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In vitro and in vivo analysis of fatty acid effects on metabolism of 17β-estradiol and progesterone in dairy cows

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ABSTRACT

Some studies have reported improved reproductive performance with dietary fat supplementation. This study examined effects of fatty acids with different lengths, or desaturation, or both, on metabolism of estradiol (E2) and progesterone (P4) in bovine liver slice incubations (experiments 1 and 2) and in vivo (experiment 3). In experiment 1, effects of fatty acids C16:0 (palmitic acid), C16:1 (palmitoleic acid), C18:1 (oleic acid), and C18:3 (linolenic acid) were evaluated at 30, 100, and 300 μ*M* on P4 and E2 metabolism in vitro. In experiment 2, stearic acid (C18:0) and C18:3 were evaluated in the same incubation conditions. In experiment 1, all of the fatty acids had some significant inhibitory effect on metabolism of P4, E2, or both (300 μ*M* C16:0 on E2; 100 μ*M* C16:1 on E2; 300 μ*M* C16:1 on both P4 and E2; 300 μ*M* C18:1 on P4; and 100 and 300 μ*M* C18:3 on both P4 and E2). In experiment 2, C18:3 (100 and 300 μ*M*) but not C18:0 decreased P4 and E2 metabolism. Overall, the most profound increase (~60%) in half-life of P4 and E2 was observed with incubations of 300 μ*M* C18:3 in both in vitro experiments. Based on these in vitro results, in experiment 3 linseed oil (rich in C18:3) was supplemented into the abomasum and acute effects on metabolism of E2 and P4 were evaluated. Cows (n = 4) had endogenous E2 and P4 minimized (corpus luteum regressed, follicles aspirated) before receiving continuous intravenous infusion of E2 and P4 to analyze metabolic clearance rate for these hormones during abomasal infusion of saline (control) or 70 mL of linseed oil every 4 h for 28 h. Linseed oil infusion increased C18:3 in plasma by 46%; however, metabolic clearance rate for E2 and P4 were similar for control cows compared with linseed-treated cows. Thus, in vitro experiments indicated that E2 and P4 metabolism can be inhibited by high concentrations of C18:3. Nevertheless, in vivo, linseed oil did not acutely inhibit E2 and P4 metabolism, perhaps because insufficient C18:3 concentrations (increased to ~8 μ*M*) were achieved. Further research is needed to determine the mechanism(s) of fatty acid inhibition of P4 and E2 metabolism and to discover practical methods to mimic this effect in vivo.
Key words: polyunsaturated fatty acid, estrogen, progesterone, steroid metabolism

INTRODUCTION

Dietary fat supplementation is a strategy that has been widely used in livestock operations to improve productivity and reproductive performance. However, results of fat supplementation on reproductive performance in beef or dairy cattle have been conflicting (reviewed by Grummer and Carroll, 1991; Staples et al., 1998; Mattos et al., 2000; Williams and Stanko, 2000; Funston, 2004). For example, some studies found a lack of response in reproductive performance when supplemental fat was fed (Carroll et al., 1990; Carr et al., 1994). In contrast, other studies indicated that effects of fatty acids on reproduction include increased conception rate (Son et al., 1996; Staples et al., 1998), increased number or diameter of follicles (Mattos et al., 2002; Robinson et al., 2002), and increased circulating progesterone (**P4**; Hawkins et al., 1995; Lammoglia et al., 1997) or estradiol (**E2**; Robinson et al., 2002) concentrations.

The observed increase in circulating P4 and E2 concentrations may be a mechanism underlying improvements in fertility following fat supplementation. Fat supplementation could enhance circulating steroids by increasing steroid hormone production through various mechanisms, including increased availability of lipopro-

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tein-cholesterol, modulation of prostaglandin synthesis, or direct stimulation of ovarian steroidogenesis (Grummer and Carroll, 1991; Staples et al., 1998; Williams and Stanko, 2000). In addition to mechanisms that alter steroid production, the circulating steroid concentrations can be regulated by changes in the rate of steroid metabolism. It has been recognized that the rate of steroid metabolism may be a critical regulator of steroid hormone concentrations and possibly of reproductive performance in lactating dairy cows (Sangsritavong et al., 2002; Wiltbank et al., 2006). More than a decade ago, a model based on data from sheep and pigs was developed that connected increases in feed intake with elevated liver blood flow and subsequent augmentation of steroid hormone metabolism (Parr et al., 1987, 1993; Prime and Symonds, 1993; Miller et al., 1999). Thus, changes in steroid metabolism could alter the reproductive physiology of any species, but may particularly alter reproduction in species with extreme increases in feed intake, such as the lactating dairy cow (Wiltbank et al., 2006).

There is evidence that fats or fatty acids may regulate circulating steroid concentrations. Feeding beef heifers from 100 d before parturition through the subsequent postpartum period approximately doubled the circulating P4 concentrations during their second or third postpartum estrous cycle (Hawkins et al., 1995) and increased the half-life of P4 (170 vs. 113 min; Hawkins et al., 1995). In addition, cows fed diets supplemented with C18:3 had increased E2 concentrations during the follicular phase (Robinson et al., 2002). The liver is the primary site for steroid hormone metabolism (Rendic and DiCarlo, 1997), with liver cytochrome P450 (**cyp**) enzymes catalyzing critical steps in this metabolism. Studies in vitro indicate that polyunsaturated fatty acids (**PUFA**) may act as competing substrate for binding sites of cyp (Hirunpanich et al., 2006, 2007; Yao et al., 2006). Thus, inhibition by PUFA of the key enzymes of steroid metabolism could increase circulating steroid hormone concentrations and partially overcome the negative effects of high steroid metabolism that have been documented in dairy cattle (Wiltbank et al., 2006).

Nevertheless, despite the substantial number of studies on the effects of fats on reproductive performance of dairy cows and the growing evidence for a role of steroid hormone metabolism in reproduction in dairy cows, no previous studies have been designed to specifically evaluate the effects of fats on E2 and P4 metabolism in dairy cattle. The present study evaluated whether fatty acids with different lengths or different amounts of desaturation could directly alter metabolism of steroids by slices from bovine liver. Further, an in vivo study evaluated the effect of linseed oil on circulating E2 and P4 concentrations during continuous infusion of these steroids. The overall hypothesis tested in these experiments was that specific fatty acids reduce E2 and P4 metabolism in vitro and in vivo.

MATERIALS AND METHODS

Reagents and Products

Progesterone and E2 were purchased from Steraloids Inc. (Newport, RI). Cell culture media (M-199, catalog # M3769) without phenol red was purchased from Sigma Co. (St. Louis, MO) and modified by adding HEPES (4.76 g/L), sodium bicarbonate (0.35 g/L), and L-glutamine (0.1 g/L), with final pH adjusted to 7.35. The intravaginal P4 devices [Eazi-Breed controlled internal drug-releasing intravaginal insert (**CIDR**) containing 1.39 g of P4] were purchased from InterAg Company (Hamilton, New Zealand), and $PGF_{2\alpha}$ (Lutalyse; 25 mg) was purchased from Pharmacia Animal Health (Peapack, NJ). Linseed oil was a gift of Archer Daniels Midland Company (Red Wing, MN).

Liver Incubations and Measurements

Bovine livers were obtained from an abattoir that primarily slaughters Holstein cattle; however, prior cow history was unknown for all livers used in this study. The livers were transported from the slaughterhouse in a thermos filled with physiological saline (36°C) and arrived at the laboratory by 4 h after slaughter. At the laboratory, the liver from each individual cow was thinly sliced (100–130 mg/slice) using a Stadie Riggs tissue slicer (Fisher Scientific Corp., Pittsburgh, PA). Each treatment (described below) was replicated in 3 different plates for each bovine liver. The steroid hormone being tested was dissolved in absolute ethanol before addition into the media with a final concentration of 0.25% (vol/vol). Each concentration of fatty acid was directly diluted into media with individual steroid hormones (unsaturated fatty acids were warmed up to allow dilution). Five milliliters of M-199 media containing the steroid hormone and fatty acids were put into 6-well tissue culture plates (Fisher Scientific Corp.). Plates were incubated at 39°C for 3 h in humidified air to equilibrate temperature before addition of liver slices. Liver slices were then added to the media containing one of the steroids [30 nM (~9.4 ng/mL) P4 or 3.5 nM (~953 pg/mL) E2] without any added fatty acids or with 10, 100, or 300 μM fatty acids. Media samples (100 μL) were taken before addition of a liver slice to account for time 0 (0 min). After addition of the liver slices, media samples were taken at 2, 5, 8, 15, and 30 min. Samples were then diluted 1:3.5 with