



## Effects of short-term administration of a glucogenic mixture at mating on feed intake, metabolism, milk yield and reproductive performance of lactating dairy ewes

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### ABSTRACT

The effects of the intra-ruminal dosing of a glucogenic mixture including 70% glycerol, 20% propylene glycol and 10% water was studied on thirty late lactation dairy ewes of Sarda breed. The animals were divided in two homogeneous groups receiving by gavage either 200 mL of water (CTR group; body weight  $40.9 \pm 1.5$  kg) or 200 mL of the above mixture (GLY group; body weight  $39.4 \pm 1.3$  kg) twice daily from d 16 to d 19 of the oestrus cycle, synchronised by “ram effect”. The ewes were then mated and their reproductive responses to the synchronised mating evaluated by scanning on d 50 and at lambing. During the treatment, the ewes were housed in an open hut, machine milked twice daily and fed concentrate and hay to meet their nutrient requirements. During the treatment, concentrate intake was markedly reduced in GLY when compared with CTR ( $P < 0.001$ ), without any effect on ewe body weight or body condition. The administration of the glucogenic mixture increased plasma osmolarity and blood volume as estimated by serum total protein concentration. Moreover, it increased plasma content of glycerol, glucose ( $P < 0.001$ ) and insulin ( $P < 0.01$ ) while decreasing plasma level of NEFA ( $P < 0.001$ ) and urea ( $P < 0.05$ ). Milk yield ( $P < 0.01$ ) and milk lactose content ( $P < 0.001$ ) were decreased by the glucogenic treatment, whereas milk protein and casein contents were increased ( $P < 0.001$ ). As for reproductive performance, the glucogenic treatment numerically increased ewe’s conception rate, but the difference was not statistically significant. Prolificacy did not change between groups. In conclusion, the administration of a glucogenic mixture to late lactation dairy ewes caused significant changes both in plasma and in milk composition during the treatment. However, reproductive performances were unaffected by the treatment.

### 1. Introduction

In the Mediterranean basin, dairy sheep breeding system typically implies one lambing per year, with the mating season starting in late spring for mature ewes and in early autumn for young ewes (Todaro et al., 2015).

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Mating of adult ewes usually starts when ewes are at the end of seasonal anoestrus period, while they are passing from mid to late lactation (5–6 months of milking). Nutritional plans applied during the mating period should meet the requirements of both the follicle and the mammary gland to increase the fertility of the flock while sustaining milk production. However, in this period pasture usually turns to reproductive phase which results in a decay of pasture nutritive value and a lower intake of nutrients (Pulina et al., 2006).

To cope with these adverse nutritional conditions and optimize reproductive performance, the short-term oral administration of a high dose glucogenic mixture can be useful. Glucogenic precursors such as glycerol and propylene glycol are rapidly absorbed by the rumen, reach the circulation, and serves directly as a substrate in the liver for glucose synthesis, thus causing a rapid and sustained rise of blood glucose (Nielsen and Ingvarsten, 2004). The administration of glycerol and/or propylene glycol has been also associated with an increase in insulin (Letelier et al., 2008) and insulin-like growth factor-1 (Porcu et al., 2017), and a decrease in NEFA and urea circulating concentrations (Habibizad et al., 2015). The follicle has a functional insulin-glucose-IGF-1 system (Scaramuzzi et al., 2010) which is affected by short-term nutritional treatments, and it is clear that components of this metabolic system are nutritionally regulated in the follicle (Dupont et al., 2014). Glucose, insulin and IGF-1 act synergistically to promote follicle growth and estradiol secretion (Downing et al., 1999). On the other hand, high circulating levels of urea and NEFA have been associated with lower fertility and negative energy balance (McEvoy et al., 1997; Aardema et al., 2011). Recent studies have shown that glucogenic-based flushing treatment improves oocyte quality in ewes submitted to ovum pick up (Berlinguer et al., 2012) and can increase ovulation rate in Manchega dry ewes (Letelier et al., 2008). These effects have been related to the modification of the plasma and follicular fluid composition during the treatment period (Porcu et al., 2017), modulated by ewe body condition score at mating (Williams et al., 2001).

All the above studies have focused on non-lactating ewes, thus the effects of short-term glucogenic dietary treatments in lactating dairy ewes have been overlooked so far.

The ovary uses glucose as its principal energy source and its well described positive effects on fertility have been related to its properties as a metabolic fuel (Scaramuzzi et al., 2010). During lactation however, glucose requirements in the mammary tissue increase dramatically, competing with those of other tissues and organs, such as the ovaries.

Starting from these premises, the present study aimed at investigating the impact of the administration of a glucogenic mixture on feed intake, metabolism, milk yield and reproductive performance of lactating dairy ewes. The hypothesis underlying the study was that the glucogenic mixture administration could create in lactating dairy ewes a hormonal and metabolic milieu favourable for follicle growth, maturation, and ovulation resulting in an improvement of sheep reproductive performance.

## 2. Materials and methods

The experiment was carried out from June 11<sup>th</sup> to July 31<sup>st</sup> 2015 (experimental period) at Bonassai research station of Agris, located in north-western Sardinia, Italy (40 °N, 8 °E, 32 m a.s.l.). Weather conditions during the experiment were assessed using an on-farm weather station and temperature humidity index (THI) was calculated according to (Johnson and Kibler, 1963).

The animal protocol and the implemented procedures described below are in accordance with the ethical guidelines in force at Agris and the University of Sassari (CIBASA 21.01.2014), in compliance with the European Union Directive 86/609/EC and the recommendation of the Commission of the European Communities 2007/526/EC.

### 2.1. Animals and treatments

On June 11 (d 0 of the experimental period), thirty Sarda dairy ewes were selected from the farm flock, homogeneous for age (mean  $\pm$  SE 3.3  $\pm$  0.26 years) and lactation stage (mean  $\pm$  SE, 155  $\pm$  5 days in milk [DIM]). The ewes were weighted, and their body condition was scored. On the same day, sheep milk yield (MY) was measured at two milkings and milk samples were collected and analysed. Thereafter, the ewes were randomly assigned to two experimental groups, homogeneous for body weight (BW; CTR group 40.9  $\pm$  1.5 kg, GLY group 39.4  $\pm$  1.3 kg;  $P = 0.43$ ), body condition score (BCS; CTR group 2.78  $\pm$  0.05, GLY group 2.71  $\pm$  0.05;  $P = 0.30$ ), MY (CTR group 1088.2  $\pm$  54.8 g, GLY group 1016.8  $\pm$  51.2 g;  $P = 0.57$ ), milk fat (CTR group 6.65  $\pm$  0.20%, GLY group 6.95  $\pm$  0.19%;  $P = 0.89$ ) and protein (CTR group 5.10  $\pm$  0.17%, GLY group 5.40  $\pm$  0.16%;  $P = 0.79$ ) concentrations. One ewe was discarded because of an acute trauma. Each group was further divided in two subgroups, used as replicates. During the experimental period, the ewes were housed overnight in an open hut, where they were machine milked twice daily.

Ram effect was used to synchronise ewes' ovulation. For this reason, the ewes were kept isolated from rams for 6 weeks before starting the experiment. At d 0 of the experimental period, vasectomised rams were introduced in the flock at the ratio of 2 rams per 15 ewes and were left in until the presumptive starting of oestrus (d 16). Thereafter, the vasectomised rams were replaced with non-vasectomized rams at the same ram per ewe ratio. The rams were removed from the flock on d 30.

Coincidentally with the onset of the follicular phase, from d 16 to d 19 (treatment period), one experimental group (glucogenic treated ewes  $n = 15$ ; GLY) received, orally twice daily 200 mL of a glucogenic mixture. The glucogenic formulation contained (v/v) 70% glycerol, 20% propylene glycol (both reagent grade (> 99% purity) chemicals from Sigma Chemical Co., St. Louis, MO, USA), and 10% water. The second group (control ewes:  $n = 14$ ; CTR) received 200 mL of water twice daily simultaneously to glucogenic mixture administration. Both the glucogenic formulation and the water were administered at 0800 and 1900 h in the evening, by oral gavage using an esophageal feeding tube. This daily dosing schedule was set as close as possible to that adopted in previous experiments by our laboratories [twice daily, every 12 h (Berlinguer et al., 2012)]. From d 15 to d 21, i.e. throughout the treatment

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