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Milk production analysis after supplementing *Calotropis gigantea* leaf silage to dairy cows

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Abstract

This study aimed at evaluating the milk production after supplementing *Calotropis gigantea* (Giant milkweed, GM) silage as a new functional feed additive for ruminants. Cows refused to eat GM plants so, we processed it into silage before feeding. After ensiling, six ruminally cannulated dairy cows were assigned to two treatment groups (GM silage supplementation treatment and control without GM silage supplementation) in a cross over design. Repeated sampling of milk and rumen fluid was carried out on the last days of the third and fourth week after treatment. Ensiling GM increased the crude proteins, neutral detergent fiber and acid detergent fiber while ash was unchanged. There was no dry matter intake (DMI) when supplementing GM forage to the cows, DMI and milk yield returned to normal conditions but feed efficiency, milk protein, milk fat and lactose slightly increased when supplementing GM silage. Rumen protozoa genera such as *Entodinium*, *Ophryoscolex, Eudiplodinium, Dasytricha* and *Isotricha* were maintained. A dose effect study remained to be carried out to identify an effective dose that could bring significant enhancement of the animal production after supplementing GM silage. This study revealed that the silage form of GM can be a new source of proteins for dairy cows and an appropriate dose could potentially induce some improvement of the milk production and composition. Therefore, the plant will not continue to be perceived as an invasive weed but as a new forage to be integrated into the cow's diet.

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INTRODUCTION

Calotropis gigantea (Giant milkweed, GM) is mostly perceived as an invasive weed, although it is constituted of an important biomass that can be valorized in an integrated agricultural system, especially livestock. We recently demonstrated *in-vitro* that GM leaves can be used as a functional feed additive to inhibit the detrimental rumen protozoa and the greenhouse gas production such as ammonia and methane[1,2]. Therefore, GM shrub could be potentially associated to animal husbandry in an agroforestry system to ensure a climate-smart sustainable animal production process.

In addition, the plant can also be valorized in the textile industry because the fruits contain high-quality fiber. GM fibers comprise up to 80%–90% hollow structures, similar to kapok (*Ceiba pentandra*) fibers[3]. Thus, they exhibited outstanding hydrophilic or oleophilic properties[4], fewer natural curls in the longitudinal direction, lighter weight[5] resulting in a smoother and softer surface. The leaves of the shrub are also used for fractionating the raw milk and making traditional cheese with positive effects on *E. coli*, yeast and mold load[6]. Therefore, GM is considered a multipurpose plant that can grow on dry land, without fertilizer constraints. The plant is native to South-East and continental Asia, introduced in the pacific islands, Australia, Central and Northern South America and Africa[7−8].

After feeding the plant in its hay form to the dairy cows, they unfortunately refused to eat and we assume that the plant may also have some toxic compounds that could have compromised the palatability and digestibility by the animals. To ensure the applicability of the *in-vitro* results at the farm level, bio-transforming GM leaves into silage before supplementing to ruminants is a prerequisite which could modulate the plant secondary metabolite composition and concentration. Ensiling has been seen as a biological way to ensure feed quality, animal metabolism, health and welfare[9]. Wang et al.[10] demonstrated how the ensiling process improved the feed palatability and avoided animal feed undergoing aerobic degradation. Therefore, we hypothesized that compared to GM hay, supplementing the silage form to dairy cows could improve the animal dry matter intake, milk yield and composition.

MATERIALS AND METHODS

Animal feeding trials supplemented with GM silage: Experimental design

Animals were cared for in compliance with the Institute of Animal Science (IAS), Chinese Academy of Agricultural Sciences (CAAS) guidelines (No. IAS20180115). The experiment was carried out in a crossover design with two groups of three cows submitted to two treatments lasting for two 28-d periods and a washout of 14 d in between the two periods[11]. The first treatment consisting of the inclusion of 0.3 Kg (dry matter 50%) of GM silage in 19.7 Kg of the total mixed ration (TMR) and the second treatment was the control (20 kg only TMR). An adaptation period of 10 d was used prior to the start of the treatments. Repeated sampling was carried out twice, on day 21 and day 28 of each period for milk and rumen fluid. Six lactating Holstein dairy cows (589 ± 37 kg of BW and 290 ± 4.5 DIM, 20 kg of feed intake per each morning and afternoon meal), permanent ruminally cannulated were paired based on milk production and randomly assigned to the two treatments within the two groups. The total mixed ration (TMR) diet was formulated using the NRC model[12] (Table 1) to supply sufficient energy and N for a 608.31 ± 95.18 kg cow producing 15 ± 5 kg/d of milk in their late lactation phase, containing 4.93% fat, 3.49% protein and 5.05% glucose. Nutritional analysis of TMR, GM silage and GM hay followed the previously described procedures[13,14].

Feed, milk and rumen fluid sampling and analysis

TMR was prepared on the daily basis using a feed mixer (Data Ranger, American Calan, Inc., Northwood, NH, USA) and offered to cows twice per day evenly at 07:00 am and 04:00 pm. The quantities of feed refused per cow were recorded daily. An amount of 1 kg of the prepared TMR was collected and stored at −20 °C for DM and basic nutritional analysis. Cows were milked daily at 06:30 am and 5:30 pm and the weights were recorded. Based on each milk yield proportion, the final sample was obtained after mixing the morning and afternoon milking. Milk samples were preserved with 2-bromo-2-nitropropane-1,3-diol (800 Broad Spectrum Microtabs II), and stored at 4 °C until laboratory analysis for fat, true protein, lactose, total solids (TS) and non-fat solids (NFS) using an infrared spectroscopy analyzer (MilkoScan 605, Foss Electric, Hillerod, Denmark). Rumen fluid was sampled before the morning feeding and four aliquots were mixed with 4% formalin to fix the protozoa cells for further microscopic observations and counting following the procedures described by Ayemele et al.[2].

Table 1. Ingredients and chemical composition of the basal diet.

Ingredients	% of TMR DM		
Soybean meal	10.42		
Cotton seed meal	5.03		
Canola seed meal	2.18		
DDGS[a]	5.45		
Feeding corn meal[b]	1.15		
Steam-flack corn	23.99		
Wheat bran	0.00		
Limestone	0.91		
Salt	0.55		
Magnesium oxide	0.36		
Dicalcium phosphate	0.42		
Fat powder	1.15		
Sodium bicarbonate	0.97		
Supplement[c]	0.67		
Corn silage	28.77		
Alfalfa hay	17.99		
Total	100		
Chemical analysis (% DM)		Ensiled GM	Hay GM
CP[d]	15.31^{A}	15.43^{A}	13.89^{B}
NDF[e]	27.69	40.65	39.81
ADF[f]	18.57	25.73	24.46
Ash	7.88	13.38	13.98
Organic matter	92.12	/	/
Ether extract	2.1	/	/
NE_L[g] (Mcal/kg DM)	1.69	/	/

[a] Distillers dried grains with solubles. [b] Flour made with corn. [c] Contained (per kg of DM) a minimum of 250,000 IU, of vitamin A; 65,000 IU, of vitamin D; 2,100 IU, of vitamin E; 400 mg of Fe; 540 mg of Cu; 2,100 mg of Zn; 560 mg of Mn; 15 mg of Se; 35 mg of I; and 68 mg of Co. [d] Crude protein. [e] Neutral detergent fiber. [f] Acid detergent fiber. [g] Net energy of lactation. Superscripts A and B refer to significance differance within the line.

Statistical analysis

Data were analyzed statistically by using PROC MIXED of SAS (version 9.1; SAS institute inc., Cary, NC) following the model:

Yijkl = μ + ti + pj + ck + εijk + al + atil + eijkl

Where: Yijkl was the cow's performance.

It was the effect of treatment, pj was the effect of period (j = 1, 2, considered random), ck was the effect of the kth cow (k = 1, 2, …, 6, considered random) and eijkl was the main plot error modeled as an interaction of cow with period and treatment. The fixed effect of amount was confounded with the passage of time over the 4 weeks of each period in which this factor was applied.

RESULTS AND DISCUSSIONS

Milk yield and composition

DMI, milk yield and milk composition after 28 d supplementing GM silage to dairy cows are presented in Table 2. Supplementing GM hay drastically decreased DMI while ensiled GM maintained the DMI to the normal situation. Milk yield and composition were maintained but a numerical increasing of the milk yield/DMI ratio, protein, fat, lactose and TS were observed when supplementing GM silage. NFS rather numerically decreased. Ensiled GM improved the palatability for dairy cows and could therefore constitute a new source of protein for cattle, with its value similar to the one of typical dairy cows' ration. Based on the *in-vitro* effective dose[1], a corresponding level of GM silage dose was used at the farm level but, this was found insignificant to observed significant changes on the milk composition and yield. Tilahun et al.[11] also found no significant difference in milk yield and composition when supplementing 0.2 kg of fresh Amla fruit to lactating cows, but when the dose increased first to 0.4 kg and then to 0.6 kg, milk true protein increased. This suggested that a dose-effect study needs to be carried out with GM leaves silage supplementation to expect observing milk production and composition difference. Meanwhile, similar to our study, quebracho or chestnut tannins did not affect the DMI or milk production of lactating cows when fed at 0.45% or 1% of diet DM[15,16]. No previous study analyzed the effects of GM or GM silage on animal production. Meanwhile, testing GM silage with its high protein content comparable to the one of lactating cows' diet, could be a new prospect to lower the feeding cost of dairy cows as GM grows naturally without farming constraints.

Rumen protozoa counts

Protozoa population was microscopically evaluated at a genus level (Table 3). *Entodinium* which constitutes the most predominant protozoa did not change when supplementing 0.3 kg GM silage to TMR. The other protozoa genera *Ophryoscolex, Eudiplodinium, Dasytricha* and *Isotricha* were also maintained. In contrast to the *in-vitro* study, the same dose of the non-ensiled GM leaves had an inhibitory effect on *Entodinium* although the other genera were also maintained[1]. This could be explained either by the low *in vivo* tested dose

Table 2. Milk yield and composition.

	Treatment			SEM	*P*-values		
	Control	GM hay	GM silage		Trt	Period	Trt × Period
DMI kg/d	12.0[a]	0.4[b]	10.6[a]	0.596	0.008	0.292	0.774
Milk yield (kg)	11.85	/	11.68	1.300	0.694	0.364	0.511
Milk yield/DMI	0.98	/	1.10	/	/	/	/
Protein (%)	4.24	/	4.25	0.205	0.957	0.523	0.891
Fat (%)	4.76	/	4.82	0.324	0.629	0.014	0.787
Lactose (%)	4.47	/	4.57	0.102	0.469	0.885	0.813
TS (%)	14.59	/	14.74	0.470	0.418	0.024	0.843
NFS (%)	9.64	/	9.52	0.346	0.682	0.461	0.703

DMI: dry matter intake, TS: total solids, NFS: non-fat solids, SEM: standard error mean, trt: treatment. Superscripts a and b refer to significance differance within the line.

Table 3. Microscopic counts of rumen protozoa.

Protozoa counts (#/mL)	Treatment		SEM	*P*-values		
	Control	GM silage		Trt	Period	Trt × Period
Entodinium	821304	842157	105171	0.663	0.883	0.463
Ophryoscolex	1688	1391	483	0.633	0.430	0.356
Eudiplodinium	2740	2337	540	0.613	0.510	0.447
Dasytricha	4967	4364	571	0.481	0.033	0.618
Isotricha	4916	5928	1058	0.242	0.066	0.393
Total protozoa	836627	855165	105939	0.695	0.938	0.466

SEM: Standart error mean; Trt: Treatment; GM: Giant milkweed leaves silage; #/mL: Number of protozoa per mL of rumen fluid.

that was not enough to inhibit *Entodinium* at the farm level or by the degradation of the inhibitory compound(s) during GM leaves ensiling. The supplementation of 0.2 kg fresh Amla fruit also did not change the protozoa population but when the dose increased to 0.4 kg, total rumen protozoa decreased[11]. Moreover, 8 g/kg DMI of gynosaponin did not affect the rumen protozoa[17]. Overall, the response to protozoa and milk characteristics are inconsistent among studies due to the source of phytochemical, plant development stage, dose, diet composition and other factors[18]. Furthermore, depending on the dose, the development of interaction of nutrients with phytochemicals, the inhibition of microbial protease activity by the latters[19] might be different.

CONCLUSIONS

Calotropis gigantea (Giant milkweed) is a rich source of metabolites that can be valorized as a new feedstock resource. It contains the same protein value with the typical diet of lactating cows and a diversity of phytochemicals. Ensiling the plant contributes to reinforcing the applicability at the farm level where new shrubs can be associated to animal husbandry to ensure an integrated and sustainable livestock production at a lower cost. Supplementing GM silage to dairy cows did not decrease DMI, milk yield and composition and rumen protozoa population. Future studies are warranted to test the dose effect of GM silage on animal production. This may enable the plant which is perceived as an invasive weed, to be viewed as a new feedstock resource at a lower cost for farmers.

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Conflict of interest

The authors declare that they have no conflict of interest.

Dates

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