analysed with a doble cross-over design.

Results: There were no significant differences between treatments for dry matter (MS), organic matter (MO) or Neutral Detergent Fibre (FDN) *in vitro* digestibility, nor in methane emissions ($P>0.05$).

Limitations on study/implications: The *in vitro* assessment of digestibility, gas production and the estimation of methane emissions of Kikuyu grass pastures and Kikuyu plus rye enable the implementation of feeding strategies for small-scale livestock production systems that do not only benefit the farmers but also the environment.

Findings/conclusions: It is concluded that Kikuyu grass pastures and Kikuyu with rye are a viable feeding option for small-scale dairy systems.

Keywords: Kikuyu grass, rye, gas production, methane.

Citation: Marín-Santana, M. N., López-González, F., Morales-Almaraz, E., & Arriaga-Jordán, C. M. (2024). *In vitro* nutritional assessment and estimation of methane emissions from Kikuyu grass pastures overseeded with rye. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i3.2638

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: July 17, 2023.
Accepted: January 19, 2024.
Published on-line: April 24, 2024.

Agro Productividad, 17(3). March. 2024. pp: 93-102.

INTRODUCTION

Agricultural production faces new challenges worldwide, such as the greenhouse gases it generates. Livestock farming contributes 14.5% of these gases [1], including methane —a greenhouse gas (GHG) with 28 times greater global warming potential than carbon dioxide [2] and a 10-year average lifetime in the atmosphere.

Since 35% of enteric methane production comes from pasture systems [3], feeding strategies that can reduce CH_4 emissions should be considered. In small-scale dairy systems, the feed is mainly based on pasture grazing. The proper management of these systems potentially improves their profitability and sustainability, enhances the quantity and quality of the forage consumed by animals, and even reduces CH_4 emissions [4].

Kikuyu grass (*Cenchrus clandestinus*) is a subtropical grass of African origin, well adapted to forage-based dairy systems in Latin America (Colombia, Brazil, and Mexico), Oceania (Australia and New Zealand), and South Africa. Properly used, it has moderate-good quality and high yield potential [4,5].

Rye (Secale cereale) is a small grain cereal, with a short growth cycle; consequently, it requires less water, is resistant to frost, and can be used for grazing, silage, or grain harvesting [6]. Small grain cereals have good forage yields and, given the current situation (low availability of irrigation, plus low precipitation and changes in rain patterns, due to increasing climate change [7]), rye is a viable option for these production systems.

The production systems that benefit from the use of these forages include small-scale dairy systems, which are considered a feasible instrument to stimulate economic growth and reduce poverty; additionally, they contribute 37% of the domestic milk production [8]. However, there are more systems that benefit from their use, such as sheep production systems.

The *in vitro* gas production technique is a method that has been widely used to assess the effect of different forages: it simulates the ruminal environment (temperature, pH, anaerobiosis, and mineral intake) to assess the fermentation of different substrates or additives [9]. The equations that estimate methane emissions have been used because they are less expensive than other *in vivo* methods [10]. Therefore, objective of this work was to assess the *in vitro* digestibility and gas production, as well as to estimate methane emissions, from Kikuyu grass pastures and Kiyuyu overseeded with rye.

MATERIALS AND METHODS

Location of the study site

The study was carried out in a small-scale dairy farm located in the municipality of Aculco in the Estado of México (between 20° 06' and 20° 17' N and 99° 40' and 100° W), at 2,440 meters above sea level. The site has a temperate-subhumid climate, a rainy season from May to October, and frost from November to February. The average annual temperature is 13.5 °C and the average annual precipitation ranges from 700 to 1,000 mm [11].

Experimental development and treatments

Two 1-ha pastures were assessed. One pasture was naturally invaded by a Kikuyu subtropical grass (*Cenchrus clandestinus*) (KY). The other one featured Kikuyu pasture which was overseeded with rye (*Secale cereale*) (KYCEN) on April 9, 2021. Both pastures were associated with white clover (*Trifolium repens*) cv. Ladino, among other unidentified grass species eaten by grazing dairy cows.

Samples were collected in June and July, 2021, during the rainy season. Three forage samplings were carried out at 14-day intervals. The experiment followed the guidelines of rural participatory research [12].

Variables assessed

Ruminal fermentation kinetics and *in vitro* digestibility

The simulated grazing technique was used to collected 200-g forage samples in different sites of the assessed pastures. The samples were then placed in an extraction oven at 55 °C until a constant weight was achieved. Subsequently, they were ground to 2.0 mm and processed to determine the digestibility, metabolizable energy, and ruminal fermentation kinetics, using the in vitro gas production technique.

The ruminal fluid from two cows was used to determine the variables of the ruminal fermentation kinetics of pasture forage. The diet of these cows was composed of grazing, maize silage, and commercial concentrate. The fluid was extracted through a nasogastric tube. According to the procedure described by [13], 990±0.01 mg of dry forage samples from each pasture were weighed and subsequently placed in 120 ml glass bottles with crimp caps. Ninety ml of buffer solution and 10 ml of ruminal fluid were added in a 9:1 (vol/vol) ratio. The solution had been previously gassed with CO_2 for 20 minutes to generate anaerobiosis.

Subsequently, the samples were incubated at 39 °C and gas production was measured using a pressure transducer (DELTA OHM, Manometer, 8804) at 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 72, 84, and 96 hours. Each sample was analyzed in sextuplicate with 96 h incubation in two repeated courses at different periods.

After 96 hours of incubation, the residues of each sample were analyzed to determine the DM, OM, and NDF digestibility [14]. In the case of the *in vitro* digestibility of neutral detergent fiber (IVDNDF), the residues from the other three bottles were removed with 50 ml of NDF solution, placed in an autoclave at 105 °C for one hour, filtered in Schott Duran® No. 1 filter crucibles, and placed in the muffle at 450 °C for 4 hours. The IVDDM was calculated based on the weight difference between the DM of the initial sample and the DM of the gas production residue, while the IVDNDF was calculated using the NDF digestibility values of the sample already incubated, divided between the NDF content of the initial sample. The ash content of the samples after 96 h incubation was used to determine the residual organic matter (OM) and the *in vitro* digestibility of organic matter (IVDOM) following the micro technique proposed by [15].

The results obtained were used to determine the *in vitro* fermentation parameters, which were estimated through the adjustment of the accumulated gas volume obtained from each bottle to the mathematical model developed for this study [16], using the following equation in the GraFit Data Analysis Software (V3) [17]:

$$PG = B\left(1 - \exp - c\left(t - lag\right)\right)$$

Where: PG=total gas production (ml gas/100 mg DM); B=asymptotic gas production from the fermentation of the neutral detergent fiber; c=degradation rate of gas production (per hour); lag=time elapsed before the beginning of the fermentation of structural carbohydrates [18].

The ether extract (EE) was determined by the immersion solvent extraction method [19], while the gross energy (GE) required to estimate methane emissions was calculated according to [10].

Enteric methane emissions

Methane emissions were estimated following the model proposed by [20]. Data from the research carried out [21] —with eight multiparous Holstein cows, similar numbers of days in milk, daily milk yield, and live weight— were used with a double cross-over design. The following equation was used:

$$\begin{aligned}\mathrm{CH}_4\left(g\,/\,day\right) = & -60.5+\left(12.4\times DMI\right)-\left(8.78\times\%EE\right)\\ & +\left(2.10\times\%NDF\right)+\left(16.1\times\%\,fat\ in\ milk\right)+\left(0.148\times LW\right)\end{aligned}$$

Where: *DMI*=dry matter intake (kg/cow/day), *EE*=ether extract of the diet, *NDF*=neutral detergent fiber of the diet, and *LW*=live weight (kg/cow).

The correction factor for methane Ym (ratio of gross energy lost as methane) was calculated based on [22].

$$Ym=100\times\left(\mathrm{CH}_4\left(MJ\,/\,day\right)/\,GE\ consumed\left(MJ\,/\,day\right)\right)$$

Where: *GE*=gross energy.

The metabolizable energy of the forages was estimated based on the digestible organic matter in the dry matter, using the following equation [23]:

$$ME=0.16*DOMD\,/\,10$$

Where: *DOMD*=digestible organic matter in dry matter.

Experimental design and statistical analysis

A split-plot design was used for the gas production and DM, OM, and NDF digestibility variables with the following statistical model:

$$Y_{ijkl}=\mu+T_i+E_j+P_k+Tp_{ij}+e_{ijk}$$

Where: μ=general mean; T=effect of the main plot (i=1, 2); E=experimental error of the main plot; P=effect of the assessment periods (k=1, 2, 3); Tp=effect of the interaction between the main plot (crops) and the split plot (assessment periods); E=residual variation.

For the methane emissions estimation variables, a double cross-over design was used with the following model:

$$Y_{ijkl} = \mu + S_i + C_{(i)j} + P_k + T_l + e_{ijkl}$$

Where: Y_{ijkl}=response variable; μ=general mean; S_i=effect of the sequence (i=1 and 2); $C_{(i)j}$=effect of the cow within the sequence (j=1... 4); P_k=effect of the experimental periods (k=1...3); T_l=effect of treatments (l=1 and 2); e_{ijkl}=experimental error [24].

RESULTS AND DISCUSSION

Table 1 includes the *in vitro* digestibility of DM, OM, and NDF results, as well as the estimation of the metabolizable energy content of the forages from the two experimental pastures. There were no significant differences ($P>0.05$) for any of the variables assessed.

Since the study was carried out during the rainy season (the optimal time for forage growth), both pastures had very similar results. Kikuyu obtained good digestibility results, due to its subtropical origin and its growing season (spring-summer).

The results are higher than those obtained by [25] in the same study area, with Kikuyu pastures, *Festulolium* cv. Spring Green, *Lolium perenne* cv. Pay Day, and *Lolium arundinaceum*

Table 1. Average *in vitro* digestibility (g/kg DM) and metabolizable energy (MJ ME kg^{-1} DM) of forage from experimental pastures during different sampling periods (PI, PII, PIII).

VARIABLE	PERIODS			Mean TX	SEMTx	P-Value	SEMExP	P-Value
	PI	PII	PIII					
IVDMD (g/kg DM)								
KY	783.83	773.42	773.16	776.80	0.13	0.947^{NS}	2.44	0.422^{NS}
KYCEN	772.55	774.75	783.70	777.00				
Mean for Periods	778.19	774.08	778.43					
Interaction SEMTx*ExP					3.16	0.06^{NS}		
IVOMD (g/kg DM)								
KY	835.93	825.68	826.42	829.34	5.35	0.114^{NS}	3.23	0.467^{NS}
KYCEN	836.78	834.12	839.85	836.92				
Mean for Periods	836.36	829.90	833.13					
Interaction SEMTx*ExP					1.82	0.479^{NS}		
IVNDFD (g/kg DM)								
KY	835.17	757.06	830.71	807.65	11.95	0.406^{NS}	22.78	0.231^{NS}
KYCEN	814.84	823.35	835.45	824.55				
Mean for Periods	825.01	790.21	833.08					
Interaction SEMTx*ExP					12.86	0.242^{NS}		
ME (MJ/kg DM)								
KY	10.65	10.51	10.51	10.56	0.002	0.929^{NS}	0.03	0.385^{NS}
KYCEN	10.50	10.53	10.65	10.56				
Mean for periods	10.58	10.52	10.58					
Interaction SEMTx*ExP					0.04	0.053^{NS}		

KY=Kikuyo; KYCEN=Kikuyo+rye; IVDMD=*in vitro* dry matter digestibility; IVOMD=*in vitro* organic matter digestibility; IVNDFD=*in vitro* neutral detergent fibre digestibility; ME=metabolizable energy; SEMTx=standard error of the mean for pasture treatments (main plots); SEMExP=standard error of the mean for experimental periods (split plot); SEMTx*ExP=standard error of the mean for the interaction between treatments and experimental periods; NS=($P>0.05$).

cv. TF-33, in the rainy season. They obtained average results of 700.70 gr, 637.90 gr, and 744.80 gr for IVDDM, IVDOM, and IVDNDF, respectively.

Some authors [26] mention that DM digestibility is an important indicator of forage quality: good quality forage has a digestibility of ≥700 g/kg DM. In this work, both pastures recorded higher values than 700 g/kg DM and therefore can be considered good quality material [14]. These authors assessed small grain cereals (including rye), obtaining higher results than 700 g/kg DM; however, metabolizable energy was higher (11.6 MJ) than in this work (10.56 MJ on average, for both pastures).

The IVDOM and IVDNDF were higher than those reported by [14], who carried out an experiment with small grain cereals (including rye), at a more advanced phenological stage, recording an average of 730 and 618.6 g/kg DM for IVDOM and IVDNDF, respectively. Likewise, these results are higher to those found by [25], who reported 637.90 g/kg DM for IVDOM and 744.80 g/kg DM for IVDNDF in Kikuyu grass pastures.

Metabolizable energy depends on the nutritional quality of forage: it is more stable in the growth period and later decreases as grain formation begins (in the case of cereals) and nutrients are mobilized towards the grain [14]. For this study, an average of 10.56 MJ was determined, similar to the results of [25] who obtained 10.34 MJ.

Table 2 shows the results of *in vitro* gas production where no significant differences were observed ($P>0.05$). *In vitro* gas production is a suitable indicator for the prediction of the carbohydrate degradation of forages [27]. This gas production is caused by the

Table 2. Averages of *in vitro* gas production parameters resulting from the fermentation of pasture forage assessed in three sampling periods (PI, PII, PIII).

VARIABLE	PERIODS			Mean TX	SEMTx	P-Value	SEMExP	P-Value
	PI	PII	PIII					
B (ml gas g^{-1} DM)								
KY	227.35	229.96	230.68	229.33	3.37	0.059^{NS}	3.06	0.123^{NS}
KYCEN	232.54	234.99	234.76	234.10				
Mean for Periods	229.95	232.47	232.72					
Interaction SEMTx*ExP					1.51	0.186^{NS}		
cB (g h^{-1})								
KY	0.03	0.03	0.03	0.03	0.002	0.253^{NS}	0.001	0.68^{NS}
KYCEN	0.03	0.03	0.03	0.03				
Mean for Periods	0.03	0.03	0.03					
Interaction SEMTx*ExP					0.0008	0.68^{NS}		
Lag (h)								
KY	6.66	6.67	6.01	6.45	0.26	0.583^{NS}	1.21	0.067^{NS}
KYCEN	6.10	8.20	6.01	6.77				
Mean for Periods	6.38	7.44	6.01					
Interaction SEMTx*ExP					0.1	0.896^{NS}		

KY=Kikuyo; KYCEN=Kikuyo+rye; B=gas production potential (ml gas/g DM) based on the insoluble but potentially degradable fraction; cB=rate of fermentation of fraction b; lag=lag time (h) before fermentation of NDF; SEMTx=standard error of the mean for pasture treatments (main plots) ; SEMExP=standard error of the mean for experimental periods (split plot); SEMTx*ExP=standard error of the mean for the interaction between treatments and experimental periods; NS=($P>0.05$).

fermentation of carbohydrates and their transformation into acetate, propionate, and butyrate; consequently, any change in carbohydrate fractions will be reflected in gas production.

The accumulated gas production (B) reached 231.75 ml, lower than the result found by [14], who obtained 258.72 ml in rye pastures, but higher than that results of [25], who obtained 209.64 ml in Kikuyu pastures. For their part, [28] assessed the nutritional value of forage species in the central Mexican Plateau and observed a higher content of accumulated gas production (215.66 ml) in Kikuyu. According to them, Kikuyu has a high hemicellulose content and a low cellulose content; therefore, hemicellulose is the NDF fraction that is completely fermented by microorganisms.

The degradation rate (c) is related to the fermentation of the substrate, which in turn is related to the type of structural carbohydrates that may indicate that there is more or less cellulose available for ruminal microorganisms [18]. This study recorded no difference between the treatments, with an average c of 0.03 for both pastures. For their part, [25] reported a fermentation rate of 0.02 for Kikuyu, as a result of the higher content of lignified cell walls, characteristics of the subtropical and tropical C4 grasses. However, in this study degradation rate was higher, due to the association between several species, which improves the nutritional quality of the pastures.

Lag (h) indicates the time in which microorganisms begin to degrade structural carbohydrates. The content of rapid degradation carbohydrates (*e.g.*, sugars, starch, and pectin) increases the lag time [18]. Lag time is important in digestibility because the presence of high amounts of fermentable carbohydrates diminishes its duration [29]. In this study, a Lag time of 6 hours was reported. Period II was the longest (7 hours), perhaps as a result of the maturation of the pastures, which is directly related to the fiber content that, as has been previously reported [21] increases over time. However, in the case of Kikuyu over seeded with rye, there was a decrease in period III, perhaps due to the increase in rains as this period approached, therefore, there was a greater growth of forage, resulting in a new decrease in Lag time. This result is similar to that found by [28] in Kikuyu grass pastures, with an average of 6 hours.

The estimated enteric methane emission is shown in Table 3. There were no significant differences per treatment for any of the variables. Nevertheless, KYCEN obtained higher numerically values for methane production (CH_4 g/kg DM) and the percentage of gross energy lost as methane (Ym).

The estimated average production of CH_4 was 298.54 g/cow/d, higher than the value reported by [8] in small-scale dairy systems (an average of 216.12 g/cow/d). In their research, [8] assessed four feeding strategies: CC=cut and carry, CC+CS=cut and carry plus maize silage, CIG=continuous intensive grazing; and CIG+CS=continuous intensive grazing plus maize silage. The farms that implemented pasture grazing as source of quality fresh forage (CIG) generated less methane than farms that implemented cut and carry and maize silage.

According to the abovementioned information, the estimated average production of CH_4 was 298.54 g/cow/d, lower than the value reported by [30] in small-scale dairy systems in the central Mexican Plateau. In their research, [30] obtained 335 g/cow/d in optimized

Table 3. Average values of estimated enteric methane emissions from Kikuyu pasture and Kikuyu overseeded with rye in small-scale dairy systems.

Variable	Treatment			P-Value	Experimental periods			SEMExP	P-Value
	KY	KYCEN	SEMTx		I	II	III		
CH_4 g/cow/day	296	301.08	4.59	0.184^{NS}	297.5	301.5	297.6	4.59	0.783^{NS}
CH_4 MJ/cow/day	16.34	16.66	0.25	0.184^{NS}	16.43	16.65	16.43	0.25	0.783^{NS}
CH_4 g/kg milk	18.72	18.28	0.49	0.994^{NS}	16.89	18.03	20.58	0.49	0.000*
CH_4 g/kg ECM	18.53	17.62	0.41	0.885^{NS}	16.7	17.62	19.92	0.41	0.000*
CH_4 g/kg DMI	23.62	23.86	0.22	0.178^{NS}	22.93	23.33	24.91	0.22	0.000*
Ym (% GE intake)	6.94	7.00	0.06	0.178^{NS}	6.74	6.85	7.32	0.06	0.000*

KY=Kikuyo; KYCEN=Kikuyo+rye; ECM=Energy-corrected milk production; DMI=Dry matter intake; GE=Gross energy. SEMTx=Standard error of the mean for pasture treatments; SEMExP=Standard error of the mean for experimental periods.

diets with a feeding mainly based on good quality forage with a metabolizable energy of 11 MJ. For their part, [10] used questions to estimate 283 g/cow/d for temperate regions in Mexico and 319.1 g/cow/d for tropical regions (results similar to the ones determined in this study), using Kikuyu grazing associated with other grass species found in the temperate regions of Mexico. Kikuyu is a plant with a subtropical origin with nutritional quality similar to temperate grasses. It has adapted very well to temperate zones [31]; therefore, the result obtained in this study is very similar to that obtained in such areas. The estimated mean emissions of CH_4 are within the normal range (77 to 447 g/cow/d) reported by [32].

The average methane emission intensity was 18.07 g CH_4/kg of ECM (energy-corrected milk). This figure is higher than the intensity recorded by [8], who obtained 15.1 g CH_4/kg of ECM. However, it is very similar to the results recorded by [33] for the Latin American region (19.9 g CH_4/kg of ECM) and with the figures estimated by [30] in the same study area (18.2 g CH_4/kg of ECM). For his part, [34] mentions that a greater intake of highly digestible foods reduces the generation of CH_4 (average of 23 g kg^{-1} DMI (dry matter intake)).

About 6-10% of the total gross energy consumed by dairy cows is converted into CH_4, which is released into the atmosphere through respiration [35]. Meanwhile, this study recorded than an average of 6.97% of the energy is converted into methane.

[36] identified that the incorporation of high-quality fresh forages can reduce CH_4 emission by 15% [8]. They found that the supply of higher quality fresh forage through grazing favored CH_4 emission per animal per day by 8.9%, compared with the cut and carry system [30]. They found that methane emissions diminish by 2% when associated pastures are used instead of single-grass pastures. Therefore, methane emissions per kg of milk produced can be reduced through the use of better feeding strategies, based mainly on good quality forage, grown in the same farm.

CONCLUSIONS

Given the lack of significant differences between both treatments, Kikuyu grass pastures and Kikuyu grass overseeded with rye are a viable feeding option for small-scale dairy systems, during the rainy season.

ACKNOWLEDGMENTS

The authors would like to thank the farmer who participated in this research for providing information and samples from his farm. Additionally, they would like to thank the Universidad Autónoma del Estado de México which funded the UAEM 4973/2020/CIB project, and the Consejo Nacional de Ciencia y Tecnología (CONACYT), for the scholarship granted to María Nayeli Marín Santana.

REFERENCES

1. Gerber, P., Steinfeld, H. Henderson, B., Mottet, A.,Opio, C., Dijkman, J., Falcucci, A. and Tempio, G. (2013). Tackling climate change through livestock. A global Assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO). Rome, Italy.
2. IPCC, Intergovernmental Panel on Climate Change. (2013). Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.
3. Vargas, J., Cárdenas, E., Pabón, M. y Carulla J. (2012). Emisión de metano entérico en rumiantes en pastoreo. *Archivos de Zootecnia*, 61: 52.
4. Marín, A., Bindelle, J., Zubieta, A.S., Correa, G., Arango, J., Chirinda, N., de Faccio-Carvalho, P.C. (2021). *In vitro* fermentation profile and methane production of kikuyu grass harvested at different sward heights. Frontiers in Sustainable Food Systems, 5. https://doi.org/10.3389/fsufs.2021.682653
5. Marín-Santana, M. N., López-González, F., Hernández-Mendo, O. and Arriaga-Jordán, C. M. (2020). Kikuyu pastures associated with tal fescue grazed in autumn in small-scale dairy systems in the highlands of Mexico. *Tropical Animal Health and Production* 52: 1919-1926. DOI:https://doi.org/10.1007/s11250-020-02216-7.
6. Vega-García, J.I., López-González, F., Morales-Almaraz, E., and Arriaga-Jordán, C.M. (2021). Grazed rain-fed small-grain cereals as a forage option for small-scale dairy systems in central Mexico. *Tropical Animal Health and Production* 53: 511. doi:10.1007/s11250-021-02958-y.
7. Thornton, P.K., van de Steeg, J., Notenbaert, A. and Herrero, M. (2009). The impacts of climate change on livestock and livestock systems in developing countries: A review of what we know and what we need to know. *Agricultural Systems* 101:113-127. https:// doi. org/10. 1016/j. agsy. 2009. 05. 002
8. Carrillo-Hernández, S., Prospero-Bernal, F., López-González, F., Flores-Calvete, G., Martínez-García, C.G., y Arriaga-Jordán, C.M. (2021). Estimación de emisión de metano entérico en sistemas de producción de leche bovina en pequeña escala bajo diferentes estrategias de alimentación. *Tropical and Subtropical Agroecosystems* 24:42 DOI:10.56369/tsaes.3306
9. Crosby-Galván, M.M., y Ramírez-Mella, M. (2018). Técnica de producción de gas *in vitro* para estimar la producción de metano. *Agroproductividad.* 11: 64-69.
10. Castelán-Ortega, O.A., Ku-Vera, J.C. and Estrada-Flores, J.G. (2014). Modelling methane emissions and methane inventories for cattle production systems in Mexico. *Atmósfera*. 27:185-191.
11. Celis-Álvarez, M.D., López-González, F., Martínez-García, C.G., Estrada-Flores, J.G. and Arriaga-Jordán, C.M. (2016). Oat and ryegrass silage for small-scale dairy systems in the highlands of central Mexico. *Tropical Animal Health and Production*. 48:1129–1134. DOI: 10.1007/s11250-016-1063-0.
12. Conroy, C. (2005). Participatory livestock research, (ITDG Publishing, Bourton on Dunsmore, Warwickshire, UK.).
13. Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J.A. (1994). Simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminants feeds. *Animal Feed Science and Technology* 48: 185–197. doi:10.1016/0377-8401(94)90171-6
14. Celis-Álvarez, M.D., López-González, F., Estrada-Flores, J.G., Domínguez-Vara, I.A., Heredia- Nava, D., Munguía-Contreras, A. y Arriaga-Jordán, C.M. (2017). Evaluación nutricional in vitro de forrajes de cereales de grano pequeño para sistemas de producción de leche en pequeña escala. *Tropical and Subtropical Agroecosystems* 20:439446.
15. Pell, A.N. and Schofield, P. (1993). Computerized monitoring of gas production to measure forage digestion *in vitro*. *Journal of Dairy Science* 76:1063-1073. https://doi.org/10.3168/jds.S0022-0302(93)77435-4
16. Krishnamoorthy, U., Soller, H., Steingass, H. and Menke, K.H. (1991). A comparative study on rumen fermentation of energy supplements *in vitro*. *Journal of Animal Physiology and Animal Nutrition* 65:28-35. https://doi.org/10.1111/j.1439-0396.1991.tb00237.x
17. Grafit, Version 3 (1992). Data analysis and graphics program. Erithacus Software LId.
18. Aragadvay-Yungán, R.M., Rayas, A.A, Heredia-Nava, D., Estrada-Flores, J.G., Martínez-Castañeda, F.E. y Arriaga-Jordán, C.M. (2015). Evaluación *in vitro* del ensilaje de girasol (*Helianthus annus* L.) solo y combinado con ensilaje de maíz. *Revista Mexicana de Ciencias Pecuarias* 6: 315-327. http://www.scielo.org.mx/pdf/rmcp/v6n3/v6n3a6.pdf

19. AOAC, Association of Official Analytical Chemists. (2000). Official Methods of analysis (17th edn), (Gaithersburg, MD, USA).
20. Niu, M., Kebreab, E., Hristov, A.N., Oh, J., Arndt, C., Bannink, A., Bayat, A.R., Brito, A.F., Boland, T., Casper, D., Crompton, L.A., Dijkstra, J., Eugene, M.A., Garnsworthy, P.C., Haque, M.N., Hellwing, A.L.F., Huhtanen, P., Kreuzer, M., Kuhla, B., Lund, P., Madsen, J., Martin, C., McClelland, S.C., McGee, M., Moate, P.J., Muetzel, S., Munoz, C., O'Kiely, P., Peiren, N., Reynolds, C.K., Schwarm, A., Shingfield, K.J., Storlien, T.M., Weisbjerg, M.R., Yanez-Ruiz, D.R., and Yu, Z. (2018). Prediction of enteric methane production, yield, and intensity in dairy cattle using an intercontinental database. *Global Change Biology* 24: 3368–3389. doi:10.1111/gcb.14094
21. Marin-Santana, M. N., Lopez-Gonzalez, F., Morales-Almaraz, E., Plata-Reyes D. A. y Arriaga-Jordan C. M. (2023). Desempeño productivo de vacas lecheras alimentadas con praderas de Kikuyo sobresembradas con centeno en sistemas de producción de leche en pequeña escala en los valles altos de México. *Tropical and Subtropical Agroecosystems* (en prensa).
22. IPCC, (2019). Emissions from livestock and manure management. In Intergovernmental Panel on Climate Change (IPCC) (ed) 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Cambridge University Press, Cambridge, United Kingdom and New York, USA.
23. AFCR, (1993). Energy and Protein Requirements for Ruminants. An advisory manual prepared by the AFRC Technical Committee on Response to Nutrients. (CAB International, Wallinford, UK).
24. Kaps, M. and Lamberson W. (2004). Biostatistics for animal science. Wallingford: CABI Publishing.
25. Plata-Reyes, D.A., Hernández-Mendo, O., Vieyra-Alberto, R., Albarrán-Portillo, B., Martínez-García, C.G., and Arriaga-Jordán, C.M. (2021). Kikuyu grass in winter–spring time in small-scale dairy systems in the highlands of central Mexico in terms of cow performance and fatty acid profle of milk. *Tropical Animal Health and Production* 53. https://doi.org/10.1007/s11250-021-02672-9
26. Chamberlain A.T., y Wilkinson. (2002). Alimentación de la vaca lechera. Acribia Zaragoza, España.
27. Danielsson, R., Ramin, M., Bertilsson, J., Lund, P., and Huhtanen, P. (2017). Evaluation of a gas *in vitro* system for predicting methane production *in vivo*. *Journal of Dairy Science* 100: 8881-8894. https://doi.org/10.3168/jds.2017-12675
28. Rayas, A.A., Estrada, J.G., Lawrence, F. and Castelán, O.A. (2012). Nutritional value of forage Species from the Central Highlands Region of Mexico at different stages of maturity. *Ciência Rural* 42:705-712. https://www.researchgate.net/publication/255703806
29. Sallam, S.M.A., Nasser, M.E.A., El-Waziry, A.M., Bueno, I.C.S. and Abdalla, A.L. (2007). Use of an in vitro Rumen Gas Production Technique to Evaluate Some Ruminant Feedstuffs. *Journal of Applied Sciences Research* 3:34-41. https://www.researchgate.net/publication/264868737
30. Velarde-Guillén, J., López-González, F., Estrada-Flores, J.G., Rayas-Amor, A.A., Heredia-Nava, D., Vicente, F., Martínez-Fernández, A. and Arriaga-Jordán, C.M. (2017). Productive, economic and environmental effects of optimised feeding strategies in small-scale dairy farms in the Highlands of Mexico. *Journal of Agriculture and Environment for International Development* 111: 225-243. doi: https://doi.org/10.12895/jaeid.20171.606.
31. Plata-Reyes, D.A., Morales-Almaraz, E.A., Martínez-García, C.G., Flores-Calvete, G., López-González, F., Próspero-Bernal, F., Valdez-Ruiz, C.L., Zamora-Juárez, Y.G. and Arriaga-Jordán, C.M. (2018). Milk production and fatty acid profile of dairy cows grazing four Grass species pastures during the rainy season in small-scale dairy systems in the highlands of Mexico. *Tropical Animal Health and Production* 50:1797–1805. DOI:10.1007/s11250-018-1621-8.
32. Ellis, J.L., Kebreab, E., Odongo, N.E., McBride, B.W., Okine, E.K., and France, J. (2007). Prediction of methane production from dairy and beef cattle. *Journal of Dairy Science* 90:3456–3467.
33. Benaouda, M., González-Ronquillo, M., Appuhamy, J.A.D.R.N., Kebreab, E., Molina, L.T., Herrera-Camacho, J., Ku-Vera, J.C., Ángeles-Hernández, J.C., Castelán-Ortega, O.A. (2020). Development of mathematical models to predict enteric methane emisión. *Livestock Science* 241. https://doi.org/10.1016/j.livsci.2020.104177
34. Hristov, A.N., Oh, J., Firkins, L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P.S., Adesogan, T., Yang, W., Lee, C., Gerber, P.J., Henderson, B., Tricarico, J.M. (2013). Special Topics. Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. *Journal of Animal Science* 91: 5045–5069. doi: https://doi.org/10.2527/jas2013-6583.
35. Eckard, R.J., Grainger, C., and de Klein, C.A.M. (2010). Options for the abatement of methane and nitrous oxide from ruminant production: A review. *Livestock Science* 130: 47-56.
36. Cameron, L., Chagunda, M.G.G., Roberts, D.J. and Lee, M.A. (2018). A comparison of milk yields and methane production from three contrasting high-yielding dairy cattle feeding regimes: Cut-and-carry, partial grazing and total mixed ration. *Grass and Forage Science* 73: 789–797. DOI: 10.1111/gfs.12353