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## Effects of reducing dietary starch content by replacing barley grain with wheat dried distillers grains plus solubles in dairy cow rations on ovarian function

E. Subramaniam,\* M. G. Colazo,† M. Gobikrushanth,\* Y. Q. Sun,\* A. L. Ruiz-Sanchez,\* P. Ponce-Barajas,\* M. Oba,\* and D. J. Ambrose\*†<sup>1</sup>

\*Department of Agricultural Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada T6G 2P5

†Livestock Research Branch, Alberta Agriculture and Forestry, Edmonton, AB, Canada T6H 5T6

### ABSTRACT

Our objective was to evaluate the effects of dietary starch content, altered by partial substitution of dietary grain with wheat dried distillers grain with solubles (DDGS), on the interval from calving to first ovulation, concentrations of hormones and metabolites in plasma and follicular fluid, and granulosa cell gene expression in preovulatory follicles. Sixty lactating dairy cows were assigned to 1 of 2 diets from calving until 84 d postpartum. Diets were formulated to contain either 17.3% rolled barley grain (29.2% starch) or 17.2% wheat DDGS (19.1% starch), with 43.0% barley silage and 21.6% rolled corn grain as the other major ingredients (dry matter basis). Transrectal ultrasonography was performed twice weekly to monitor ovarian dynamics from 7 ± 2 d postpartum until ovulation or until 56 d in milk, whichever occurred earlier. Plasma concentrations of insulin and insulin-like growth factor-1 (IGF-1) were determined in all 60 cows, and that of glucose, fatty acids, and urea in a subset of 24 cows, representing those in which the first ovulation occurred spontaneously within 5 wk postpartum. Estradiol (proestrus) and progesterone (12 d postovulation) in plasma were also measured. Concentrations of insulin, IGF-1, glucose, fatty acids, and urea were determined in follicular fluid (wk 9), and the expression of LH receptor, estrogen receptor  $\beta$ , cytochrome P450 aromatase, and plasma type glutathione peroxidase genes measured in granulosa cells obtained from the preovulatory follicles at wk 9 postpartum in the subset of 24 cows. Diets did not alter the interval from calving to first ovulation (32.3 ± 2.5 d), but a significantly lower proportion of cows on the DDGS diet (20%) ovulated multiple ( $\geq 2$ ) follicles at the first ovulation than those on the barley grain

diet (40%). The incidence of multiple ovulations tended to be lower at first insemination (10 vs. 21% for cows fed DDGS and barley grain diets, respectively). Mean plasma concentration of insulin was higher in cows fed the barley grain diet (2.5 vs 1.6 IU/mL), and a diet by time interaction was noted, with cows on the barley grain ration having higher insulin from wk 6 to 12 postpartum; however, mean plasma IGF-1 concentration did not differ between dietary groups. In the subsets, mean plasma concentrations of metabolites or estradiol and progesterone were not affected by diet, parity, or diet by parity interactions. Cows on the DDGS diet had lower concentrations of IGF-I (69 vs. 108 ng/mL) and higher fatty acids (222 vs. 149 mEq/L) in the follicular fluid obtained from preovulatory follicles. Diet, parity, and diet by parity interactions did not affect the concentrations of insulin, glucose, urea, estradiol, and progesterone in follicular fluid. Diets did not alter the expression profiles of *LHr*, estrogen receptor  $\beta$ , *CYP19*, and *Gpx3* genes in granulosa cells. In summary, diets did not affect the interval from calving to first ovulation or granulosa cell gene expression. However, reducing dietary starch content by a partial replacement of dietary grain with wheat DDGS increased fatty acids in follicular fluid and reduced the concentrations of insulin in plasma, IGF-1 in follicular fluid, and the incidence of multiple ovulations.

**Key words:** starch, dried distillers grains with solubles, lactating dairy cow, multiple ovulations, follicular fluid

### INTRODUCTION

Lactating dairy cows are often in a state of negative energy balance for up to 6 to 8 wk postpartum, which has been associated with an increase in the interval from calving to first ovulation (Butler and Smith, 1989) and a decrease in conception rates (Butler, 2000). Mobilization of fat reserves associated with negative energy balance increases plasma concentrations of fatty acids and may interfere with availability of hormones

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<sup>1</sup>Corresponding author: [divakar.ambrose@gov.ab.ca](mailto:divakar.ambrose@gov.ab.ca)

such as insulin and IGF-1 in early lactation (Bossaert et al., 2008). Insulin and IGF-1 have been shown to play critical roles in follicular cell proliferation and steroidogenesis in vitro (Spicer and Echtenkamp, 1995). Armstrong et al. (2001) reported that heifers fed a high-energy (816 kJ/kg) diet had higher plasma concentrations of insulin and IGF-1 compared with those fed a low-energy (408 kJ/kg) diet. The same research group (Armstrong et al., 2002) also reported that dietary-induced changes in circulating insulin and IGF-1 concentrations have a direct effect on the steroidogenic potential of bovine granulosa cells obtained from small follicles, where cows were given either maintenance or twice maintenance diets during the experimental period. In addition, raising the circulating concentrations of insulin by increasing dietary starch content from 10 to 26% resulted in a greater proportion of cows ovulating before 50 d postpartum (Gong et al., 2002). In a more recent study (Dyck et al., 2011), resumption of cyclicity occurred sooner in cows fed diets containing 27% starch compared with those fed diets containing either 25 or 23% starch, although plasma concentrations of insulin and IGF-1 did not differ among dietary treatments. Cows ovulating sooner postpartum are more likely to conceive to first insemination (Butler, 2000; Ambrose and Colazo, 2007). Therefore, feeding high-starch diets during the early postpartum period could be a strategy to improve reproductive performance of lactating dairy cows. However, an association between high-starch diets and increased incidence of multiple ovulations has been reported (Dyck et al., 2011). Double ovulation is the main reason for twin pregnancies in cattle (Wiltbank et al., 2000) and twinning is an undesirable outcome as it often increases the risk of postpartum disease, increases average days open and services per conception during the subsequent lactation, increases the probability of culling, and reduces the productive lifespan of cows calving twins than that of cows calving singletons (Andreu-Vázquez et al., 2012).

The expansion of ethanol fuel production in western Canada in recent years has increased the supply of ethanol by-products, such as wheat dry distillers grain with solubles (DDGS). Due to its increased availability at a reasonable cost and high NDF of about 37.4%, DDGS is being used as an alternative feedstuff in postpartum dairy cows (Zhang et al., 2010). Whereas DDGS can be used to partially substitute grain, the effects of including wheat DDGS in dairy rations, to reduce dietary starch, on ovarian function of lactating dairy cows have not been investigated.

We hypothesized that reducing dietary starch content by replacing barley grain with wheat DDGS would affect plasma concentrations of insulin, IGF-1, and ovarian function, and alter the intrafollicular milieu.

Specific objectives were to evaluate the effects of dietary starch content, altered by partial substitution of dietary grain with wheat DDGS, on the interval from calving to first ovulation, concentrations of hormones and metabolites in plasma and follicular fluid, and gene expression in granulosa cells of preovulatory follicles.

## MATERIALS AND METHODS

### *Animals and Diets*

The study was conducted in a tiestall barn at the University of Alberta. Experimental protocols were approved by the Animal Care and Use Committee for Livestock at the University of Alberta and animals were cared for in accordance with the requirements of Canadian Council on Animal Care (1993).

Sixty lactating Holstein cows (22 primiparous and 38 multiparous) were assigned to this study. Cows were individually housed in a tiestall barn and let out for approximately 2 h of exercise on weekdays. Body condition score was determined based on a 5-point scale (Wildman et al., 1982) 1 wk before expected date of calving. Cows, blocked by parity and date of calving, were assigned to 1 of the 2 experimental diets starting on the day of calving until 84 DIM (Figure 1). Diets were formulated according to NRC (2001) to meet the requirements of a 650-kg lactating cow producing 45.0 kg of milk/d with 3.5% milk fat and 3.0% milk protein. Following calving, cows were assigned to 1 of the 2 experimental diets containing either 17.2% wheat DDGS or 17.3% rolled barley, and 43.1% barley silage. Ingredient composition of the diets has been previously published in a companion paper (Sun and Oba, 2014; Table 1). Cows were offered the assigned diets once daily as TMR at 105 to 110% of the expected intake at 0730 h and had access to water ad libitum. The amounts of feed offered and refused were recorded individually to assess daily DMI for each cow. Cows were milked daily in their stalls at 0400 and 1600 h, and daily milk production was recorded.

### *Ultrasonography and Timed AI*

Transrectal ultrasonography (Aloka-500V scanner equipped with a 7.5-MHz linear transducer, Aloka Co., Tokyo, Japan) was performed twice weekly to monitor ovarian dynamics from  $7 \pm 2$  d after calving until ovulation was confirmed or until 56 DIM, whichever occurred earlier. Cows that did not ovulate by 56 DIM were considered anovular. Measurement of large and small follicles was performed as previously reported by Pierson and Ginther (1984). Ovulation was presumed if the large follicle ( $\geq 10$  mm) detected at one examina-

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