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Effect of different growth stages of rapeseed (*brassica rapa* L.) on nutrient intake and digestibility, nitrogen balance, and rumen fermentation kinetics in sheep diets

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ABSTRACT

The objective of the present study was to determine the dietary effect of different growth stages of rapeseed (*Brassica rapa* L.) on nutrient intake and digestibility, nitrogen balance, and rumen fermentation kinetics in sheep. Four dietary treatments were utilised. A basal control diet based on alfalfa hay, oat hay, soybean meal and corn grain. Then alfalfa hay was replaced with 300 g/kg DM of rapeseed forage harvested at three different growth stages: Vegetative, Flowering and Pod. *In vitro* gas production was determined using three rumen cannulated Suffolk sheep in a completely randomised design, and nutrients intake and digestibility of each diet were determined using four Suffolk sheep in a 4×4 Latin square design with 21 d periods consisting of 14 d for diet adaptation and 7 d for sample collection. Feed intake and excretion of faeces and urine were recorded. Dry matter intake was higher for control and Pod compared to Vegetative and Flowering. The digestibility of dry matter, organic matter, neutral detergent fibre and acid detergent fibre were similar among treatments. Nitrogen intake was higher for control and Pod and lower for Vegetative and Flowering. *In vitro* gas production was similar among treatments ($P > .05$). *In vitro* gas yield at 24 h was higher ($P < .05$) for control than the rest of the treatments. Overall, inclusion of 300 g/kg DM of rapeseed forage harvested at pod stage as a substitute for alfalfa hay is an alternative source of protein without affecting nutrient intake and digestibility.

HIGHLIGHTS

- Effect of different growth stages of rapeseed on nutrient intake and digestibility was determined
- Nutrient digestibility was similar between growth stages of rapeseed
- *In vitro* gas production was similar between growth stages of rapeseed

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Introduction

Sustainable animal diets (StAnD) is a concept based on three dimensions: planet, people and profit, which among other objectives, promotes the use of local feed resources (Makkar and Ankers 2014). In this regard, the use of *Brassica* species in temperate grazing systems (Seguel et al. 2020) can be an alternative forage source (Barry 2013). These crops can be from *Brasicca rapa* and *Brassica napus* which are characterised by their high contents of crude protein (around 30% CP), high contents of metabolisable energy (around 12 MJ/kg DM) and low contents of neutral detergent fibre (around 17% NDF) compared to more common forage crops for ruminant feed such as perennial ryegrass pastures (Kaur et al. 2009). In the case of *Brassicas,* its chemical composition changes depending on its growth stage, year season and cultivars (Cartea et al. 2008). For example, rapeseed follows dry matter (DM) and nitrogen (N) uptake patterns

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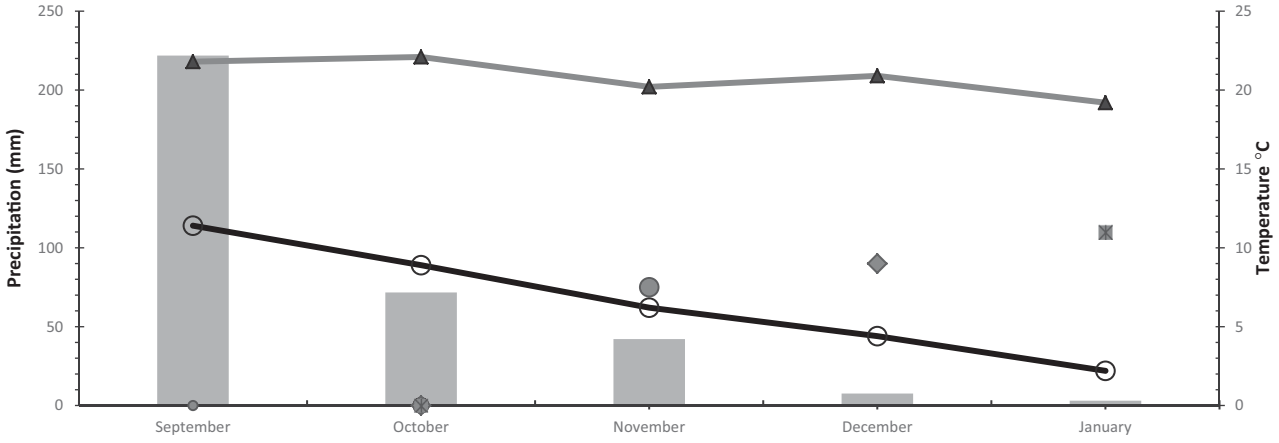

Figure 1. Temperature (min ○, max ▲) and precipitation (grey bars) in San Pedro Tlanixco, Mexico, during the growing season of forage rapeseed collected at Vegetative (●), Flowering (◆) or Pod (■) growth stages.

similar to wheat, where the higher contents of DM and N accumulation will be observed between the beginning of stem elongation and/or branching and the end of flowering (Koenig et al. 2011). Until now, feeding rapeseed forage at different stages of digestibility or maturity has not been fully described in ruminant nutrient partitioning.

It is important to note that *Brassica* species contain compounds known to cause erythrocyte oxidant damage, including methaemoglobin formation in ruminants (Smith 1978). This occurs as a result of sulphur-containing compounds in plant tissues and their conversion to toxic products by rumen microorganisms. Also, *Brassica* species by-products such as canola meal (7-25 μmol/g) and rapeseed meal (7–210 μmol/g) contain glucosinolates (Barry 2013; Cartea et al. 2008). Glucosinolates have been shown to reduce feed intake and amino acid digestibility in animals (Tripathi and Mishra 2007).

In temperate regions, feeding cows with forage *Brassica* species has been shown to be an effective alternative to the use of perennial grass pastures such as rye grass maintaining milk yield and without impairing milk components (Seguel et al. 2020). In Australia, feeding forage *Brassica* to sheep was reported (Williams et al. 2016) to be an alternative to the use of perennial ryegrass, as it promoted increases in milk yield, and increased contents of milk fat and milk protein. Also, from an environmental point of view, feeding sheep with forage rape could be an alternative to reduce methane emissions (Sun et al. 2016).

Until now, no reports have been published on the use of *Brassica* species and their different vegetative stages, more specifically forage rapeseed in central Mexico, where this forage material is readily available and can be a low-cost and viable alternative for sheep feeding. In temperate regions of Mexico, farmers traditionally produce *Brassica* species (Vieyra-Odilon and Vibrans 2001), which are harvested at the beginning of flowering for collection of tender leaves, tender stems and flowers (Bye and Linares 2000). Moreover, effects on rumen fermentation kinetics and nutrient utilisation from sheep fed with rapeseed at different growth stages remains unclear. Thus, the objective of the present study was to determine the effect of different growth stages of rapeseed (*Brassica rapa* L.) as hay on nutrient intake, nutrient digestibility, nitrogen balance and rumen fermentation kinetics in sheep diets. This objective was achieved by replacing part of the forage in the diets with forage rapeseed at different growth stages. We hypothesised that early vegetative stages of forage rapeseed will result in higher nutrient intake and higher digestibility compared to late stages of growth.

Materials and methods

Rapeseed crop and sampling conditions

Forage samples of *Brassica rapa* L. (rapeseed) were collected from San Pedro Tlanixco, municipality of Tenango del Valle, State of Mexico. The location is 2840 m above sea level, between the coordinates 19°04′ north latitude and 99°32′ west longitude. According to the Köppen climate classification, the climate is humid temperate, Cb (w2) (w) (i′) (g), with summer rains and little thermal oscillation (Figure 1) (CONAGUA 2015).

The soil from the study site had a clay soil texture with 62% sand, 10% silt and 28% clay. The land had a slope of 2 to 6%. The dominant rocks were of volcanic

Table 1. Chemical composition (g/kg DM) of the ingredients used in the diets with inclusion of rapeseed (*Brassica rapa)* at different growing stages.

	Rapeseed Vegetative	Rapeseed Flower	Rapeseed Pod	Alfalfa hay	Oat Hay	Soybean meal	Corn grain
Dry matter	881	897	924	858	897	886	884
Organic matter	924	927	887	875	927	929	750
Crude protein	192	159	119	216	38	431	84
Ether extract	88	50	232	27	43	17	43
aNDF	369	267	452	357	534	147	96
ADF	201	182	289	213	313	59	21
ADL	165	140	192	63	92	70	7
NFC	275	451	84	275	312	324	527
Metabolisable energy, Mcal	2.15	1.91	2.39	2.40	1.43	3.20	3.40

aNDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: acid detergent lignin; ME: metabolizable energy; Non fibre carbohydrates (NFC) = [100-(NDF + CP + Ash + ether extract)].

and clastic type. Soils were pelic vertisol type and haplic phaeozem (WRB 2006), and they were characterised by being very compact and clayish; similarly, they exhibited wide and deep cracks in the epoch of drought and showed a layer of tepetate (interstratified volcanic banks) between 10 and 50 cm of depth. It had a pH of 5.8, 23.8 $cmol^{+}$/kg of capacity of cationic interchange, 0.31% of total nitrogen, 7.56% of organic matter and 0.8 dS/m of electrical conductivity (Vaca García et al. 2014).

The seeds used for rapeseed were harvested in May 2013 from maize and potato fields, where the plant grew as native forage (Vieyra-Odilon and Vibrans 2001). Rapeseed seeds were planted in October 2013. Sowing was done manually using 6 kg/ha of rapeseed seed, for a dry matter (DM) yield of 8132 kg/ha, with a DM concentration in the forage of 320 g/kg and an estimated germination percentage between 70 and 80%. A total area of 530 m^2 was divided into three plots previously fertilised with sheep manure. For the study, the vegetative stage (Vegetative) refers to the first flower buds and tender stems harvested at 70 d after cropping. The flower (Flowering) was harvested with 80% plants flowering at 90 d, and pods (Pod) were harvested when the plants contained 80% of their pods reaching their final size at 110 d. Samples from Vegetative, Flowering and Pod stages (100 kg of each stage) were collected from the whole plot and dried for 5 days at room temperature. Then, samples were ground in a hammer mill (5 mm Ø) for later use.

Experimental procedures

In vivo *trial: animals and diets*

Animal care and procedures were carried out according to the guidelines of the Animal Care and Use Committee of Universidad Autónoma del Estado de México (project code UAEM 4335/2017). Four Suffolk sheep were used, with a mean age of 10 months and 47.8 ± 4 kg of body weight. Animals were arranged in a 4 × 4 Latin square design with 21 d periods consisting of 14 d for diet adaptation and 7 d for sample collection. Animals were weighed at the beginning of the study and at the end of each experimental period.

Individual ingredients (Table 1) and four dietary treatments were evaluated (Table 2). A basal or control diet was based on alfalfa hay, oat hay, soybean meal, and corn grain (Table 2). Then, alfalfa hay was replaced with 300 g/kg of dried forage rapeseed at three different growth stages and fed as Vegetative, Flowering and Pod. Control diet was formulated to contain 150 g/kg CP and 2.7 Mcal ME/kg DM according to the nutritional requirements of growing sheep (NRC 2007) (Table 2). Animals were housed in individual metabolic cages (1.20 × 0.80 m), fed individually twice a day (08h00 and 15h00), with free access to water.

Feed intake, refusals and water intake was measured daily but only data from the last 7 days of each period were accounted for statistical analysis. During the last 7 d of each period, samples of faeces and urine were collected daily and 10% of the total samples for faeces and urine were frozen at −20 °C for further analysis.

In vitro *trial: gas production and fermentation kinetics*

Animal care and procedures for extraction of rumen inoculum were approved by the Ethics Committee for Animal Experimentation (UAEM 4335/2017). Care and management of animals was according to UAEMex Policy for Animal protection of animals used for experimental and scientific purposes.

Three fistulated sheep (40 ± 3 kg of body weight) were used as donors of rumen fluid and fed with the same experimental basal diet (Table 2) with free access to water. Ruminal contents (500 mL of liquid and semisolid phases) were placed in thermos flasks and immediately transferred to the laboratory for incubation. To determine the kinetics of ruminal fermentation, three incubation runs (96 h) were carried out, in

Table 2. Contents of ingredients and chemical composition (g/kg DM) of dietary treatments from sheep fed with 300 g/kg DM of forage rapeseed at vegetative, flowering or pod growth stages.

	Control	Vegetative	Flowering	Pod
Rapeseed	0	300	300	300
Alfalfa hay	300	0	0	0
Oat hay	200	200	200	200
Soybean meal	100	100	100	100
Corn grain	380	380	380	380
Vitamin and minerals premix[a]	20	20	20	20
Chemical composition				
Dry matter	881	893	888	901
Organic matter	844	861	860	849
Crude protein	156	130	140	118
Ether extract	35	42	53	96
Non fibre carbohydrates	384	430	378	320
Neutral detergent fibre	268	238	269	294
Acid detergent fibre	142	131	137	163
Acid detergent lignin	48	70	78	86
Metabolisable energy, Mcal/kg	2.7	2.4	2.5	2.5

[a]Containing in 1.0 kg DM the following: 25 mg of antioxidant, 4.5 g of calcium carbonate, 6 g of salt, 30 g of ionophore, 50 g of zinc oxide, 6 g of sodium bicarbonate, 6 g of copper sulphate, 20 g of ferrous sulphate, 125 g of sodium sulphate, 18000 IU of vitamin E, 3 000 000 UI of vitamin A, 3 750 000 IU of vitamin D, 140 g of potassium chloride, 0.500 g of E.D.D. I ethylene-dynamine, 0.090 g of cobalt carbonate, 500 mg of magnesium oxide, 36 g of manganese oxide, and 0.090 g of selenium.
Non fibre carbohydrates = [100-(NDF + CP + Ash + Ether extract)].

a water bath at 39°, according to Theodorou et al. (1994).

After rumen fluid extraction, samples were mixed and filtered through a triple layer of gauze, and homogenised with CO_2 for five minutes. A total of 16 bottles per run were incubated, with three bottles per treatment, two bottles without substrate (considered as blanks of inoculum), and two bottles with known substrate (barley hay) (considered as standard for correction purposes). Incubation data where differences between the mean gas production of standard obtained and the standard value were higher than 10% were not included in the analysis. Then 800 g/DM of each diet mixture were incubated in glass bottles of 125 mL, and incubation runs were performed. In each incubation run, the bottles were filled under anaerobic conditions with 10 mL of rumen inoculum and 90 mL of a buffer solution (Theodorou et al. 1994). In 1 L, this solution consisted of 238 mL/L of buffer solution (14 g $NaHCO_3$ and 1.5 g $(NH_4)HCO_3$ per L), 238 mL/L of a macro mineral solution (5.7 g Na_2HPO_4, 6.2 g KH_2PO_4 and 0.6 g $MgSO_4.7H2O$ per L), 474 mL/L of distilled water, 0.1 mL/L of micro minerals (13.2 g $CuCl_22H_2O$, 10.0 g $MnCl_24H_2O$, 1.0 g $CoCl_26H_2O$, 8.0 g $FeCl_26H_2O$ and made up to 100 mL with H_2O) and 50 mL/L of a reduction solution (47.5 mL distilled water, 2 mL of 1 N NaOH and 313 mg HCl-cysteine), and resazurin (phenoxazine dye).

Bottles were filled with the incubation solution under a CO_2 stream, sealed and incubated for 96 h in a water bath at 39 °C. The gas volume was recorded at 3, 6, 9, 12, 24, 36, 48, 72 and 96 h of incubation in three different runs for incubations. The pressure produced in each bottle was measured with a HD8804 manometer provided with a TP804 pressure gauge (Delta OHM, Caselle di Selvazzano, Italy). Readings corrected for atmospheric pressure were converted to volume (mL) using a pre-established linear regression developed in our laboratory [mL gas = (2.7384x) − 0.0243, n = 45, R^2 = 0.994, where x = psi hour] and expressed as a unit of incubated dry matter.

Calculations

The accumulated gas volume of each of the samples (diets) was adjusted to the model proposed by France et al. (1993):

$$Y = A\ [1 - \exp\ (-B\ (t\ - T)\ -\ C\ (\sqrt{}\ t - A/T))].$$

where: "Y" is the cumulative gas production (mL), "t" is the incubation time (hours), A is the asymptote curve (total gas produced, mL), B (h^{-1}) and C ($h^{-1/2}$) are the gas production constants, T is the time of delay (hours) for microbial colonisation in order to begin fermentation.

After *in vitro* incubation periods, these bottles were opened, and the whole incubation content was filtered and dried (48 h, 60 °C) to measure the dry matter disappearance (DMD96h), and gas yield production at 24 h (GY24) was determined. The volume of gas (mL gas/g DM) produced after 24 h of incubation was estimated by dividing the amount of DMD (g): Gas production (GY24) = mL gas 24 h/g DMD (González Ronquillo et al. 1998).

The partitioning factor at 96 h of incubation (PF96; a measure of fermentation efficiency) was estimated as the ratio of *in vitro* DMD (DMD, mg) to the volume (mL) of GP at 96 h (i.e. DMD/total gas production (GP96)) according to Blümmel et al. (1997).

Analytical procedures

All chemical analysis were performed in triplicate. Feed orts, faecal samples, individual feedstuffs and diets were dried in a forced air oven (60 °C, 48 h) an analysed for dry matter (DM), then ground in a mill (Wiley Mill, 2 mm Ø Arthur H. Thomas Philadelphia, PA) to determine organic matter (OM; 942.05) AOAC (1990). Total nitrogen (N; 954.01) was determined by the Kjeldahl method (AOAC 1990) multiplied by 6.25 for the crude protein content, acid detergent fibre (ADF) and lignin were determined according to Van

Table 3. Intake (g/LW$^{0.75}$/day) and digestibility coefficient from sheep fed with 300 g/kg DM of forage rapeseed at vegetative, flowering or pod growth stages.

	Control	Vegetative	Flowering	Pod	SEM	*P-value*
Live weight, kg	47.8	48.0	47.7	48.0	0.38	.093
Metabolic live weight, LW$^{0.75}$	18.17	18.22	18.15	18.25	0.86	.090
Intake, g/kg LW$^{0.75}$						
Water, ml/kg LW$^{0.75}$	21.5	20.8	16.6	19.2	2.67	.579
Dry matter	90.4^{a}	71.1^{b}	70.4^{b}	83.7^{ab}	1.76	.005
Organic matter	82.5^{a}	65.0^{b}	64.2^{b}	75.5^{ab}	3.80	.001
Ether extract	8.3^{b}	5.7^{c}	6.5^{c}	11.3^{a}	0.39	.001
Non-structural carbohydrates	22.8^{a}	21.7^{ab}	17.7^{bc}	16.3^{c}	1.08	.003
Neutral detergent fibre	40.0^{a}	29.3^{b}	31.1^{b}	39.1^{a}	1.83	.005
Acid detergent fibre	23.5^{a}	18.0^{b}	18.3^{b}	23.9^{a}	1.08	.001
Metabolisable energy, Mcal/d	3.3^{a}	2.6^{b}	2.5^{b}	3.0^{a}	0.10	.001
Digestibility coefficient						
Dry matter	0.76	0.71	0.77	0.77	0.031	.413
Organic matter	0.77	0.72	0.78	0.78	0.031	.446
Neutral detergent fibre	0.69	0.63	0.69	0.73	0.043	.444
Acid detergent fibre	0.67	0.62	0.67	0.71	0.045	.336

^{a,b,c}Means with different literal in the same row are statistically different ($p < .05$); SEM: standard error of the mean.

Soest et al. (1991). Neutral detergent fibre (aNDF) content was determined as described by Van Soest et al. (1991) using thermostable alpha-amylase without sodium sulphite and corrected for residual ash (Mertens 2002) using an Ankom 200 Fibre Analyser (Ankom Technology, New York). Ingredients and total mixed rations components were analysed for ether extract (method 920.39, AOAC 1997), while non-structural carbohydrates (NSC) were calculated as follows: NSC = [100-(NDF + CP + Ash + Fat)]. Chemical composition of diets is shown in Table 2.

Faecal and urine samples were used to determine nitrogen excretion. Faecal and urine samples were subjected to nitrogen (N; 991.20) determination (AOAC 1990). Nutrient digestibility coefficients (Wiseman 2018) were determined as: Digestibility = (nutrient intake – nutrient excreted)/(nutrient intake)

Statistical analysis

For the *in vivo* experiment, a 4 × 4 Latin square design (SAS, Statistical Analysis System Institute 1999) was used with the following model:

$$Yijk = \mu + A_i + Txj + Pk + \varepsilon ij$$

where Yij = is each observation, μ is the general mean; Ai is the effect due to the animal, Txi (i = 4) is the treatment effect; Pk is the effect due to the period and εij is experimental error. A $P < 0.05$ value was considered as a significant difference between treatments. The Tukey test was used when significant differences were observed between treatments $p < .05$.

A completely randomised design was used to measure chemical composition, *in vitro* gas production parameters and *in vitro* fermentation. Diet ($n = 4$) was considered as factor and the incubation series ($n = 3$) as a block. For total gas production (96 h) and DMD, the experimental unit was the average of the three bottles per treatment incubated for 96 h within the same run.

$$Yij = \mu + Txi + \varepsilon ij$$

where Yij = is each observation of treatments it; μ is the general mean; Tx (i = 4) is the treatment effect; and εij is the experimental error. Tukey test was used when significant differences were observed between treatments $p < .05$.

Results

Intake, digestibility, and nitrogen balance

Nutrient intake and digestibility are shown in Table 3. Live metabolic weight were marginal ($P = .09$) for Vegetative and Pod, compared with the rest of the treatments. Water intake was similar between treatments ($P > .05$). Intakes of dry matter, organic matter and neutral detergent fibre were higher $P < .05$) for control and Pod, compared with Flowering and Vegetative. Ether extract intake was greater ($P < .05$) in Pod than the rest of the treatments. Intake values of non-structural carbohydrates, neutral detergent fibre, acid detergent fibre and metabolisable energy were higher ($P < .05$) in control and Pod. Digestibility coefficients of dry matter, organic matter, acid detergent fibre and neutral detergent fibre were similar among treatments. Nitrogen intake was higher ($P < .001$) for control. Nitrogen excretion in faeces and urine and N balance was similar between treatments (Table 4).

In vitro *gas production and fermentation kinetics*

With regard to *in vitro* fermentation parameters for dietary treatments (Table 5), fermentation rates B (h^{-1})

Table 4. Nitrogen retention (g N/day, %) from sheep fed with 300 g/kg DM of forage rapeseed at vegetative, flowering or pod growth stages.

	Control	Vegetative	Flowering	Pod	SEM	*P*-value
Nitrogen balance						
N intake, g/d	33.0[a]	23.9[b]	25.6[b]	25.4[b]	0.978	.001
Faecal N excretion						
g/d	7.0	6.9	5.8	5.9	0.54	.360
% of N intake	21.4	28.8	22.5	23.6	2.979	.346
Urinary N excretion						
g/d	13.4[a]	8.1[ab]	7.6[b]	10.9[ab]	1.46	.090
% of N intake	40.7	33.9	29.8	42.5	5.01	.294
N retention						
g/d	12.7	8.9	12.2	8.6	1.94	.147
% of N intake	37.9	37.2	47.6	33.8	5.19	.317

[a,b,c]Means with different literal in the same row are statistically different ($p < .05$); SEM: standard error of the mean.

Table 5. *In vitro* rumen gas kinetics (mL gas/ g DM) and fermentation profile from sheep fed with 300 g/kg DM of forage rapeseed at vegetative, flowering or pod growth stages.

Parameters	Control	Vegetative	Flowering	Pod	SEM	*P-value*
A	319	283	336	337	30.0	.579
B	0.02	0.01	0.09	0.01	0.03	.444
C	−0.01	0.026	0.003	0.01	0.01	.169
Lag time	0.96	0.19	0.31	0.20	0.41	.531
Gas production, mL gas/g DM						
6 h	30.6	28.7	33.0	33.6	5.18	.900
12 h	84.4	76.9	84.2	83.7	6.58	.840
24 h	132	112	135	129	7.16	.193
48 h	212	163	206	197	12.2	.089
72 h	262	206	263	248	17.5	.147
96 h	281	233	284	281	19.3	.262
GY24h	297[a]	218[b]	209[b]	227[b]	14.2	.009
PF96	633	456	439	494	46.1	.064
DMD96	0.445[c]	0.519[b]	0.648[a]	0.570[ab]	0.0226	.001

[a,b,c]Means with different literal in the same row are statistically different ($p < .05$); SEM: standard error of the mean.
A = total gas production (ml gas/g incubated DM); B = fermentation rate (h^{-1}); C = fermentation rate ($h^{-1/2}$); Lag time = time in which fermentation starts; GY24 = gas yield at 24 h (mL gas/g DMD); PF96 = partition factor (ml gas/g DMD 96 h); DMD96h = DM disappearance at 96 h (mg/100 mg).

and C ($h^{-1/2}$), and lag time were similar between treatments. Dry matter disappearance at 96 hours was lower ($p < .05$) for control compared with Flowering and Pod. Gas production at 48 hours (GP48) tended ($P = .08$) to be higher for control and Flowering, whereas gas yield at 24 h (GY24h) was higher ($p < .05$) for control (297 ml gas/g DMD) than the rest of the treatments (218 ± 9.0 ml gas/g DMD).

Discussion

With regard to dietary treatments chemical composition, Pod had the lowest crude protein content compared to Vegetative and Flowering, and this agrees with previous research (Liu et al. 2016; Espinoza-Canales et al. 2017) showing that rapeseed seeds and husk increases fibre content and decreases the concentration of crude protein and amino acids. Also, ether extract contents in Pods were higher, which was expected as with flowering, the formation and grain filling begins. It is important to consider that the decline in nutritional quality is linked to advance growth where the leaf:stems ratio decreases and lignin and cell wall in stems increases in *Brassica napus* (Kaur et al. 2011).

Crude protein contents were similar in Vegetative and Flowering; however, in Pod, CP contents were slightly lower. Although, control diet was theoretically formulated to fulfil the nutritional requirements of growing sheep with an average daily gain of 250 g/d (NRC, 2007), replacing alfalfa hay with 300 g/kg of forage rapeseed in the treatment diets was not enough to balance CP in Pod treatment, with less protein (38gPC/kg DM) than the control diet. It is known that CP varies depending on the growth stage of forage rapeseed (Koenig et al. 2011). The CP content of rapeseed at their different vegetative stages decreases across maturity stage, compared with alfalfa or other leguminous plants this could range from 160 to 190 g/kg DM (NRC 2007), except for rapeseed pod with CP ranged in 119 g/kg DM, similar to other forages as ryegrass or barley hay at maturity stage (NRC 2007). Therefore, our data demonstrated that rapeseed forage could be used to supply adequate CP to meet maintenance or growth requirements in sheep diets (NRC 2007).

In vivo *trial: intake, digestibility, and nitrogen balance*

Previous studies have reported and observed reduced growth in sheep fed with high contents of glucosinolates in sheep fed with mustard oil (Tripathi et al. 2004), rapeseed and safflower meals (Thomas et al. 1984). The content of glucosinolates varies according to the development of the plant parts, such as roots, flowers, leaves, pods (Suzuki et al. 2006; Mohn et al. 2007, Barry 2013). In early vegetative states, more glucosinolates are retained in the reproductive organs, including seeds, flowers and pods, which are expected to have the highest concentrations of these defense compounds (Brown et al. 2003). Newkirk et al. (2003) found that in addition to the toxic effect of glucosinolates, these are found in greater quantity in wild plants of this genus and their bitter taste causes a reduction in feed intake. This was also noted in the present study; however, DM intake was higher than that reported by Bastida Gacia et al. (2011), who used other alternative forage sources such as field pea hay or barley hay. This may be partly explained by the fact that in the present study, the diets contained more

than 12% CP, which allowed a higher ingestion of N and a greater synthesis of microbial protein production, and thereby improving nutrient utilisation and intake.

In this study, rapeseed forage increased concentrations of NDF, ADF and lignin, and this was related to advanced growth stages from vegetative to pod. This is because NDF digestibility is a function of the potentially digestible fraction of dietary fibre and its rate of digestion and rate of passage (Oba and Allen 1999). The increase in DMI in Pod enhanced NDF digestibility, because forages with high NDF digestibility have shorter rumen retention times, allowing greater DMI at the expense of NDF digestibility. Although ADF and NDF are indicators of fibre contents in forages, they do not directly indicate how digestible the dietary fibre is. In general, an increased NDF digestibility will result in higher digestible energy and forage intake. In this regard, it has been suggested that a one-unit increase in NDF digestibility will be associated with a 0.17 kg increase in DMI in dairy cows (Oba and Allen 1999).

Our data showed that compared to control Vegetative, Flowering and Pod decreased N intake while Pod had the highest content of microbial protein. According to Nousiainen et al. (2004), the increase of dietary protein is the most important nutritional factor influencing milk urea N efficiency and this could be used as a diagnostic of protein feeding in ruminants and used to predict urinary N excretion. In this study, based on N balance, rapeseed at Pod and Flowering stage could be used to supply dietary crude protein, which later is used for microbial protein production and secreted as milk protein or growing performance.

In vitro *gas production and fermentation kinetics*

In vitro digestibility parameters were similar to Kaur et al. (2011) who reported that the age of brassicas such as *Brassica napus*, is a factor that affects rumen degradation due to lignification. Similarly, Chapoutot and Sauvant (1997), who determined the nutritional value of raw and extruded pea-rapeseed blends in ruminants, reported decreases in organic matter digestibility with extrusion. Sun et al. (2012) obtained lower values for apparent total tract nutrient's digestibility, when sheep were fed fresh brassicas (*Brassica* spp.) compared to perennial ryegrass. They reported that there was a functional alteration of the rumen, which led to high concentrations of propionate, affecting acetate:propionate ratio, and thereby reducing available hydrogen for the methane production. In the present study, compared to the control group, the different vegetative stages of forage rapeseed yielded lower gas production (GY24h) and therefore, one promising research line is to analyse at which growth stage of rapeseed will be best for reducing methane production without disturbing overall animal performance.

Glucosinolates are present in all brassicas and are responsible for the decrease in feed intake (Barry 2013) which coincides with the vegetative and flowering diets in the present study. Vincent et al. (1990) did not find an effect on dry matter intake in brassica-fed sheep, but observed an increase in thyroid weight, which is a symptom of iodine deficiency caused by glucosinolates (Tripathi and Mishra 2007). These effects could be due to a longer exposure time in the consumption of these compounds and the amount ingested, especially at vegetative and flowering stages, thus, results from the present study should be taken with caution and it is suggested to further investigate their long-term effects.

Conclusions

Based on the digestibility and estimated nutrient supply, the inclusion (up to 300 g/kg DM) of forage rapeseed at the pod stage was a suitable replacement for alfalfa hay in sheep diets. Therefore, inclusion of rapeseed pods in sheep diets can be a viable alternative for small-scale farmers and thereby optimise the use of fodder resources since rapeseed pods have no negative effects on nutrient intake and digestibility. Data from this study will be useful for farmers looking for the precise growth stage of forage rapeseed for sheep feeding.

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Ethical approval

The protocol used in this experiment approved by the Animal Care and Use Committee of Universidad Autónoma del Estado de México (project code UAEM 4335/2017).

Disclosure statement

The authors declare there are no conflicts of interest.

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