

***In vivo* Digestibility of Honey-Locust Seeds (*Gleditsia triacanthos* L.) Administered as a Dietary Complement to Ruminants and their Effects on Weight Gain**

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Abstract: Information on nutritional complementation for cattle is necessary for the implementation of feed management systems capable to maintain or improve yield production. In order to determine the effectiveness of the use of *Gleditsia* seeds as feed complement a group of 6 castrated Merino sheep were employed to measure the *in vivo* seed digestibility from 4 varieties: Milaud (MIL), Total (TOT), Super-M (SUM) and Voié-Ferré-Merchandise (VFM). Daily records of the amount of distributed, ingested, digested and rejected seeds as well as urine and feces were obtained for the calculation of the dry and organic matter balance, digested seeds balance, nitrogen balance and the coefficient of digestive utilization. The mean daily ingestion value per animal varied between 57-80 g of the dry matter of seeds/live weight^{0.75}; the digestibility values varied considerably between individuals (33-99% of the DM; average 76%). The animals with a higher ingestion rate had a lesser digestion (CDU = 57.3%) than others with lesser ingestion (CDU = 67%). The nutrient content and digestion parameters of honeylocust seeds varied among sheep and varieties, being MIL and TOT higher in protein content than SUM and VFM. The animal weight gain was higher during the consumption period in the variety VFM (128 g day⁻¹) compared to the SUM variety (96 g day⁻¹). The seeds of honeylocust should be considered as different entities than other ingredients of the traditional diets when characterizing nutrient quality.

Key words: *Gleditsia triacanthos*, seed digestibility, feed complementation, Merino sheep

INTRODUCTION

Great loss episodes in the production of cattle in summer pastures during severe winter periods at the end of the XIX century motivated the necessity to complement the diet of the ruminants. The complement consisted of native grass. Since that time knowledge on animal food requirements and the methods and materials to carry out the complementation have been improving (Foroughbakhch *et al.*, 2001; Ramirez, 2003). The reasons to complement feeding include: increase yield gain, proper forage use to compensate the deficiencies of nutrients to maintain the animal condition and to keep suitable production when a forage shortage occurs (Knox, 1976). Complementation with protein compounds improves the ruminal fermentation due to the increase in feed consumption and allows the animals to take sufficient forage to satisfy their energy requirements to maintain development, since the capacity of rumen can limit the ruminal fermentation (Nelson, 1971; Boling, 1971).

The nutritive value of the different components of trees and shrubs has been studied with ovine livestock and goat cattle (Chadhokar, 1982; Cooper and Owen, 1985; Smith and Van, 1987; Cooper *et al.*, 1988; Rodriguez, 1992). Thick grounding of dry seeds and their incorporation to feed concentrates enhances the quantity and quality of the animal diet. However, this practice may be combined with others to preserve the resources and increase the economical output (Hentgen, 1985; Ramirez, 1995; Webb, 1988).

Honeylocust is considered as a nutritional complement which has a high cold tolerance, up to five weeks in the end winter and earlier spring growth, higher forage yield (leaf, pods and seeds), tolerance to long periods of drought (during summer) and good nutritional quality (protein, fiber, sugar and vitamins) as mentioned by Genin (1990) Rodriguez-Soto (1992).

Owing to the importance of honeylocust trees as feed for livestock, certain metabolic and chemical data, its *in vivo* digestibility and effect on animal weight gain were

obtained after administration of 4 varieties of honeylocust seeds to a group of Merino sheeps held in digestibility cages.

MATERIALS AND METHODS

To carry out the experiments on *in vivo* seed digestibility a batch of six male castrated Arles Merino sheeps were selected. Two year-old specimens of 41.5 kg weight in average and with a good corporal state (2.25-2.50) were selected. Each specimen was considered as an experimental unit.

Sheeps were maintained in individual cages with dimensions of 1.2×2.0 m (0.026 m³ kg⁻¹ live weight) designed specially for accurate measure of ingestion, urine and fecal excretion for specimens fed with compound feed rations. A period of adaptation of 17 days was necessary for the feeding with the seed complemented diets. The varieties of honeylocust trees employed were Milaud (MIL), Total (TOT), Super-M (SUM) and Voie-Ferré-Marchandise (VFM).

The feed ration was supplied once a day at 9:00 a.m. At the onset of the period the diet contained about 500 g of dry seeds from *G. triacanthos*, 500 g hay, 285 g alfalfa concentrate and 30 g of mineral and vitamins concentrate. The adaptation to the new diet was surprisingly fast since at 4:00 p.m. most of the animals had finished the ingestion of the diet ration.

During the whole *in vivo* digestibility measuring phase, the drinking water and the supplementary mineral salts were distributed voluntarily (*ad libitum*).

For each variety the experiment had a duration of seven days.

The data recorded in the digestibility cages for each measure period were:

- Daily distributed DM, rejected DM, litter quantity and the urine volume per animal. The ingestion was determined by the difference between the total supply and the quantity rejected by each animal.
- Determination of the DM balance (incubator 103°C, 72 h) and organic matter content (furnace 575 °C, 9 h) from the distributed feeds, food rejection and littering.
- Digested seed balance determined from the difference between the total weight of the distributed seeds and the weight of the rejected seeds plus the weight of the intact seeds found in littering:

$$W_{\text{digested seeds}} = W_{\text{distributed seeds}} - (W_{\text{rejected seeds}} + W_{\text{intact seeds in littering}})$$
- Fixed Nitrogen balance: the total nitrogen ingested, excreted in feces and urine was calculated according to Kjeldahl (AOAC, 1997):
 Fixed Nitrogen = TN ingested-TN in feces-TN in urine

- Digestible protein and the Coefficient of Digestive Utilization (CDU):

$$CDU_{\text{Nitrogen}} = \frac{\left(\begin{matrix} \text{Total ingested} \\ \text{nitrogen matter} \end{matrix} \right) - \left(\begin{matrix} \text{Nitrogen matter} \\ \text{in feces} \end{matrix} \right)}{\text{Total ingested nitrogen matter}}$$

$$CDU_{\text{Seeds}} = \frac{\left(\begin{matrix} \text{DM of} \\ \text{ingested seeds} \end{matrix} \right) - \left(\begin{matrix} \text{DM of seeds} \\ \text{in feces} \end{matrix} \right)}{\left(\begin{matrix} \text{DM of ingested} \\ \text{seeds} \end{matrix} \right) - \left(\begin{matrix} \text{DM of intact} \\ \text{seeds in feces} \end{matrix} \right)}$$

RESULTS AND DISCUSSION

Balance analysis of ingested and digested seeds: During the *in vivo* digestibility evaluation periods the daily distributed seeds and the daily seed intake and daily seed rejection per animal and variety were monitored. Quantification of intact and swollen seeds in feces allowed the calculation of the digested seeds by the difference with the ingested seeds (Table 1).

According to the results on Table 1, the seeds of the MIL and VFM varieties, with a rejection daily average of 35.5g (7.9%) and 74,3 g (17.6%), respectively were more preferred and showed a greater digestibility in comparison to the varieties TOT with 126,8 (22.7%) and SUM with 182,2 g (27.4%). The smoother surface of the seed coat and softer properties of the varieties MIL and VFM conferred higher palatability and preference by cattle. Nevertheless, the results indicate that despite the high intake of the seeds of the variety VFM, their digestion in rumen was lower than other varieties.

In general terms, 76% of the ingested seeds were digested by the animals at variety level. The MIL variety seeds (88%) and the TOT variety (81%) showed a higher digestibility than the varieties SUM (71%) and VFM (62%).

At animal level, there is an enormous variation between the quantities of digested seeds. This proportion varied from 33 to 99%. For some animals which in average consumed 88% of the seeds it was found that 59% of those ingested seeds were rejected during rumination while the rest of the animals practically did not reject seeds. For the whole animal group we have observed that 4% of the seeds of the MIL, VFM and SUM varieties were rejected. This percentage is more elevated for the TOT variety (11%).

The quantity of ingested seeds appearing intact in feces was found in inverse relationship with the quantity of digested seeds. Those animals having a low percentage of digested seeds excreted a high percentage of intact seeds in feces (14 to 67%). Taking into account that those

Table 1: Daily dry matter quantity (g) of ingested, digested, rejected and excreted seeds by sheeps in the digestibility cages

Seed variety	Dist.(1)	Rejected (R) (2)	Ingested (I) (3)	Excreted (4)	Digested (D) (5)	%D/I (6)	%Intact in feces (7)	%R/D (8)
MIL	447	35.5±5.2	411.5±18.8	50.8±8.3	360.7±20.8	88.2±5.5	11.8±3.9	8.6±1.6
TOT	558	126.8±36.5	431.2±53.6	92.2±4.1	339.0±40.1	81.3±7.5	18.5±5.7	36.2±5.4
VFM	422	74.3±8.8	347.7±41.2	137.2±20.7	210.5±36.7	62.2±8.8	37.8±8.8	31.8±3.1
SUM	665	182.2±58.3	483.0±88.4	157.6±32.5	325.2±52.6	71.0±10.2	29.0±3.6	32.4±1.9

(1) Quantity (g) of distributed seeds; (2) Seeds (g) not digested and rejected in rumination; (3) Seeds (g) ingested; (4) Seeds (g) excreted in feces; (5) digested seeds (g); (6) % of seeds digested/ingested; (7) % of ingested seeds intact in feces; (8) % of seeds rejected during rumination/digested seeds

Table 2: CDU values of the dry ingested seeds per varieties and animals in the digestibility cages

	CDU-Ingested seeds
Variety level	MIL 62.9
	TOT 60.5
	VFM 60.2
	SUM 56.0
Animal level	1 61.8 ab*
	2 59.8 ab
	3 57.3 ab
	4 67.0 a
	5 55.0 b
	6 61.3 ab

* Different letters in columns indicate significant differences (p<0.05)

Table 3: Quantity of not ingested seeds per Kg of DM of feces (n = 23)

Variety	No. of intact seeds	No. of swollen seeds	Total No. of not digested seeds	Weight of not digested seeds	TNM of seeds (g) kg ⁻¹ feces
MIL	96.67±21.6	295.0±22.6	391.7±31.2	67.3±35.1	20.2±15.1
%	25	75			
TOT	298.3±131.8	283.3±19.6	581.7±183.4	118.4±81.6	36.0±11.3
%	51	49			
VFM	870.0±241.7	118.3±52.3	988.3±242.1	176.8±51.0	52.9±25.2
%	88	12			
SUM	702.0±191.2	126.0±39.1	825.0±59.3	189.1±49.3	56.6±24.1
%	85	15			

animals were the best seed consumers, they showed a percentage of digested seeds (63 to 56%) far inferior to the average value (76%) with 37 to 44% of ingested seeds found in the feces. On the contrary, animals that showed the best percentage of digested seeds (superior to 96%) showed a very low percentage of intact seeds in feces (<2%).

Considering the coefficients of digestive utilization, the *Gleditsia* seeds seem to be more digestible than other parts of the plant (the entire pods for example). The CDU values for the digested seeds vary 56 to 63% between varieties and 55 to 67% between animals (Table 2).

The results derived from the determination of intact seeds in feces are shown in Table 3.

The amount of seeds in feces varied noticeable between varieties and animals. We have found from 10 to 1720 seeds per Kg of dry feces being VFM, SUM and TOT the varieties with higher quantity of intact seeds (88, 85 and 51%, respectively). On the contrary, the MIL variety showed contrasting results with a percentage of swollen seeds (75%) against 25% of intact seeds. The results about the seed balance have indicated the noticeable clear preference of the animals for the consumption of seeds with soft tegument (varieties MIL and TOT). These results are presented in perfect

concordance with the digestibility values where the varieties MIL and TOT represented the highest values compared to those showed by the varieties VFM and SUM.

In vivo nitrogen balance: The nitrogen content value in the food is expressed by the total nitrogen content matter, which is estimation by excess of the accurate nitrogen value of the food. In effect, this estimation is a measure of the quantity of food that is absorbed in the digestive tract. In this sense, the quantity of TNM in seeds varies between 105g kg⁻¹ of DM for the VFM variety and 130 g kg⁻¹ of DM for the SUM variety which is considered to be elevated.

The nitrogen balance (g of TNM retained each day) for the animals in the digestibility cage was calculated taking into account the amount of TNM retained and excreted (g TNM in feces + g TNM in urine per day) on the following equation:

$$\text{Fixed nitrogen} = \text{Ingested TNM} - \left(\begin{matrix} \text{TNM} \\ \text{in feces} \end{matrix} \right) + \left(\begin{matrix} \text{TNM} \\ \text{in urine} \end{matrix} \right)$$

Table 4: Determination of the nitrogen balance (g of TNM digested per day) and CDU of the animals subjected to a diet based on seeds from *G. triacanthos*

Variety	TNM ingested (g day ⁻¹)	TNM in feces (g day ⁻¹)	TNM in urine (g day ⁻¹)	Fixed nitrogen	CDU
MIL	216.9±17.6	115.2±25.0	39.9±9.4	61.8±26.2	27.2±7.1
TOT	190.4±43.2	107.3±30.1	55.0±11.6	39.6±19.1	20.0±7.5
VFM	190.2±33.9	115.6±26.3	42.6±6.7	33.7±18.1	21.7±12.1
SUM	205.5±41.7	133.7±36.6	39.3±8.5	32.5±11.7	19.8±1.9

Table 5: Daily average weight gain (g) of the merino sheeps during the different periods of measuring.

Animal number	Measuring period						Average gain/day
	Adaptation	MIL	TOT	VFM	SUM	Ad-lib.	
1	123	143	371	171	129	260	199.5±97.8
2	59	242	28	271	186	460	207.7±157.2
3	150	186	100	414	57	820	287.8±246.1
4	135	186	128	256	120	281	184.3±69.6
5	144	196	20	243	271	245	186.5±93.1
6	85	71	271	257	14	480	191.3±122.3
Average/day/period	116.0±36.2	170.7±58.1	153.0±140.1	268.6±79.5	129.5±91.4	419.3±218.1	

Taking into account the inability to distinguish the quantity of nitrogen coming from the seeds, hay and alfalfa we implemented the following hypothesis: considering the ingested quantity of ingested seeds, hay and alfalfa as well as the quantity of the TNM of hay and alfalfa we have been able to set up that about 2/3 of the TNM of feces and urine come from seeds while only 1/3 come from alfalfa and hay. On the basis of this hypothesis we could also calculate the CDU of the seeds (Table 4).

In general terms, the fermentable feeds show low protein values. However, we have noticed that the higher the fermentation in rumen the higher the digestible nitrogen value in almost all animals (personal observation). Thus, it might be affirmed that most of the animals took advantage of the TNM in a proper way during the whole measure period.

A comparison can be made between the animal which best consumed the seeds with animals with a low seed acceptance and we could set up that an animal which ingested many seeds digests a low fraction while the animal which ingested few seeds digests most of them. This statement goes in accordance with observations reported about the foliar biomass by other authors describing the leaves and stems as a forage of ligneous origin of excellent quality (Baertsche *et al.*, 1986; Fierro *et al.*, 1989; Tolla *et al.*, 2001).

Live weight gain in animals subjected to a diet with seeds from *G. Triacanthos*: The results on the corporal state and the animal weight were determined at the onset and the end of every measure period.

The variations in weight were determined in comparisons with the metabolic weights of the animals during different periods of measuring.

The results on the daily average gain in weight are shown in the Table 5.

The results obtained indicate that almost all the animals developed a gain in live weight after the ingestion of *Gletsidia* seeds. The gain in weight was higher during the periods of consumption in the variety VFM (268 g day⁻¹) and the voluntary intake (419 g day⁻¹).

The weight gain was minimum (129 g day⁻¹) during the consumption period of the SUM variety. It was found that the coefficients of digestive utilization of seeds from this variety (56%) was inferior to the MIL (63%) and TOT (61%) varieties.

At the end of the measure periods it was registered an increase in weight of 8.0 to 14.5 g/animal. These quantities corresponded to a daily weight gain of 184 to 287 g day⁻¹ for these animals.

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