



# Short term dietary propylene glycol supplementation affects circulating metabolic hormones, progesterone concentrations and follicular growth in dairy heifers



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## ABSTRACT

This study was designed to determine the effects of dietary propylene glycol (PG; Propypact<sup>®</sup>, DIFAGRI, France) on blood metabolites, metabolic and reproductive hormones and follicular growth in 10 dairy heifers. Treatments consisted of (1) 1.1 kg of sugar beet pulp (Control), (2) 150 g PG (PG150) and (3) 300 g PG (PG300). Each heifer received the three treatments in different randomized orders. A standard hay/concentrate diet, formulated to allow a daily liveweight gain of 900 g/day, was given at 8:00 and the dietary treatments were given at 16:00 from Days 1 to 13 of the oestrous cycle following induced oestrus (Day 0). Oestrus induction treatment consisted of a subcutaneous 3 mg norgestomet implant inserted for 9 days combined with GnRH treatment (i.m.) at implant insertion. Two days before implant removal, 500 µg cloprostenol was administered i.m. Blood samples were collected by jugular venipuncture every 2 h for 24 h on Days 0 and 13 to measure plasma insulin, glucose, β-hydroxybutyrate (BHB) and urea concentrations. Blood samples were also collected to measure insulin-like growth factor-1 (IGF-1), oestradiol, progesterone concentrations on Days 2, 6, 9 and 12 and AMH (Anti-Müllerian hormone) on Days 0, 2, 6, 9 and 12. On Days 2, 6, 9 and 12 ovarian follicular growth was evaluated; the total number of follicles and their diameters were recorded and classed (2–3 mm, 4–7 mm, and > 8 mm). Results were analysed by repeated-measures ANOVA. There were no treatment, day and interaction effects on average urea concentrations while there were some differences between Days 0 and 13 for insulin, glucose and BHB. Insulin and glucose concentrations were higher on Day 13 compared to Day 0 and the opposite was observed for BHB. There were treatment, time and interaction effects on glucose and BHB concentrations measured over 24 h on Day 13; glucose concentrations were higher ( $P < 0.05$ ) at 4:00, 8:00, 12:00, 16:00 and 20:00 h, whereas BHB concentrations were lower ( $P < 0.05$ ) at 20:00 and 22:00 h in the PG300 group compared to the control and PG150 groups. There were treatment, day and interaction effects on IGF-1 and progesterone concentrations, and the number of small follicles. PG150 resulted in higher

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progesterone concentrations on Days 9 and 12, and more small follicles on Day 2 compared to Control. AMH concentrations were unaffected by day of oestrous cycle and dietary treatment. However a negative correlation was observed between pre-PG distribution insulin and AMH ( $r = -0.47$ ,  $P < 0.05$ ). These results indicate that short-term dietary PG supplementation affects circulating concentrations of metabolites and metabolic hormones, increases progesterone concentrations and the number of small follicles. Propylene glycol supplementation may be effective in improving oocyte production when combined with hormonal treatments to stimulate follicular growth for super-ovulation or ovum-pick up.

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## 1. Introduction

It has long been recognised that the nutritional and metabolic statuses of domestic ruminant females are associated with reproductive success, but the underlying mechanisms remain poorly understood (Gutierrez et al., 1997; Gong, 2002; Adamiak et al., 2005). Several authors have proposed that metabolic signals, such as circulating concentrations of insulin, growth hormone (GH), leptin and the insulin-like growth factor system (IGF) interact at the central level to modulate the release of gonadotrophins (Schneider, 2004; Garnsworthy et al., 2008). In vitro studies have highlighted the fact that insulin and IGF-1 are important mediators of follicular development, steroidogenesis, oocyte maturation and embryonic development (Gong et al., 1993; Totey et al., 1996). Exogenous insulin administration increases the recruitment of follicles in response to gonadotropin (Cox et al., 1987) and also rescues follicles from atresia and therefore increases the number of ovulatory follicles (Matamoros et al., 1991). Therefore, an increase in the levels of insulin during the oestrous cycle may enhance follicle numbers. However, Freret et al. (2006) observed that diets that increased insulin concentrations decreased oocyte quality. Nevertheless, an increase in insulin concentrations over a short lapse of time has been shown to have a positive effect on the growth of small follicles prior to superovulatory treatment and exerts a beneficial influence on subsequent embryonic development (Adamiak, 2005; Freret et al., 2006).

Plasma concentrations of glucose and insulin are known to increase in response to dietary propylene glycol (PG) (Studer et al., 1993; Formigoni et al., 1996; Miyoshi et al., 2001). PG is a gluconeogenic precursor widely used in the prevention and treatment of ketosis. It increases the molar percentage of ruminal propionate in postpartum dairy cattle and reduces plasma non-esterified fatty acids (NEFA, Christensen et al., 1997; Grummer et al., 1994). After oral administration, a portion of PG is fermented in the rumen to propionate, but the majority of PG escapes the rumen untransformed and is converted to glucose by the liver (Rizos et al., 2008). High levels of glucose stimulate pancreatic insulin secretion which in turn reduces plasma NEFA if animals are mobilising adipose tissue reserves. However, there is another mechanism of action described for PG, involving the production successively of propionate together with propanol and with the latter being converted to propanol in the rumen which in turn is converted to propionate in the liver and thereafter glucose (Kristensen and Raun, 2007). In addition they proposed that propanol may create a relative 'insulin-resistance' thereby

blocking the uptake of glucose by insulin-sensitive tissues and therefore causing glucose concentrations to rise. PG is commonly administered as an oral drench however supplying PG with concentrate is more practical because this method requires less labour than drenching (Christensen et al., 1997). Studies on ewes have shown that oral glucogenic supplements can stimulate follicle growth and ovulation rate (Letelier et al., 2008a, 2008b) and improve oocyte quality, as estimated by in vitro development and blastocyst output (Berlinguer et al., 2012). The authors concluded that the effects were mediated by a transient improvement in energy balance.

The aim of this study was to determine the effects of short-term dietary propylene glycol on metabolic hormones (insulin, IGF-1), on subsequent follicular growth together with blood metabolites (glucose,  $\beta$ -hydroxybutyrate (BHB), and urea) and reproductive hormones (progesterone, oestradiol, AMH) in dairy heifers.

## 2. Materials and methods

### 2.1. Animals

All experimental work was performed in the experimental Station of the Regional Union of Breeding Coops for Genetic Improvement and Animal Service (MIDAT-EST), in the south-west of France. Ten Holstein dairy heifers were used for the study. At the start of the experiment the heifers were:  $14 \pm 1$  months old, weighed  $332 \pm 26$  kg and had a body condition of score of  $2.8 \pm 0.7$  (BCS; on scale from 0=thin to 5=fat, Bazin, 1984). Heifers were confirmed to be cyclic by rectal palpation before being introduced into the experimental station. Prior to the experimental period they were housed together indoors and fed a maintenance diet composed of natural prairie hay and a commercial concentrate. During the experiment, the heifers were housed individually and given the experimental treatments. Water was available ad libitum. The present study was carried out according to French legislation on animal experimentation (code rural: articles R 214-87–R214-94) in line with the European Convention for the Protection of Vertebrates used for Experimental and other Scientific Purposes (European Directive 86/609).

### 2.2. Experimental protocol

After a dietary transition and adaptation period of 3 weeks, each group of heifers received individually a commercial

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