Scientific Paper

Substitution of *Medicago sativa* L. by *Ricinus communis* L. leaf, in pregnant ewes

Luis Antonio Ramírez Navarro[1] https://orcid.org/0000-0003-4872-6460, Alejandra del Viento Camacho[2] https://orcid.org/0000-0001-9564- 7971, José Manuel Zorrilla Ríos[1] https://orcid.org/0000-0003-0323-3320 and José Manuel Palma García[2] https://orcid.org/0000-0001-6061-546X

[1]*Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara.* [2]*Centro Universitario de Investigación de Desarrollo Agropecuario (CUIDA), Universidad de Colima. E-mail: palma@ucol.mx*

Abstract

Objective: To evaluate the effect of the substitution of *Medicago sativa* L. by *Ricinus communis* L. leaf blade on the feeding and health of pregnant ewes.

Materials and Methods: A behavior test was conducted with 10 primiparous ewes in the last third of pregnancy, with initial live weigh of 45 ± 3 kg. They were homogeneously distributed, individually, and two groups of five animals each were formed. The animals were randomly allocated. The integral rations included 20 % of *M. sativa* or *R. communis.* The total dry matter intake, crude protein and metabolizable energy intake, duration of pregnancy, weight of the litter at birth, individual weight of the lamb at birth and clinical signs of intoxication, abortions or deaths, were evaluated.

Results: The dry matter, protein and energy intake was higher in *M. sativa* with regards to *R. communis* until a week before parturition. There after intake of both treatments was similar. There were no statistical differences in the litter weight (5,53 *vs* 7,80 kg), weight per lamb (3,57 *vs* 3,25 kg) and duration of pregnancy (146 *vs* 147), for *M. sativa* and *R. communis*, respectively. The ewes that consumed *R. communis* did not show clinical signs of intoxication and neither abortions nor deaths occurred.

Conclusions: The *R. communis* leaf meal is a nutritional alternative in the last third of pregnancy for sheep, without detriment to the productive indicators or the generation of intoxication signs, abortion or deaths. Besides, it does not affect lamb viability..

Keywords: trees, intake, leaf meal, leaf protein

Introduction

During the last third of ewe pregnancy, fetal growth negatively influences the dry matter intake capacity of the ewes by limiting the space available for the rumen (Faverdin *et al.*, 2011). In this context, the ruminal degradability of the ration, related to the particle size, as well as the fiber quantity and quality, among others, are determinant characteristics in the feeding strategy of the ewe. For such reason, the *Medicago sativa* L. meal is used as high-quality component in the diet of ewes in this productive stage.

Recently, Ramírez *et al.* (2017) proposed as forage of high nutritional value the leaf blade of *Ricinus communis* L., based on its high crude protein content (27,6 to 32,0 %) and its low fiber concentration (25,2 % of NDF and 22,5 % of ADF, respectively), with lower values than the report for *M. sativa* (44,2 and 35,2 %, respectively). Lara *et al.* (2016) indicated that it shows higher levels than 2,7 Mcal ME/kg DM and *in situ* ruminal degradability of DM at 48 h of 93,2 ± 4,06 %.

Regarding health, there is contradictory evidence about the ingestion of *R. communis* leaf blades. Some authors refer the absence of intoxication, death, or both, in growing sheep (Lara *et al.* 2016) and pregnant ewes (del-Viento and Palma, 2016), different from what was found experimentally (Bezerra and Brito, 1995; Döbereiner *et al.*, 1981) or by accidental ingestion (Aslani *et al.*, 2007; Bianchi *et al.*, 2018)..

The objective of this study was to evaluate the effect of the substitution of *M. sativa* by *R. communis* leaf blade on the feeding and health of ewes in the last third of pregnancy.

Materials and Methods

Locality. The trail was conducted in the Borregueros Ramírez farm, located in the Ahualulco de Mercado municipality, Jalisco, georeferenced at 103°58'W, 20°41'N and 1,321 m.a.s.l. of altitude (Google, 2015). The climate of the locality is subhumid semi-warm, with rainfall from June to September, and temperature range from 14 to 22 ºC. The mean annual rainfall is 900 mm (IIEG, 2018).

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Treatments and experimental design. A complete randomized design was used, with two treatments:

- *M. sativa* (control) in: a daily integral ration, with the inclusion of 20 % of *M. sativa* hay meal.
- Leaf blades of *R. communis* (experimental) in: a daily integral ration, which included 20 % of leaf blade meal of *R. communis* in substitution of *M. sativa* hay meal.

Experimental animals. Ten primiparous ewes Katahdin breed, 45 ± 3 kg live weight and 11 months of age were used. They were individually confined in 1,5-m^2 pens, with dirt floor and distributed in two groups of five animals each, according to experimental treatment. At the beginning of the trial, the ewes were in the last third of pregnancy. As prophylaxis strategy of the herd they received selenium (sodium selenite), vitamin A and 8-path bacterin[1]. The animals received as prophylactic dose 0,2 mg, 500 mg per kg/LW and 2,5 mL intramuscular, respectively. The pregnancy diagnosis was carried out 45 days post-mating through a Metvet ultrasound device.

Management of the used forages. The leaf blades of *R. communis* (LBRc) were harvested manually from wild plants of the region, which were between 1,5 and 2,0 m high. The blades were dehydrated under shade during 10 days, and were then ground (Azteca mill) to a particle size of 3,0 mm. The resulting meal was used to substitute in the diet the *M. sativa* hay meal, of equal particle size.

Experimental procedure. The essay lasted 52 days and was divided into four periods: the first one from day 1 to 15, the second from 16 to 30, the third from 31 to 45, and the last one since day 45 until parturition.

The rations, designed to cover the nutritional needs of the animals (Martín and Palma, 2017), were offered twice per day (8:00 a.m. and 4:00 p.m.) *ad libitum*, without previous adaptation stage. For the ration offer a refuse of 5 % daily was estimated. The estimation of feed intake was obtained through the difference between that offer and the refuse, daily. The water was available *ad libitum* throughout the experimental period.

The inclusion percentages and the chemical composition of the diets are shown in table 1. The variables total dry matter intake (kg/animal), DM intake per metabolic weight (g DM/$LW^{0,75}$), protein intake (g) and metabolizable energy (Mcal), were evaluated.

In the productive environment, the duration of pregnancy (days) and the number of lambs per ewe at parturition, the weight of the litter at birth (kg) and the individual weight of the lamb (kg) were controlled. Regarding health, signs of intoxication (Tokarnia *et al.*, 1975), abortions or deaths, were recorded.

Statistical analysis. A variance analysis was carried out for the data processing. The statistical package Statistix version 8 (Statistix, 2003), was used, with the weight of the animals at the beginning of the experiment as covariable. For the mean comparison Tukey's test ($p < 0,05$) was used. Meanwhile, for the presence of intoxication signs, abortions, or deaths of the animals, non-parametric test (X^2) was applied. The trend in dry matter and crude protein intake, based on the metabolic weight with regards to time, was subject to linear regression analysis per animal and treatment, according to the following formula:

$$Y = a + bx, \text{ where:}$$

Y = response variable
a = value of the ordinate at the origin, where the regression line is intercepted with the Y axis.
b = mean variation of the response variable, when x increases one unit (slope of the straight line)

With the parameters of the regression straight line "a" and "b" per animal and treatment, a variance analysis was carried out.

Results and Discussion

The *R. communis* leaf blade intake by pregnant ewes during 52 days (equivalent to the last third of pregnancy) did not generate clinical signs of intoxication, abortions or deaths in the ewes nor in their lambs.

Table 2 shows the DM intake analysis, divided into four periods. In all of them, the treatment with *M. sativa* proved to have higher intake with regards to *R. communis*, except in the last period, of six days before parturition.

Regarding the intake dynamics, the performance was variable, with trend to decrease as the ewes were closer to parturition (figure 1). In the initial stage, in the treatment with *R. communis*, a less marked intake was recorded, perhaps due to the process of adaptation of animals to this diet.

[1] Medicine that contains complete cells and toxoid of inactivated cultures of *Clostridium chauvoei, Clostridium septicum, Clostridium novyi, Clostridium sordelli* and *Clostridium perfringes* C and D, as well as inactivated complete cells of *Pasteurella multocida*, types A and D and *Mannheimia* (*Pasteurella*) *haemolytica* A-1.

Table 1. Inclusion percentages and chemical composition of the experimental diets.

Ingredient	Leaf blade of *R. communis*, %	*M. sativa*, %
R. communis leaf blade meal	20,00	0,00
M. sativa hay meal	0,00	20,00
Saccharum officinarum L. (sugarcane) top	38,44	38,00
Zea mays L. (ground corn)	31,00	30,31
Molasses	5,40	4,00
Minerals	1,00	1,00
Tallow	3,00	4,60
Common salt	1,00	1,00
Urea	0,16	1,09
Proximal chemical analysis, %		
Dry matter	87,65	87,44
Ethereal extract	6,04	5,49
Ash	9,86	10,25
Nitrogen-free extract	48,90	54,53
Crude protein	13,13	13,20
Total digestible nutrients	78,32	79,04
Neutral detergent fiber	42,73	31,08
Acid detergent fiber	31,51	21,99
Metabolizable energy (Mcal)[¥]	2,82	2,85

[¥]Estimated values

Table 2. Dry matter intake per ewe during the last third of pregnancy, divided into periods.

Period		LW, kg	kg DM/ewe/day	Live weight, %	GDM/kg LW
First (1-15 days)	*M. sativa*	45,2	1,484	3,31	86
	LBRc	46,6	1,213	2,62	68
	SE ±	0,6	0,050	0,12	3,0
	P - value	0,024	0,001	0,001	0,001
Second (16-30 days)	*M. sativa*	46,5	1,372	2,98	78
	LBRc	47,4	1,312	2,80	73
	SE±	0,7	0,028	0,08	2,0
	P - value	0,143	0,033	0,028	0,031
Third (31-45 days)	*M. sativa*	47,3	1,225	2,62	68
	LBRc	50,2	1,152	2,36	62
	SE ±	0,8	0,034	0,09	2,0
	P - value	0,001	0,035	0,005	0,006
Fourth (46-52 days)	*M. sativa*	47,3	1,189	2,53	60
	LBRc	50,2	1,166	2,27	61
	SE ±	0,7	0,062	0,30	8,0
	P - value	0,001	0,708	0,091	0,155

LBRc: leaf blade of *R. communis*, LW: live weight/ewe, SE: standard error, P: probability

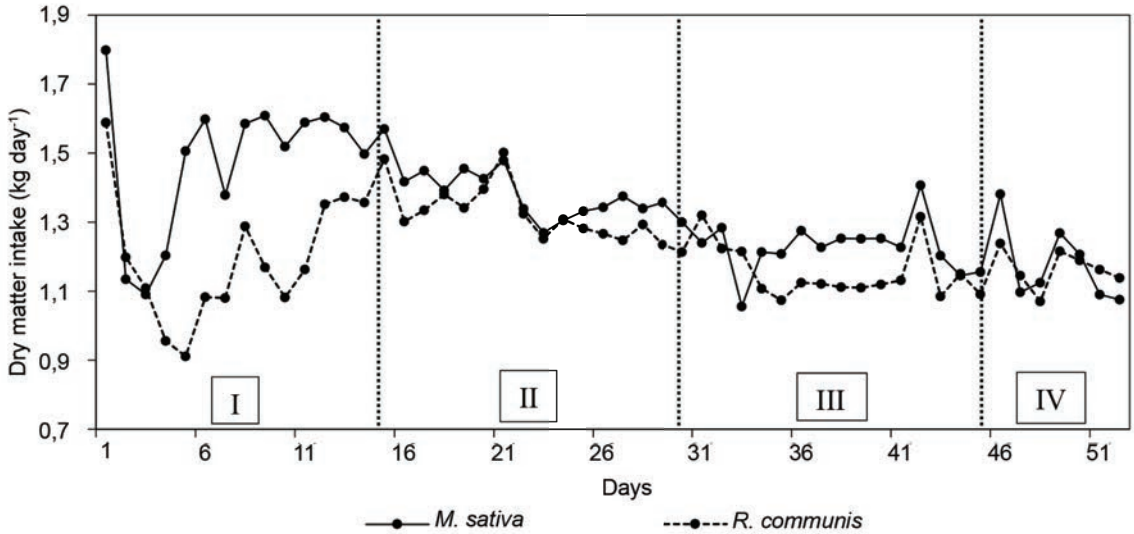

Figure 1. Dry matter intake dynamics per periods, with the inclusion of leaf blade of *R. communis* or *M. sativa* in rations for ewes in the last third of pregnancy.
I- First period (1-15 days), II- Second period (16-30 days), Third period (31-45 days) and IV- Fourth period (46-52 days)

This behavior of the animals is controversial, because, generally, *R. communis* is considered as a toxic plant, although it is still necessary to determine whether the toxicity is due to the seed, leaf or both. Most of the studies report toxicity due to seed ingestion (Fonseca and Soto-Blanco, 2014; Mondal *et al*., 2019), propitiated by the conditions of forage scarcity and feed deficit, particularly in the dry season (Albuquerque *et al*., 2014). In this situation, intoxication generates digestive signs associated to the consumption of a toxalbumin called ricin (Alburquerque *et al*., 2014), which is present in the seeds.

Other authors, such as Döbereiner *et al.* (1981) and Bezerra and Brito (1995), referred intoxication and death due to consumption of *R. communis* leaves in experimental sheep. Meanwhile, Brito *et al.* (2019) reported spontaneous intoxication because of access to pruning; although it is indicated that this phenomenon is unusual.

The clinical manifestations are of nervous type, related to the consumption of an alkaloid, known as ricinine, which is abundant in the leaf (Tokarnia *et al*., 1975). According to Sánchez *et al.* (2016); Riet-Correa *et al.* (2017) and Brito *et al.* (2019), these clinical manifestations can be persistent, from two to 16 h. Then, the animals can recover or die, depending on the quantity of ingested leaves. These authors indicated that the experimental intoxication appears with doses of 10 to 20 g of consumed leaves/ kg live weight.

Aslani *et al.* (2007) and Bianchi *et al.* (2018) emphasized in the intake leaves and seeds together in the face of the appearance of spontaneous intoxication signs, and even death, in sheep and goats, respectively. In addition to ricinin, the leaf of *R. communis* is known to contain several flavoniodes (Mamoucha *et al*., 2016).

Table 3 shows the performance of protein and energy intake, which turned out to be higher in the treatments with *M. sativa* hay meal ($p < 0,05$) with regards to that of *R. communis* meal, except at the end of pregnancy. In this stage there were no statistical differences, which is associated to the total DM intake of both rations.

Table 4 shows the values of the linear regression per treatment for the dry matter and crude protein intake per kg $LW^{0,75}$. In both cases, there was higher initial intake of dry matter ($p = 0,060$) and protein ($p = 0,048$) in favor of the treatment with alfalfa of the ordinate at the origin (a), without difference in the change rate (b) between treatments during the last third of pregnancy, which comprised 52 days.

In this study, the use of the leaf blade was incorporated in integral rations aimed at sheep, because it is considered an option of energy-protein contribution (Palma, 2018). Because its incorporation in integral diets for confined, grazing animals, or in its use as additive did not cause intoxication signs, abortions or deaths in ewes during the last third of pregnancy and it did not affect their lambs, these results coincide with the report by del-Viento and

Table 3. Crude protein and metabolizable energy intake by ewes during the last third of pregnancy, divided into periods.

Period	*M. sativa*	LBRc	SE ±	P - value
Crude protein intake, g				
First (1 to 15 days)	196	159	7,0	0,001
Second (16 to 30 days)	181	172	4,0	0,017
Third (31 to 45 days)	162	151	5,0	0,022
Fourth (46 to 52 days)	157	153	8,0	0,636
Metabolizable energy intake, Mcal				
First (1 to 15 days)	4,23	3,42	0,14	0,001
Second (16 to 30 days)	3,91	3,70	0,08	0,009
Third (31 to 45 days)	3,49	3,25	0,10	0,014
Fourth (46 to 52 days)	3,39	3,29	0,35	0,565

LBRc: leaf blade of *R. communis*, SE: Standard error of the mean, P: Probability

Table 4. Variance analysis for the linear regression parameters of dry matter and crude protein intake in the last third of pregnancy.

Treatment	Dry matter intake/kg $LW^{0,75}$		Crude protein intake/kg $LW^{0,75}$	
	Ordinate at the origin	Change rate	Ordinate at the origin	Change rate
M. sativa	88,45	-0,49	11,69	-0,07
R. communis	72,27	-0,19	9,47	-0,02
SE ±	7,41	0,24	0,95	0,03
P - value	0,060	0,244	0,048	0,214

SE: standard error, LW: live weight

Palma (2016) for pregnant ewes and growing male sheep (Lara *et al*., 2016; Zamora *et al*., 2018).

Based on the experience by Lara *et al.* (2016), who offered *R. communis* with progressive adaptation to its consumption, an intake of up to 527 g of dry matter at 31 days was recorded. This meant 37 % of the total intake, without intoxication. For such reason the incorporation of 20 % of *R. communis* in integral rations aimed at pregnant animals was proposed.

This approach allowed to develop a proposal that ensures not intoxicating the animals, because the ingestion of the leaf does not reach the toxic limit (Döbereiner *et al*., 1981), and exceeds the proposal made for cattle by Tokarnia *et al*. (1975), who suggest 5 g DM/kg of live weight. A factor to be considered in the innocuousness of the *R. communis* blade used in this work was its dehydration and storage during one month before the beginning of the experiment. Tokarnia *et al.* (1975) made reference to this process and indicated that dehydration and storage of the leaves reduce toxicity.

The limited voluntary intake of the ration, corresponding to the treatment with *R. communis* blade mainly in the first 15 days of exposure, can be associated to the presence of the potentially toxic components of this forage. Nevertheless, a progressive habit was later shown regarding the digestion and metabolism of the animals, because the intake was matched in the next evaluation periods. However, the intake was always lower ($p < 0,05$) than the treatment with *M. sativa* hay meal and, only in the period following parturition both treatments could be matched.

In general, decrease was shown in the dry matter intake of the ewes as the animals approached parturition. This could have been associated with the fetal exponential growth in this stage of pregnancy (from 70 to 80 %), which reduces the space in the abdominal cavity and, in turn, decreases the ingestion capacity of the animal (Herrera *et al*., 2010; Cal-Pereyra *et al*., 2011; Vicente-Pérez *et al*., 2015).

This became evident in the treatment with *R. communis* blade, in which the number of lambs per parturition was higher ($p < 0,05$) (2,40 *vs* 1,60 for blade of *R. communis* and *M. sativa*, respectively), with higher weight of the litter at birth (table 5). For such reason, it is stated that the initial intake of dry matter as well as protein, in the treatment with

R. communis blade, could have acted as catalyst of ruminal degradation, causing better utilization of the integral ration by the animal in a period of high nutritional demand. This phenomenon can be explained by the high degradation rate of the *R. communis* blade, with value of 95 %, according to the report by Palma *et al.* (2015), Ramírez *et al.* (2017) and Palma (2018).

There is little literature in which forage diets with 60 % of forage are evaluated in this productive stage; thus, it is necessary to continue the studies on this topic, where the impact of these diets on the intake capacity of dry matter and its main components.

Table 5 shows the productive indicators of the ewes at the moment of parturition with regards to the pregnancy days, litter weight at birth and individual lamb weight, variables in which there were no statistical differences between treatments. The number of lambs/parturition was higher in the treatment with *R. communis.* The total fetus weight was higher for the *R. communis* in the last stage of pregnancy, in which the fetus development limits the capacity of rumen filling. This possibly explains the intake difference between the treatments.

The weight of the lambs in both treatments (> 3,0 kg) was in the range reported by Vicente-Pérez *et al.* (2015) and Lucio *et al.* (2018). Regarding the days of pregnancy, the substitution of *M. sativa* by *R. communis* blades in the ration of the ewes did not alter the duration of this stage.

The approach developed in this work not only shows the energy-protein contribution of *R. communis*, but also proves that its incorporation in ruminant feeding allows to generate options of forage associations, which favors the development of feeding options in critical periods and under adverse conditions. This is the case of the association of *R. communis* with the *Saccharum officinarum* L. top.

This work is a contribution to the development of agroforestry systems with the use of *R. communis*, topic that was referred by Sánchez *et al.* (2016). Specifically, it aims at the design of silvopastoral systems in the tropical area, where it is combined with grasses such as sugarcane (*S. officinarum).* In this case, it is associated with sugarcane top, as reported by Palma *et al*. (2019) when referring to the integration of agricultural and agroindustrial waste in the generation of resilient animal husbandry systems.

Conclusion

The *R. communis* leaf meal could substitute *M. sativa* in the feeding program for pregnant ewes in their last third of their pregnancy without promoting health hazards nor jeopardizing lamb viability.

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Authors' contribution

- Luis Antonio Ramírez-Navarro. Data collection, data analysis and interpretation, paper writing and revision.
- Alejandra del Viento-Camacho. Data analysis and interpretation, paper writing and revision.
- José Manuel Zorrilla-Ríos. Design, data analysis and interpretation, paper writing and revision.
- José Manuel Palma-García. Conception, design, data analysis and interpretation, paper writing and revision.

Conflict of interests

The authors declare that there is no conflict of interests among them.

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Table 5. Productive indicators of the ewes at the moment of parturition.

Indicator	Treatment		SE ±	P - value
	M. sativa	*R. communis*		
Days of pregnancy	145,80	147,40	1,09	0,182
Litter weight at birth	5,53	7,80	1,34	0,129
Average weight/lamb	3,57	3,25	0,40	0,450
Number of lambs/parturition	1,60	2,40	0,35	0,049

SE: Standard error, P: probability

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