Utilization of Medium-Chain Triglycerides by Neonatal Pigs: Effects of Emulsification and Dose Delivered1,2

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ABSTRACT: Four trials were conducted using 86, 24-h-old pigs to evaluate the utilization of medium-chain triglycerides (MCT). Effects of emulsification and amount of MCT fed were examined. After a 4-h period during which feed was withheld, pigs were force-fed MCT (containing 75% octanoate and 25% decanoate), marking time 0 of the experiment. Blood samples were obtained at 1 and 2 h for subsequent medium-chain fatty acid (MCFA) analysis. In Trials 1 (six pigs/treatment) and 2 (four pigs/treatment) the response to three emulsifying agents was compared to a nonemulsified (NE) control. Twenty milliliters of a 30% (vol/vol) emulsion of MCT or 6 mL of NE MCT was administered. Concentrations of MCFA at 1 h in pigs receiving a Tween 80 (polyoxy-ethylene [20] sorbitan monooleate) emulsion were 3- to 19-fold higher than concentrations in animals administered a gum arabic/gum tragacanth emulsion, a lecithin emulsion, or NE MCT. Trials 3 (eight pigs/treatment) and 4 (six pigs/treatment) were conducted to determine the plasma MCFA concentrations resulting from feeding increasing levels of NE (3, 6, 9, or 12 mL of MCT) or emulsified MCT oil (2, 4, 6, or 8 mL in a 30% Tween 80 emulsion). Plasma octanoate concentrations measured at 1 h increased linearly (P < .05) with increasing MCT dosage through 9 mL of NE and 6 mL of emulsified MCT. A transient narcosis was observed in 8 of 12 animals that received 6 or 8 mL of emulsified MCT and was most pronounced 1 to 2 h after feeding, which roughly corresponded to peak plasma MCFA concentrations. Collectively, these studies indicate that emulsification increases the rate of digestion/absorption of MCT; Tween 80 was a more effective emulsifier than gum arabic or soy lecithin under the conditions employed. However, administration of excessive emulsified MCT may induce coma by increasing the concentrations of circulating fatty acids to toxic levels.

Key Words: Pigs, Neonates, Medium Chain Triacylglycerols, Fatty Acids, Emulsification, Toxicity

Introduction

Morbidity and mortality of newborn pigs result in significant economic loss to the swine industry. Preweaning mortality varies considerably among production units, ranging from 5 to 30% of pigs born alive. A survey conducted by the USDA, including 1,661 farms, estimated preweaning mortality at 12% (USDA, 1991). Fifty percent of deaths were reported to occur within the first 3 d. Energy insufficiency was identified as one of the major causes for these losses. If unable to suckle, the neonatal pig rapidly depletes its supply of glycogen, thereby increasing susceptibility to hypothermia, disease, and crushing by the sow.

To remedy the energy insufficiency, researchers have attempted to conserve the endogenous fuels of the 0- to 3-d-old pig through supplementation with medium-chain triglycerides (MCT, containing fatty acids with 6 to 12 carbon atoms). Research (Odle et al., 1989, 1991; Chiang et al., 1990) has indicated that pigs can effectively digest, absorb, and oxidize MCT. In most studies reported to date, MCT have been fed to pigs in nonemulsified form. However, for pancreatic lipase to hydrolyze triglyceride effectively, a water-oil interface must be present. Consequently, if bile secretion by the newborn pig is low, then emulsification may be a limiting factor to small intestinal hydrolysis of MCT. The objectives of the present studies were, therefore, to evaluate the relative efficacy of three emulsifying agents in enhancing MCT utilization by neonatal pigs (Trials 1 and 2) and to determine the dose-response relationship between the

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amount of nonemulsified and emulsified MCT administered and the resulting plasma concentration of medium-chain fatty acids (MCFA) (Trials 3 and 4).

Materials and Methods

Four experiments were conducted using a total of 86 crossbred (Yorkshire × Duroc × Hampshire) pigs. All animal management and handling procedures were approved by the University of Illinois Laboratory Animal Care Committee. The sows were fed a standard corn-soybean meal diet, were moved into farrowing crates at 109 d of gestation, and were allowed to farrow naturally. Pigs were allowed to suckle for 24 to 36 h then were removed from the sow, weighed, and housed at 30°C.

After a 4-h period during which feed was withheld, the pigs were gastrically intubated with MCT (Captex 300, containing 75% octanoic and 25% decanoic fatty acids; Karlshamns Lipid Specialties, Columbus, OH). Flexible plastic tubing attached to a glass syringe filled with the liquid MCT was passed through the esophagus into the pig’s stomach and the triglyceride was administered. This marked time 0 of the experiment. Heparinized blood samples (3 mL) were obtained by jugular venipuncture at 1 and 2 h. It was considered unnecessary to draw a 0-h blood sample because previous work (Odle et al., 1989, 1991) has shown that MCFA are undetectable in pigs from sows fed corn-soybean meal diets. After the 2-h blood sample was taken, pigs were returned to the sow. The blood was centrifuged at 4°C and plasma was stored at −20°C for subsequent MCFA analysis.

Trials 1 and 2. The response to various emulsifying agents was studied in two experiments. All emulsifying reagents were obtained from Sigma Chemical (St. Louis, MO). Initial tests were done to determine the type and concentration of emulsifying agents and concentration of oil that would yield stable emulsions with droplets of < 50 μm, when measured with a calibrated microscope-stage micrometer. Two percent (weight/volume) aqueous solutions of the emulsifying agents Tween 80 (polyoxyethylene [20] sorbitan monooleate), soy lecithin (L-α-phosphatidylcholine), or gum arabic were used. Gum tragacanth was added as the emulsifying agent because it is normally secreted by the gall bladder and acts as an emulsifier in the gastrointestinal tract.

Trials 3 and 4. Two experiments were conducted to determine the plasma MCFA concentrations resulting from feeding increasing levels of nonemulsified and emulsified MCT. In Trial 3, 32 pigs weighing 1.64 ± .23 kg (± SD) were blocked by litter and gavaged with 3, 6, 9, or 12 mL of nonemulsified MCT. Trial 4 was also conducted as a randomized complete block design, containing six replications of four treatments. Animals averaging 1.59 ± .14 kg (± SD) received either 2, 4, 6, or 8 mL of MCT oil in a 30% Tween 80 emulsion, which was equivalent to 6.66, 13.33, 20, or 26.66 mL, respectively, of the 30% emulsion. Tween was chosen as the emulsifying agent because it gave the greatest response compared with gum arabic and soy lecithin in Trials 1 and 2 (Table 1).

Medium-Chain Fatty Acid Analysis. Plasma concentrations of octanoate (C8) and decanoate (C10) were measured by a modification of the method described by Odle et al. (1991). Briefly, nonanoate (C9, Sigma Chemical) or radiolabeled octanoate (1-14C, 55 μCi/μmol, American Radiolabeled Chemicals, St. Louis, MO) were added to the .25 mL of plasma as an internal standard, and MCFA were extracted as described by Bligh and Dyer (1959), with the exception that the physiological saline used to induce phase separation also contained .2 mol/L of HCl and bromothymol blue pH indicator. The final chloroform fraction was evaporated and fatty acids were derivatized using phenacyl bromide and a crown ether catalyst (Phenacyl-8, Pierce, Rockford, IL). The fatty acid-phenacyl esters were separated using HPLC. A Beckman System Gold HPLC (Beckman Instruments, Palo Alto, CA), using a Beckman Ultrasphere C18 reversed-phase column (25 cm × 4.6 mm; 5 μm particle size), a convex acetonitrile gradient (from 43 to 100% over 32 min), and a flow rate of 1.5 mL/min
Table 1. Plasma octanoate (C8) and decanoate (C10) concentrations in 1-day-old pigs at 1 and 2 hours after gastric intubation of 6 mL of medium-chain triglyceride\( ^a \) in a 30% (vol/vol) Tween 80, gum arabic, or lecithin emulsion, normalized relative to nonemulsified control values

<table>
<thead>
<tr>
<th>Fatty acid, Trial 1</th>
<th>Fatty acid, Trial 2</th>
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<tbody>
<tr>
<td>C8</td>
<td>C10</td>
</tr>
<tr>
<td>Item</td>
<td>1h</td>
</tr>
<tr>
<td>NE Control(^a)</td>
<td>100(^f)</td>
</tr>
<tr>
<td>TWEEN</td>
<td>1,435(^f)</td>
</tr>
<tr>
<td>GUM</td>
<td>409(^f)</td>
</tr>
<tr>
<td>LECI</td>
<td>409(^f)</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>117</td>
</tr>
</tbody>
</table>

\(^a\)Captex 300, containing 75% C8 and 25% C10 on a molar basis.

\(^b\)= 6 pigs/treatment.

\(^c\)= 4 pig/treatment.

\(^d\)NE = nonemulsified control, TWEEN = Tween 80 (polyoxyethylene sorbitan monooleate), GUM = gum arabic/gum tragacanth, LECI = soy lecithin.

\(^e\)Absolute plasma concentrations measured were, from left to right, .141, .072, .042, .044, .090, .095, .017, and .023 mM.

\(^f\)Means within a column lacking a common superscript differ \( (P < .05) \).

was used to separate the MCFA esters. Peaks were detected by UV absorption at 242 nm, and the radioactivity in the phenacyl-C8 peak was determined by scintillation counting using a Beckman LS6000IC scintillation counter. For quantification of MCFA, peak areas were integrated, compared with standards, and corrected for recovery of internal standard that ranged from 71 to 95% in Trials 1 and 2, using radiolabeled C8 and from 91 to 111% in Trials 3 and 4, using C9.

Statistical Analysis. Data were analyzed using the GLM procedure of SAS (1985). In Trials 1 and 2, treatment means were compared using the protected LSD multiple comparison procedure, and, in Trials 3 and 4, the dose-response relationships were evaluated using orthogonal polynomials (Steel and Torrie, 1980).

Results

In Trial 1, plasma C8 concentrations in pigs given the 30% TWEEN emulsion (Table 1) were 14- and 20-fold higher than the concentrations measured in the NE controls and were 3.5- and 3.3-fold higher than concentrations measured in pigs fed the GUM emulsion at 1 and 2 h after the force-feeding of MCT \( (P < .05) \), respectively. Plasma C10 concentrations at 1 and 2 h were also two- to threefold higher for the TWEEN than for the NE and GUM treatments \( (P < .05) \). Plasma MCFA concentrations were not different between pigs given the GUM emulsion and the NE control animals \( (P > .1) \). In Trial 2, at 1 h, C8 concentrations in pigs given the TWEEN emulsion were 7.7- and 5.4-fold higher than levels in pigs given NE MCT and LECI emulsion \( (P < .05) \), respectively. Decanoate concentrations at 1 h, in pigs given the TWEEN emulsion, were approximately 1.7- and 1.4-fold higher than levels in pigs receiving NE MCT and LECI emulsion \( (P < .05) \), respectively.

Figure 1. Plasma octanoate (C8) concentrations \( (\text{mM}) \) in 1-d-old pigs at 1 and 2 h after intubation with 3, 6, 9, or 12 mL of nonemulsified medium-chain triglyceride oil [MCT, Captex 300, containing 75% C8 and 25% C10 on a molar basis; \( n = 8 \) pigs/treatment]. Linear effect of MCT dosage at 1 and 2 h \( (P < .05) \). Quadratic effect of MCT dosage at 1 h \( (P < .1) \).
MCT dosage was observed at 1 h, C10 plasma concentrations were not different; was greatest (P < .05) at 2 h (Figure 1). Linear effect of MCT dosage at 1 and 2 h after intubation with 2, 4, 6, or 8 mL of medium-chain triglyceride oil (MCT, Captex 300, containing 75% C8 and 25% C10 on a molar basis) in a 30% (volume/volume) Tween 80 emulsion (n = 6 pigs/treatment). Linear effect of MCT dosage at 1 and 2 h (P < .05). Cubic effect of MCT dosage at 2 h (P < .05).

Figure 2. Plasma octanoate [C8] concentrations [mM] in 1-d-old pigs at 1 and 2 h after intubation with 2, 4, 6, or 8 mL of medium-chain triglyceride oil (MCT, Captex 300) at 1 mL. Octanoate concentration at 2 h increased linearly (P < .05) with increasing dose (Figure 1). Octanoate concentration was greatest (.7 mM) in pigs given 9 mL of MCT and was lower (.4 mM) in pigs given 12 mL of MCT (quadratic tendency, P < .1). At 2 h, a linear effect of MCT dosage was observed (P < .05). There were no differences in plasma C10 concentrations (P > .1; data not shown). In Trial 4, pigs fed emulsified MCT showed a response similar (Figure 2) to that of pigs fed nonemulsified MCT (Trial 2, Figure 1). Specifically, plasma C8 concentrations at 1 and 2 h after feeding increased linearly (P < .05) with increasing dose of emulsified MCT oil up to 8 mL. In addition, a cubic effect of dosage level (P < .05) was seen at 2 h. At 1 h, C10 plasma concentrations were not different; however, linear and cubic effects of MCT dosage (P < .05) were detectable at 2 h (data not shown). In 8 of 12 pigs given 6 or 8 mL of emulsified MCT, a transient comatose state was observed that began as early as 40 min after feeding and lasted for up to 2 to 3 h. During maximal sedation, the pigs gave no response to toe-pinch, but all animals eventually recovered.

Discussion

In Trials 1 and 2 the relative efficacy of three emulsifying agents in enhancing MCT utilization was evaluated. One- and two-hour blood sampling times were chosen based on research by Odle et al. (1989, 1991), wherein the transient increases in plasma MCFA concentrations were highest at 1 h after an oral dose of 12 mL of nonemulsified MCT. Similarly, we used pigs that were older than 24 h of age because Odle et al. (1989) reported developmental increases in response between 6 and 18 h with no further change through 48 h. Food-grade emulsifiers were chosen based on their use in previous research (Hagerman et al., 1969; Desnuelle and Savary, 1963). Under the conditions studied, Tween 80 was more effective than gum arabic or soy lecithin in elevating plasma MCFA after MCT feeding. Emulsification with Tween 80 increased plasma octanoate concentrations over NE control values by 7- to 20-fold. Although plasma C8 concentration in pigs fed the GUM emulsion seemed to be higher than observed in the NE controls, it, along with the concentration in pigs given the LECI emulsion, was not statistically different from the concentrations observed in NE controls.

Tween 80, a nonionic detergent consisting of fatty acid esters of polyoxyethylene sorbitan, emulsifies by surrounding oil droplets and alters the availability of substrate to lipase (Wills, 1965). In a study conducted by Hagerman et al. (1969), Tween 80 completely inhibited the hydrolysis of MCT by pancreatic lipase in vitro. Addition of taurocholate and linoleate restored pancreatic hydrolysis of MCT. A companion in vivo study using rinsed rat intestine (rinsing lowered lipase and bile salt levels) also showed that MCT digestion and/or absorption was impaired by Tween 80. The amount of MCT absorbed was 25% lower in rats fed Tween 80 emulsions than in rats given gum arabic emulsions. Because gum arabic did not inhibit the action of pancreatic lipase in the presence or absence of bile salts, the authors concluded that the 25% decrease associated with the Tween 80 emulsion was due to the absence of bile salts. Thus, it was surprising in our study that the Tween 80 emulsion increased MCFA concentrations more than NE and GUM treatments. The reduced efficacy of soy lecithin may be explained by the observation that lecithin favors formation of water-in-oil emulsions (Fox, 1986), and thus the amount of oil (30% in Trial 2) may have been too low for optimal emulsion conditions.

Little is known about the early ontogeny of bile acid excretion or gastrointestinal lipase activity (including co-lipase) in the pig. Walker (1959) reported that the volume of bile was small in the 1-d-old pig (generally < 1 mL) and increased slowly over the first 3 wk of life, and then rapidly thereafter. Although pigs are able to utilize finely dispersed milk lipid efficiently, digestibility coefficients exceeding 95% at 2 d of age (Frobish et al., 1967), others have reported benefits to adding lecithin or monoglyceride to diets containing lard for weanling pigs (Jones et al., 1992).

Emulsification of fats is essential for their digestion by pancreatic lipase, because this enzyme works at the oil/water interface of emulsions (Borgstrom and Erlander, 1971). Furthermore, co-lipase is necessary to remove the inhibition imposed by bile salts. Emulsifi-
cation also decreases the size of the lipid droplets, thereby increasing the interfacial area of lipid exposed to the lipase. Emulsification aids lipid absorption as well. To reach the enterocyte for absorption, the products of triglyceride digestion must traverse the unstirred water layer adjacent to the brush border (Wilson et al., 1971). Micelles and liposomes, stabilized by the negative charges carried by bile salt molecules, serve as vehicles for diffusion of nonpolar substances across this water environment (Rombeau and Caldwell, 1990). Short- and medium-chain fatty acids are more soluble than long-chain fatty acids in the watery chyme and can diffuse through the unstirred water layer without the assistance of the micelle. However, even within the family of short- and medium-chain fatty acids, Sallee and Dietschy (1973) observed a twofold increase in the diffusion coefficient (square centimeters per second $\times 10^{6}$) as chain length decreased from C12 (7.6) to C2 (16.7). The authors suggested that increased partitioning into micelles occurs as chain length increases. In the neonatal pigs, endogenous sources of bile salts, lecithin, and cholesterol, which act as emulsifying agents and are essential components of micelles and liposomes, may be limited because bile output is decreased. Therefore, we hypothesized that administration of emulsified MCT would be an effective way to increase digestion and absorption of MCT. The 7- to 20-fold increase in MCFA concentrations for TWEEN vs NE is evidence that emulsification was indeed beneficial.

Trials 3 and 4 were conducted to determine the dose-response relationship between the amount of nonemulsified and emulsified MCT administered and the resulting plasma concentration of MCFA. Plasma concentrations at 1 h were linear through the 9-mL dosage of nonemulsified MCT. The quadratic components of the dose-response relationships (Figures 1 and 2) were in general agreement with previous reports. Specifically, Chiang et al. (1990) found that maximal absorption and oxidation of [1-$^{14}$C]trioctanoin occurred at a dose of approximately 6 g/kg$^{75}$, compared with dosages of 3 and 11.5 g/kg.$^{75}$ Assuming an average 24-h-old pig weighs 1.5 kg, this equates to approximately 8 mL of MCT. Thus, according to their estimate, optimal digestion, absorption, and oxidation of nonemulsified MCT should occur at dosages of approximately 8 to 9 mL for animals of the size (i.e., 1.5 kg) used in our studies. Benevenga et al. (1989) likewise showed that 12 mL of MCT fed to newborn pigs had no effect on colostrum consumption, whereas 24 mL reduced intake, suggesting that an upper limit had been exceeded.

The 6- and 8-mL doses of emulsified MCT oil also resulted in vomiturition, lethargy, and narcosis in approximately 40 and 65% of the animals, respectively. Pigs typically recovered between 2 and 3 h after MCT gavage. The comatose state seemed to be deepest during the interval (0 to 3 h after feeding) when plasma MCFA levels are typically at their highest. This may explain in part the report by Lepine et al. (1989), who conducted a field study to evaluate the efficacy of MCT to increase survival rates of pigs. Supplementation with two 25-mL intubations of MCT during 24 h caused pigs to become lethargic and reduced survival by 25% at 30 h and by 12% at 21 d compared with saline-dosed controls. This was likely due to the excessive dose of MCT that provided energy (> 400 kcal) well beyond the pigs’ daily energy needs (i.e., 90 kcal/d; Odle et al., 1992). These observations suggest that high dosages and emulsification of MCT may result in accumulation of systemic MCFA concentrations to toxic levels. Researchers reported as early as 1956 that intravenous and intraperitoneal injections of the salts of short- and medium-chain fatty acids produce reversible unconsciousness in rats, chicks, mice, dogs, and guinea pigs (Samson et al., 1956; Sanbar et al., 1965). In fact, an octanoate-induced, reversible coma has been previously used as a model for Reye’s syndrome (Trauner and Huttenlocher, 1978). Samson et al. (1956) showed that the amount of fatty acid anion that produced narcosis in 50% of a sample of rats decreased (23 to 5 mmol/kg) as chain length increased from C4 to C8. More recently, the mechanism underlying MCFA-induced cerebral coma has been investigated. Trauner (1980) suggested that fatty acids produce coma and hepatic encephalopathy by inhibition of cerebral Na$^{+}$K$^{+}$ATPase activity with resultant disruption of normal transport across neuronal and glial membranes. In addition to effects from i.v. and i.p. injections, Greenberger and Skillman (1969) warned that oral feeding of MCT may cause indigestion and lethargy.

**Implications**

The results herein suggest that emulsification increases the rate of digestion and/or absorption of medium-chain triglycerides by pigs of normal birth weight. Under the conditions studied, Tween 80 was a more effective emulsifying agent than gum arabic or soy lecithin, suggesting that it may be the emulsifier of choice under practical conditions. Although emulsification could prove beneficial by improving the efficiency of medium-chain triglyceride utilization, caution must be exercised in selecting safe and adequate dosages because greatly elevated plasma medium-chain fatty acid concentrations may be intoxicating and could result in increased pig mortality. Whether supplementation of newborn pigs with medium-chain triglyceride will reduce preweaning mortality remains to be determined.
Literature Cited


