Scientific Paper

Effect of the substitution of corn by crude glycerol on dry matter intake, in grazing Holando cows

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Abstract

In order to evaluate the effect of the substitution of corn by crude glycerol on the DM intake, a study was conducted in which 18 Holando cows (16 multiparous and 2 primiparous ones) in the first 60 days of lactation, were used. The intake of the diet components and the intake of total dry matter were determined, through the double-marker technique; in addition, a feeding balance was made. The cows were divided in two treatments, and were incorporated to a feeding system consisting in night grazing and consumption of a partially mixed ration (PMR) in feeding trough, in the morning. The diet was composed by sorghum silage and concentrate feed, the latter defined the treatments. In the control treatment a concentrate feed that contained corn (38,6 % on fresh basis) was used, and in the other one, corn was substituted by crude glycerol with 76,5 % of purity. For the statistical processing a mixed model of measures repeated in time was used. The treatment influenced the intake of PMR, with an increase of approximately 2 kg DM day^{-1} in the control; however, there was no effect on the total dry matter intake. It is concluded that this proportion of glycerol, in substitution of corn in the concentrate, did not affect the dry matter intake in highly-producing cows at the beginning of lactation. Likewise, its use is recommended, due to the benefits that can be obtained from the economic and environmental point of view.

Keywords: lactation, grazing, dairy cows

Introduction

One of the most critical stages of the productive cycle of dairy cows is the beginning of lactation, because the ingested feed is not capable of contributing the required energy; this is known as negative energy balance (Grummer and Rastani, 2003), and it is more stressed under certain circumstances. In grazing, for example, there is an extra demand of energy compared with confined systems; this increase is originated by the physical activity of taking the cows to grazing and by the act of grazing itself, constituted by the pasture search, selection and harvest.

The use of concentrate feeds in the diet helps to attenuate the nutrient deficiencies; nevertheless, the high price of most of its components indicates the need to search for alternatives of substitution by cheaper sources, such as crude glycerol. The increase of biodiesel production brings about higher availability of this byproduct and, thus, a reduction in its price (Molinero, 2013). On the other hand, the utilization of those large glycerol volumes prevents it from becoming an environment contaminant.

Glycerol can be used to substitute cereal grains, because both contribute similar levels of energy (Castañeda-Serrano *et al.*, 2014). In this sense, Syahniar *et al.* (2016) refer to glycerol as an apparently adequate ingredient to be used as energy source in the diets of ruminants, precisely as it does not have noxious effect on the environment and it does not affect rumen fermentation. In addition, it is a normal component of animal metabolism that is produced by lipolysis of the adipose tissue or the blood lipoprotein. Nevertheless, there is no consensus about the metabolic implications of the administration of exogenous glycerol supplements in the diet (Silva *et al.*, 2014), although it has been widely used in dairy cows (Werner *et al.*, 2013). There is not much information either about works conducted in grazing systems. Thus, the objective of this study was to evaluate the effect of the substitution of corn by crude glycerol on the dry matter intake of grazing cows.

Materials and Methods

Location and experimental method. The study was conducted in the Research Station Mario A. Cassinoni (EEMAC), located on route 3, km 363,

School of Agronomy, Paysandú Department, Uruguay (32,5º South latitude and 58° West longitude); and lasted for 74 days.

Animals. Eighteen clinically healthy Holando cows were used, 16 multiparous and two primiparous ones; with an average pre-parturition body condition of 3,04 (± 0,26), determined according to the methodology proposed by Edmonson *et al.* (1989), and an average pre-partum live weight of 687,87 kg (± 102,26).

Management and feeding. Since 10 days of lactation the cows were placed in individual pens with access to water and to the corresponding partially mixed ration (PMR), in the morning. After the afternoon milking they were taken to grazing, in independent plots per treatment. The occupation time of each plot was determined according to the dry matter availability, and more than 60 kg cow^{-1} day^{-1} were guaranteed.

Experimental design and treatments. A repeated measures design was used, with a mixed model, in which the effects treatment and date were included. For the allocation of the cows to the treatments, the number of lactations, probable parturition date, body condition and live weight were taken into consideration. Feeding was based on fescue (*Festuca arundinacea*), bird's-foot trefoil, (*Lotus corniculatus*) and white clover (*Trifolium repens*) pastures, with a PMR that defined the treatments:

- Control, control T: pasture + 22,0 kg of sorghum silage + 7,5 kg of concentrate feed A (38,6 % of corn)
- Glycerol, glycerol T: pasture + 22,0 kg of sorghum silage + 5,0 kg of concentrate feed B (without corn) + 3,0 kg of crude glycerol (76,5 % of purity).

Crude glycerol was used, from the biofuel and alcohol producing plant of Uruguay (Alur), whose characteristics are shown in table 1.

Determinations in the feedstuffs. The feedstuff samples for the chemical analysis were collected in weeks six and nine of the experiment. Similar quantities were taken in the case of the offered feedstuff, and approximately 10 % of the refuse, to form weekly samples per animal and per determination period. The pasture was sampled through the grazing simulation or hand plucking technique (Le Du and Penning, 1982), and a composed sample was obtained per treatment.

The feed samples were dried in forced air stove at 60 ºC, until obtaining constant weight, and were ground in a Willey mill with 1-mm mesh. Afterwards, they were stored in duly identified nylon bags, and sent to the animal nutrition laboratory of the School of Agronomy (UDELAR) to determine the ash content (ASH, to estimate the organic matter), ether extract (EE) and crude protein by the Kjeldahl method. In the case of neutral detergent fiber, acid detergent fiber and lignin (AOAC International, 2005), a fiber analyzer (ANKOM 220) was used. Non-fibrous carbohydrates were calculated by difference between dry matter and the other fractions (NRC, 2001). The chemical composition of the feedstuffs is shown in table 2.

Determinations in the animals. Feed intake in trough (FIT_{PMR}) was daily measured for each cow, by the weight difference between offer and refuse. On the other hand, the total dry matter intake (DMI_t)

Table 1. Characteristics of crude glycerol.

Characteristic	Unit	Value	Essay method
Flash point	ºC	40	ASTM D9307
Water content (Karl Fischer)	% (m m^{-1})	3,8	UNIT ISO-12937
Viscosity at 20 ºC	cSt	450	ASTM D445
Density at 20 ºC	g ml^{-1}	1,15	ASTM D4052
Ash	% (m m^{-1})	4,5	ASTM D5468-95
Crude calorific value	Mcal kg^{-1}	4,45	ASTM D5468-95
Glycerol content	% (m m^{-1})	76,5	USP 32 Glycerin
NGOM[1] (fat matter)	% (m m^{-1})	14	ISO 2464
Methanol content	% (m m^{-1})	2,7	Mod. EN 14110

[1]Non-glycerin organic matter

(m m^{-1})= masa/ masa

Table 2. Chemical composition (%) .

Indicator	PMR		Pasture	
	Control T	Glycerol T	Control T	Glycerol T
Dry matter	55,00	55,80	20,60	21,90
Organic matter	93,40	92,70	88,30	88,30
Crude protein	11,70	10,60	16,10	14,60
NDF	31,70	29,70	53,10	55,20
ADF	14,50	13,70	23,80	27,90
Ether extract	0,50	4,60	1,40	0,90
NFC	49,50	47,90	17,60	17,40

NDF: neutral detergent fiber, ADF: acid detergent fiber, NFC: non-fibrous carbohydrates.
NFC = 100 - (% NDF + % CP + % EE + % ASH), PMR: partially mixed ration

was estimated through the addition of dry matter intake in trough and dry matter intake of the pasture (DMI_p). Pasture intake was determined twice, using the double marker technique recommended by Zorrilla (1979). Chromium oxide III (Cr_2O_3) was used as external indicator in the estimation of the fecal production, and indigestible acid detergent fiber (ADFi) as internal indicator, for estimating the diet digestibility. Feed sample taking coincided with the intake determinations.

Feces collection was performed in both milking times, through direct sampling of the rectum in the last four days of Cr_2O_3. The samples were dried in forced air stove at 60 ºC, until constant weight, and were ground. Then compound samples were formed in each determination, and were sent to the soil laboratory of the School of Agronomy for determining Cr by atomic absorption spectrophotometry. On the feces, offered and refused feed and pasture samples the content of *in situ* indigestible acid detergent fiber (ADFi*is*) was determined according to the description made by Correa *et al*. (2009).

To estimate the dry matter intake of the pasture the following equation was used, adapted from Correa *et al*. (2009):

$DMIp\ (kg\ cow^{-1}\ day^{-1}) = ([ADFih] \times H/0,8 - [ADFi_{PMR}] \times DMI_{PMR})/ [ADFip]$

Where:

ADFih: percentage of ADFi in the feces

H: feces production

0,8: ADFi recovery in the feces

$ADFi_{PMR}$: percentage of ADFi in the partially mixed ration

ADFip: percentage of ADFi in the pasture

To calculate the fecal production H ($kg\ day^{-1}$) the daily dose of the marker (D, g) was divided between the corresponding fecal concentration of the marker (C, $g\ kg^{-1}$ of dry matter).

H = D/C (Correa *et al.,* 2009).

Feeding balance was carried out, and for the energy balance the methodology proposed by NRC (2001) was used. In the case of the protein balance, the crude protein requirements were related to the contribution of this nutrient by the diet. The requirements were obtained from the nutritional value tables proposed by García-Trujillo and Pedroso (1989).

Statistical analysis. The dry matter intake was analyzed through mixed models of repeated measures in time, using the Mixed procedure of SAS 9.2 (SAS, 2010). The model included the fixed effects of the treatments, date date x treatment interaction, and random effect of cow nested within the treatment and residual error. The cow was the experimental unit on which the repeated measures were carried out. The means were compared using Tukey and Kramer test (Kramer, 1956), and an effect was reported as significant when $p < 0,05$.

Model:

$$Y_{iJk} = \mu + T_i + D_j + (TD)_{ij} + \beta(C)_k + \varepsilon_{iJk}$$

Where:

Y_{iJk}: dependent variable (DMIt, DMIPMR, DMIp)

μ: general mean

T_i: effect of the i-eth treatment (i:1,2)

D_j: effect of the j-eth date (j:1,2)

$(TD)_{ij}$: treatment by week interaction

$\beta(C)_k$: effect of cow nested within treatment

ε_{iJk}: residual error, with zero mean and variance σ_e^2

Results and Discussion

Table 3 shows the feed intake in Holando cows at the beginning of lactation. The total dry matter intake was not affected by the inclusion of glycerol in the diet; this could have been influenced by the high feed availability and the time of access to it. In that sense, Tarazona *et al.* (2012) define voluntary intake as the quantity of forage that an animal can consume during one day, without limitations of time or availability. Thus, intake in grazing could be deficient if the access time was limited. However, in this study the animals had approximately eight hours of grazing, and five of consumption in feeding trough. In general, cows should remain eating approximately eight hours, eight hours ruminating and eight hours resting, as long as there is sufficient feed availability.

Another factor that influences intake is the diet quality, which was similar for both treatments (table 1). Among the components CP percentage is important, which directly intervenes in digestibility and, thus, in passage rate; because it is essential for microbial protein synthesis. Non-fibrous carbohydrates contribute energy and carbonated chains for the synthesis of this protein, and neutral detergent fiber determines the diet digestibility. Only the percentage of ether extract was affected in the case of the partially mixed ration. In this sense, Tarazona *et al.* (2012) stated that the nutritional composition of the diet influences directly the intake levels and has effect on the physical-chemical and neuro-hormonal regulation. In addition, they emphasize the importance of the NDF content, due to its direct relation with the effect of rumen fill.

The studies related to the use of glycerol have shown variable results in this indicator. Thus, Zymon *et al.* (2012) and Werner *et al.* (2013) did not find difference when incorporating glycerol to the diet. A similar performance was reported by Eiras *et al.* (2014), when using it in bulls up to 17,8 %; Vongsamphanh *et al.* (2017), when including it in diets destined for calves; and D 'Aurea *et al.* (2017) in the finishing of Nelore cows.

On the other hand, Shin *et al.* (2012) found an increase in intake when including glycerol at a rate of 5 % of the dry matter in the diet. The same occurred in the study conducted by Dias *et al.* (2016), with equal percentage of glycerol, but with fattening goats.

In turn, higher levels of glycerol can affect intake, as observed in the results obtained by Paiva *et al.* (2016) when including it in 21 % of dry matter, and Del-Bianco-Benedeti *et al.* (2016) with 15 % of inclusion in the diet of calves.

Total intake, of 25,54 and 22,75 kg DM day^{-1} for the treatments control and with glycerol, respectively, was similar to the one reported by Zymon *et al.* (2012), of 22,00 and 23,14 kg day^{-1}. On the other hand, the forage intake was also similar for both treatments; while the intake of the totally mixed ration showed a decrease of 1,80 kg DM cow^{-1} day^{-1} (p = 0,0369) in the treatment with glycerol, which could have been due to the rejection of the product by some cows. In this regard, Zymon *et al.* (2012) stated that the salts and methanol can affect the taste of the final product. This decrease in intake could have been also related to a higher utilization of the available nutrients in the diet and, consequently, a lower need of ingestion of the ration.

The decrease in the intake of the partially mixed ration and, thus of concentrate feed is feasible as long as the production levels are maintained. This was reported by Delgado *et al.* (2016), who also appreciated a reduction in the feeding costs when substituting corn by crude glycerol.

An adequate evaluation of intake implies evaluating the nutrients that enter the organism and the nutritional requirements of the category under study. Table 4 shows the feeding balance of the evaluated period, integrated by protein balance, energy balance and protein-energy ratio. The crude protein requirements were similar for both treatments, which did not occur with the contribution that was higher in the control. The difference in the crude protein

Table 3. Feed intake in Holando cows at the beginning of lactation.

Variable	Treatment		± SE	Effect P - value		
	Control T	Glycerol T		Treatment	Date	Treatment x Date
DMIt[1], kg day^{-1}	25,54	22,75	1,49	0,2044	0,3170	0,0911
DMIp[2], kg day^{-1}	12,44	11,09	1,15	0,4157	0,7799	0,3221
DMI_{PMR}[3], kg day^{-1}	13,58	11,78	0,56	0,0369	0,2255	0,1829

[1]Total dry matter intake, [2]pasture dry matter intake, [3]dry matter intake of the partially mixed ration.

Table 4. Feeding balance for the evaluated treatments.

Indicator	Treatment	
	Control T	Glycerol T
Total CP requirements, g cow^{-1} day^{-1}	2 978,53	2 888,44
Pasture contribution, g cow^{-1} day^{-1}	2 002,84	1 615,14
PMR contribution, g cow^{-1} day^{-1}	1 588,86	1 248,68
Difference[1], g.cow^{-1} day^{-1}	613,17	-20,62
Total requirement of NE_L[2], Mcal cow^{-1} day^{-1}	30,19	29,57
PMR contribution, Mcal cow^{-1} day^{-1}	19,40	19,13
Pasture contribution, Mcal cow^{-1} day^{-1}	13,67	10,42
Difference[3], Mcal cow^{-1} day^{-1}	2,88	-0,02
Protein:energy ratio, g cow^{-1} Mcal-1	108,61	96,91
Dry matter intake of pasture by NE, kg DM day^{-1}	11,22	11,08

[1]$Dif = PBA_p + PBA_{PMR} - RPBt$

[2]$RNE_L = NE_{mant} + NE_{L(prod)} - NE_{L(MRC)}$

[3]$Dif = A_pNE_L + A_{RPM}NE_L - REN_L$

contribution of the ration (340,18 g cow^{-1} day^{-1}) is related, mainly, to the increase in the dry matter intake of the partially mixed ration (table 3). Such increase suggests decreasing the content of sources of this nutrient in the control.

Regarding the net energy, a balance was observed between the requirements and contributions in the treatments with glycerol; while in the control the contribution by the diet was increased. This surplus could have been used in the synthesis of urea by the liver, and it is related to the high protein levels in the diet. Such process occurs in order to eliminate ammonia from the organisms, which can be highly toxic. The quantity of ammonia absorbed through the rumen walls is in correspondence with its availability within the organ. According to Reynolds and Kristensen (2008), the ammonia produced in the rumen is used by microorganisms for the synthesis of microbial protein or absorbed to be converted into urea by the liver.

For the synthesis of microbial protein an adequate relation between ammonia and energy at rumen level, besides the contribution of carbonated chains, is necessary. Van Cleef *et al.* (2014) referred that the energy/protein balance in the diet of ruminants is essential for the good utilization of ammonia. Thus, the wider relation for this indicator in the control treatment could signify higher ammonia absorption through the rumen walls.

In general, the use of glycerol reduces the ammonia concentration at rumen level. The studies conducted by Avila-Stagno *et al.* (2014) showed a linear reduction of this indicator as the glycerol content in the diet was increased. This could suggest a higher utilization of ammonia, related to the high degradation rate of glycerol in rumen.

Conclusions

The substitution of corn by glycerol in the diet of highly-producing dairy cows, at a rate of 10 % of dry matter, did not affect total dry matter intake. The use of this proportion of glycerol in the diet of dairy cows is recommended, and the study with other levels of inclusion and purity is suggested.

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