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Abstract

Inadequate milk production by sows often limits the growth of piglets. A successful lactation requires prolactin (PRL)-induced differentiation of the alveolar epithelium within the mammary glands of sows between days 90-110 of gestation. We hypothesized that induction of late gestational hyperprolactinemia in primiparous sows by oral administration of the dopamine antagonist metoclopramide (MET) would enhance mammary epithelial differentiation, milk yield, and piglet growth rate, and that these effects would carry over into a subsequent lactation. Twenty-six gilts were assigned to receive either MET (n = 13, 0.8 mg/kg) or vehicle (CON, n = 13) twice daily from days 90-110 of gestation. The same sows were followed into their second lactation without additional treatment. On day 90 of gestation, circulating PRL concentrations peaked 45 min after feeding MET ($P < 0.001$) then returned to baseline 3 h later. This response occurred daily out to day 104 of gestation ($P < 0.05$). Compared to CON, MET-treated gilts had enlarged alveoli on gestation day 110 ($P < 0.05$). Treatment with MET did not affect feed intake, body weight, or body fatness during pregnancy or lactation. Piglets born to MET-treated sows had both increased body weights and average daily gain on lactation days 14 and 21 ($P < 0.05$). Milk intake by piglets was estimated from deuterium oxide dilution. While milk intake by piglets nursing MET sows was not statistically different from those nursing CON sows on day 21 of lactation ($P = 0.18$), there was a greater increase in milk consumption by piglets born to MET-treated sows between days 9 and 21 of lactation than for those in CON litters ($P < 0.001$). In one group of second parity sows (n=11) that were treated with MET during their first gestation, milk yield increased by 21% during their second lactation ($P < 0.05$) in association with a 14% decline in body fatness across lactation compared with a 7% decline in CON sows ($P < 0.05$). These findings demonstrate that MET-induced hyperprolactinemia in primiparous sows during late pregnancy can increase milk yield and piglet growth rate, setting the stage for further large-scale studies.

1. Introduction

Milk production by sows often limits the growth of their piglets, as evidenced by their increased average daily gain during the first 10-30 days of life in response to supplemental milk [1]. Given that postweaning growth of pigs also reflects nutrient availability during the first three weeks of life [2], there is a clear need and opportunity to increase the lactation performance of sows. While insufficient milk production by sows can be mitigated by providing creep feed to piglets, or a high-energy diet to sows, these strategies are not always successful or optimal [3].

Successful lactation requires differentiation of the mammary alveolar epithelium during late gestation [4], which is mediated by the actions of pituitary-derived prolactin (PRL). The critical period for PRL action on the differentiation of mammary epithelial cells was narrowed to days 90-109 of gestation (G90-109), where bromocriptine-induced activation of dopamine type II receptors (D_2R) diminished PRL secretion, mammary development, and subsequent milk production, and led to stunted piglet growth [5]. By targeting this same PRL-sensitive period, VanKlompenberg *et al*. [6] demonstrated that pharmacologic induction of low-level hyperprolactinemia by the D_2R antagonist domperidone (DOM) led to precocious alveolar differentiation during late gestation. Those gilts subsequently produced more milk during their first lactation and weaned piglets that were 21% heavier than controls.

Domperidone is not approved for use by humans in the United States due to risk for sudden cardiac death, thus limiting its potential extension to livestock production. Another D_2R antagonist, metoclopramide (MET), is FDA-approved [7] but, unlike DOM, crosses the blood-brain barrier. Previous studies have demonstrated that MET can increase milk yield when administered to dogs, humans, and horses during pregnancy or lactation [8-10]. Given the positive effect of DOM [6], the objective of this study was to test the effects of MET on the pharmacokinetics of PRL secretion,

68 mammary differentiation and milk yield, subsequent piglet growth and sow body composition, and to
69 determine whether these effects were conserved in the subsequent parity.

70 *2. Methods*

71 *2.1 Animals and treatments*

72 A pilot study using 3 primiparous pregnant gilts (211.3 ± 13.0 kg) was performed to determine
73 the optimal dose of MET required to induce a hyperprolactinemic state similar to that previously
74 induced by domperidone (DOM), which was administered at 0.4 mg/kg [6]. Hereafter, all doses of MET
75 are reported as those calculated based on G89 live bodyweight, and remained the same throughout the
76 treatment period. Dosing rate is expressed per dose, which was administered twice daily. For the pilot
77 study, MET was fed at either 0.4 mg/kg (n = 1) or 0.8 mg/kg (n = 2) twice daily from G90-110 prior to
78 the collection of plasma (collected on G89 and daily at 0745h until G110) for assay of porcine PRL
79 (pPRL). The conclusion from the pilot study was that MET administered twice daily at 0.8 mg/kg
80 induced a level of hyperprolactinemia similar to that induced by DOM.

81 An experiment to assess the effect of MET on subsequent lactation performance was conducted
82 using two blocks, in the fall of 2017 (n = 8 CON, n = 8 MET; Yorkshire-Hampshire crossbred) and the
83 fall of 2018 (n = 5 CON, n = 5 MET; PIC maternal line in the 2018 group). Average body weight of
84 these primiparous gilts at the start of the study was 212.8 ± 7.08 kg (CON) and 215.8 ± 7.14 kg (MET),
85 where all females were from littermate pairs that were assigned randomly to the CON or MET group.
86 Gilts were bred to a Yorkshire-Hampshire crossbred boar in 2017, and a PIC boar in 2018 (PIC-359,
87 Birchwood Genetics, Manchester, OH).

88 Gilts received MET (0.8 mg/kg; Teva Pharmaceuticals, North Wales, PA, USA, suspended in 1:1
89 corn syrup:water excipient) every 12h. The average mass of MET administered throughout the treatment
90 period was 172.7 ± 7 mg. Control gilts received excipient only. Gilts were dosed via drenching at 0800

and 2000h from G90-110 relative to expected farrowing date (G114). Gestating gilts consumed 2.8 ± 0.08 kg (CON) or 2.8 ± 0.12 kg (MET) of a standard gestation diet daily (13.8% crude protein, 0.65% lysine). Sows consumed 3.4 ± 0.24 (CON) kg or 3.3 ± 0.27 kg (MET) per day of a standard lactation diet (17.88% crude protein, 1.01% lysine) for the first 2 days after farrowing, then were fed the same diet *ad libitum* until weaning. Feed intake in both blocks was measured daily at 0800 and 2000 h during lactation. All sows had *ad libitum* access to water.

Gilts were weighed on G89 and on day 21 of lactation (L21). In 2017, gilts were housed in individual pens from G90 until weaning. In 2018, gilts were group-housed within treatment until G112 when they were moved into farrowing crates. Backfat thickness was measured via ultrasound at the 10th rib on L1 and L21 in all sows, allowing body fat percentage to be estimated using an equation adapted from Shields *et al*. [11].

The day of parturition was considered L1, and piglets were processed by L3. Litters were standardized to n = 10 piglets in 2017 and n = 12 piglets in 2018 within 72 h of farrowing. Piglets were weighed weekly until the end of lactation. No creep feed was provided. In one group of piglets weaned from sows treated in the fall of 2017 (n = 10 CON, n = 15 MET piglets), body weights were obtained throughout the grower phase until slaughter, with chilled carcass weight and backfat thickness (measured using calipers by a treatment-blinded individual) obtained post-slaughter.

2.2 Biopsies and mammary tissue processing

Mammary tissue was collected on G110 and L21 using a vacuum-assisted biopsy device [12]. At each time point, the same gland was sampled for each animal (G110, left 3rd mammary gland; and L21, right 3rd mammary gland), and that gland was only biopsied once. Two cores of tissue were collected; one was snap frozen and stored at -80°C, while the other was fixed in 10% formalin overnight at 4°C, then transferred to 70% ethanol. Fixed tissue was embedded in paraffin then sectioned at 4 µm. One

114 section per animal was stained with hematoxylin and eosin to assess morphological changes between
115 treatments. Alveolar lumen diameters were calculated from perimeters measured on n = 10 alveoli/field
116 in images captured at 10X using ImagePro Express (Media Cybernetics, Rockville, MD). Measurements
117 were made by an individual blinded to treatment [6].

118 *2.3 Blood sampling*

119 In 2017, n = 16 gilts (n = 8 CON, n = 8 MET) were anesthetized on G88 and fitted with a jugular
120 catheter (14 G, 16 cm, 0.32 in diameter, Arrow International, Reading, PA) for intensive blood sampling
121 during the treatment period. Blood samples (5 ml) were collected into sodium heparin tubes at 0745h on
122 G89, 90, 91, 97, 104, 110, and L1, 7, 14, and 21. To evaluate PRL kinetics during MET administration,
123 serial blood sampling via the jugular catheter was performed on G90 at -15, 0, 15, 30, 45, 60, 120, 180,
124 360, 540, and 720 min relative to the first dosing with MET or CON. All other samples were collected
125 via jugular venipuncture. Blood samples were immediately placed on ice prior to centrifugation at 3,700
126 x *g* for 10 min at room temperature to yield plasma that was stored at -20°C until analysis.

127 The MET-induced PRL kinetics on G90 in the 2017 cohort indicated that plasma PRL peaked
128 approximately 45 min post-dosing. Subsequently, in 2018, blood samples were obtained from gilts 45
129 min after the evening dosing with MET (n = 10) to determine the persistency of the short-term MET-
130 induced PRL release. Blood samples were collected in the fall of 2018 at 2045h on G89, 90, 91, 97, 104,
131 110, and L1, 7, 14, and 21.

132 *2.4 Radioimmunoassay (RIA) and non-esterified fatty acids (NEFA)*

133 The concentration of NEFA in plasma was measured in duplicate using a commercial kit
134 according to the manufacturer's instructions (NEFA-HR, Wako Chemicals USA Inc., Richmond, VA).
135 Plasma PRL was measured using an RIA for pPRL [6]. Purified pituitary pPRL (2 ug, National
136 Hormone and Peptide Program, Torrance, CA) was labeled with 1 mCi Na-I^{125} using chloramine-T

137 (Sigma-Aldrich). Plasma and standards (100 µl each) were included with a rabbit α-pPRL antibody (250
138 µl of a 1:10000 dilution; National Hormone and Peptide Program) overnight followed by incubation
139 with ^{125}I-pPRL (50 µl of 20,000 counts per minute) at 4°C for 48 h at 4°C, and precipitation with anti-
140 rabbit-antiserum (1 ml, 1:200, Sigma-Aldrich, St. Louis, MO) and 50 µl of rabbit gamma globulin (0.2
141 mg/ml, Sigma-Aldrich). After centrifugation (3400 rpm, 4°C for 30 min), bound ^{125}I-pPRL was
142 measured via gamma counter and analyzed using a log/Logit standard curve of pPRL. Assay sensitivity
143 was 0.25 ng/ml, intra- and inter-assay variation was 7.2 and 11.3%, respectively. Recovery of 1 ng of
144 pPRL spiked into diluted serum was 125%. Linearity was assessed by dilutional parallelism of serum.
145 Observed to expected ratios for serial dilutions ranged from 73% to 151% (average of 108%).
146 *2.5 Milk production*
147 Milk production was measured on days L7-9 and L19-21 in three piglets per litter, with another
148 piglet serving as an internal control, using the deuterium oxide dilution (D_2O) method described by
149 Theil *et al.* [13]. Colostrum was also collected from all sows within 3h of the start of farrowing, without
150 the use of oxytocin. Milk consumption was determined over 48 h, with the same schedule followed for
151 all piglets. After a 2 h fast, a serum sample was obtained for background deuterium levels, followed by
152 an injection of D_2O that was equilibrated with piglet body water for 1h prior to injection (0.5 mg/kg of
153 20% D_2O, 99.9% purity, Thermo Fisher, San Diego, CA, in 0.9% saline) or 0.9% saline. Deuterium was
154 allowed to equilibrate in the piglet for 1 h, prior to collection of a second blood sample to measure
155 maximum enrichment. Piglets were replaced with the sow and left undisturbed for 48 h. Thereafter,
156 piglets were fasted for 2 h before a third blood sample was collected to determine total body water
157 turnover. Milk samples were collected on the last day of D_2O measurements (on L9 and L21) by
158 oxytocin-induced milk ejection (1ml IM, 20 IU/ml; VetOne, Boise, ID). Each milk and colostrum
159 sample was a composite from the anterior, middle, and posterior glands. Milk samples were frozen

160 immediately at -20°C until analysis for protein, fat, lactose, solids, and water content using Fourier-
161 transformed infrared spectroscopy (LactoScope Advanced, Delta Instruments, Norwood, MA). Serum
162 samples were analyzed for the atomic fraction of deuterium via Laser Water Isotope Analyzer V2 (Los
163 Gatos Research, Inc., Mountain View, CA, USA) at the UC Davis Stable Isotope Facility. Milk
164 production was estimated from the atomic fraction of deuterium in the serum samples from piglets after
165 adjustment for the milk composition of each sow, using the equations developed by Theil *et al.* [13].

166 *2.6 Second lactation*

167 After weaning, all sows (n = 10 CON, n = 9 MET) were synchronized with Matrix (Merk
168 Animal Health, Madison, NJ), re-bred to a PIC boar (PIC-359, Birchwood Genetics, Manchester, OH),
169 then followed into their second lactation that occurred in the Spring of 2018 and 2019 (Fall 2017 and
170 2018 cohorts, respectively) without additional treatment. Litters were standardized to n = 12 piglets per
171 litter, and were weighed weekly. Mammary biopsies were performed as above on G110 (Spring 2019
172 only, n = 5 CON, n = 4 MET), and on L21 (Spring 2019 n = 5 CON, n = 5 MET, Spring 2018 n = 5
173 CON, n = 5 MET), with samples subsequently analyzed for alveolar lumen diameter as described above.
174 Milk samples were collected from all sows for standard composition analysis on L21, and milk yield
175 was determined on L19-21 in both groups via D_2O dilution as described above. Body fatness was
176 estimated based on ultrasound measurements as described above.

177 *2.7 Statistical Analyses*

178 *2.7.1 First lactation statistics*

179 During the first lactation, PRL concentrations measured throughout gestation and lactation, milk
180 yields, milk and colostrum composition, piglet body weights, piglet average daily gain, gilt body
181 weights, circulating NEFA concentrations, grower pig live weights, and lumen diameter were analyzed
182 by a linear mixed model with repeated measures. Gilt and block (2017 or 2018) were the random effects,

and treatment and time of sampling were fixed effects. Grower pig carcass weights, backfat thickness, and circulating pPRL on G104 before and after MET treatment were analyzed using an unpaired t-test.

2.7.2 Second lactation statistics

Lumen diameter and piglet body weights throughout lactation were analyzed by a linear mixed model. Gilt and block were the random effects, and treatment and time of sampling were fixed effects. Milk yields and sow body fatness percentages were analyzed using an unpaired t-test. Normality was verified by a nonsignificant D'Agostino-Pearson omnibus normality test and a Shapiro-Wilk normality test, and all data were analyzed using Prism8 (Graphpad Software). Statistical significance was declared at $P < 0.05$.

3. Results

3.1 Plasma prolactin

To test the effect of *per os*-administered MET on circulating PRL, gilts were dosed from G90 to G110 (Figure 1A). After 30 min of MET there was a tendency for elevated PRL, and after 45 min, PRL concentrations had significantly increased from 7 ng/ml to 43 ng/ml ($P < 0.01$) and remained elevated for 2 h ($P < 0.05$, Figure 1A).

The effect of daily MET from G90-110 on circulating PRL every week throughout gestation and lactation (by sampling at 0745h, 12 h after the evening dose, Figure 1B) allowed us to compare the MET-induced PRL response to that induced by DOM as reported by VanKlompenberg *et al*. [6] and Farmer *et al*. [14]. On day G90, PRL concentrations measured 12 h after dosing were greater in MET-treated than CON gilts ($P < 0.001$), but then remained similar for both groups until G104 (Figure 1B, treatment x time $P < 0.001$). When plasma PRL levels were measured 45 min after the evening dose of MET, circulating PRL remained greater in MET gilts than CON gilts from G90 to G104 ($P < 0.05$, Figure 1C, treatment x time $P < 0.001$). To determine whether PRL was reduced to baseline

206 concentrations similar to what we observed in Figure 1B, we analyzed circulating PRL before and after
207 MET dosing in n = 2 gilts on G104. The concentration of PRL 15 min before MET administration on
208 G104 (12 h after the morning dose) was 17.4 ng/ml, then was 24.1 ng/ml at 45-min after MET dosing (P
209 = 0.07, Figure 1D).

210 *3.2 First lactation phenotype*

211 Bodyweight of piglets in CON and MET litters was not different on L1 and L7 (1.36 $\pm$ 0.06 kg
212 versus 1.42 $\pm$ 0.06 kg, $P > 0.10$, Figure 2A). The average co-efficient of variation (CV) for CON and
213 MET litters on L1 was 13.7% and 14.0%, respectively. By L14 and L21, piglets raised by MET-treated
214 sows were 6% and 11% heavier than their CON counterparts (Figure 2A, $P < 0.001$), where the average
215 daily gain of piglets nursing MET-treated sows was 16% greater than for piglets from CON litters
216 (Figure 2B, $P < 0.05$). At weaning, average bodyweight of piglets in CON and MET litters was 5.55 $\pm$
217 0.28 kg (CV 17.7%) and 6.11 $\pm$ 0.26 kg (CV 14.4%), respectively.

218 Piglet milk intake was measured indirectly using D_2O dilution. On days L7-9, piglets raised by
219 CON and MET sows consumed an average of 475.9 g and 455.2 g of milk per day, respectively ($P >$
220 0.10, Figure 3). On days L19-21, piglets from MET-treated sows consumed an average of 655.3 g/d
221 while those nursing CON-treated sows consumed 561.2 g/d (Figure 3, $P = 0.18$). Milk intake by piglets
222 nursing MET-treated sows on L21 was 44% higher compared to that on L9 ($P < 0.001$), whereas there
223 was only an 18% increase in milk intake by CON piglets during the same period ($P = 0.17$, Figure 3).
224 There was no difference ($P > 0.10$) between groups for the composition of sow colostrum (Table S1) or
225 milk (Table S2), nor was there any difference in sow feed intake (Figure S1), sow body weight or body
226 fatness during gestation or lactation (data not shown).

227 On G110, the average lumen diameter of mammary gland alveoli was 21% greater in MET-
228 treated compared to CON gilts (Figure 4, $P < 0.05$). On L21 the average lumen diameter did not differ

between the two groups (Figure 4). There were no treatment effects on circulating NEFA in sows (Figure S2). Growth and feed intake of piglets from the Fall 2017 litters, from weaning to slaughter, are depicted in Figure 5. There was no treatment effect on either weekly feed intake (data not shown), or body weight gain ($P > 0.10$; Figure 5A). At slaughter, the carcass weight and backfat thickness did not differ between groups ($P > 0.10$, Figure 5B and 5C).

3.3 Second lactation phenotype

On L21 of the second lactation, average body weight of piglets from sows treated with MET during the first parity (fall 2017 and 2018 groups) was 9% greater than that for piglets from CON sows ($P = 0.10$; Figure S3). Milk yield for second parity sows from the fall 2017 group was not affected by treatment (Figure 6A). By contrast, the second parity sows in fall 2018 that were previously treated with MET during their first gestation had piglets that ingested 21% more milk on days L19-21 than CON piglets ($P < 0.05$, Figure 6B). Milk composition was not different between CON and MET sows ($P > 0.10$, data not shown). In fall 2018, the second parity sows lost 14% of their estimated body fatness between days L1 and L21 compared with 7% for CON sows ($P < 0.05$; Figure S4). There were no treatment differences in the alveolar lumen diameter in mammary tissue from second parity sows on L21 (Figure S5).

4. Discussion

Administering MET to gilts from G90-110 improved piglet weight at weaning and increased their subsequent milk production between days L7-9 and L19-21 of the first lactation. These responses mimic those we recorded previously when first-parity gilts were treated with DOM using a similar treatment regimen in late gestation [6]. They also align with histological evidence of precocious alveolar expansion within the mammary glands, consistent with the increased and earlier differentiation of the mammary epithelium [6]. The similar response to oral-dosing with either MET or DOM highlights that

252 improved lactation performance by first parity sows can be stimulated by 20 days of pharmacologically-
253 induced low-level hyperprolactinemia. This finding builds on the recognized importance of PRL for
254 growth and differentiation of the mammary epithelium for first-parity gilts during the same period [5].
255 These combined data warrant further corroboration via larger studies in a commercial setting.

256 In an interesting contrast to these outcomes, others found that sustaining high levels of PRL
257 during late gestation negatively impacted aspects of lactogenesis and galactopoiesis in gilts. When King
258 *et al.* [15] administered a high dose of recombinant pPRL (15 mg I.M.) to pregnant gilts each day
259 between G102 until weaning, serum PRL concentrations reached 1963 ng/ml. Those PRL-treated sows
260 produced 22% less milk, coincident with clear evidence of precocious lactogenesis at G110. More
261 recently, we administered daily I.M. injections of DOM to primiparous gilts for either 7 or 20 days
262 during gestation to induce sustained and higher serum PRL concentrations relative to those induced by
263 oral therapy here [16]. That approach led to precocious lactogenesis within the mammary glands by
264 G110, which was also associated with clear signs that the mammary parenchyma had started to regress,
265 almost certainly due to milk stasis.

266 Taken together, these past and present findings indicate that mild hyperprolactinemia during the
267 first parity can stimulate early lactogenesis [6], a phenotype that may be sustained into the subsequent
268 lactations (as demonstrated by one of the two cohorts in this study). On the contrary, high levels of PRL
269 during late pregnancy have an inhibitory effect on first lactation milk yield by virtue of the precocious
270 lactogenesis, extensive milk stasis and subsequent alveolar regression [5,15]. The benefit from short-
271 term hyperprolactinemia appears to be due to earlier differentiation of the mammary epithelium, rather
272 than increased mammary growth [6]. The galactopoietic benefit following low level hyperprolactinemia
273 during gestation also aligns with other physiological situations. For example, ewes bearing twins or
274 triplets have elevated PRL levels and produce more milk than those bearing singletons [17]. Similarly,

goat does bearing twins or triplets had respective milk yields that were 29 and 47% greater than those bearing singletons [18]. Given that the placenta of sheep and goats (but not pigs; [19]) is a rich source of non-pituitary lactogenic hormones (e.g. placental lactogen), these data further support the prospect for enhancing lactogenesis in swine via low level hyperprolactinemia during late gestation. How factors such as the pulsatility or frequency of elevated PRL during the period from G90-110 fully impact the galactopoietic outcome remain to be explored. Likewise, the dose response range and timing of DOM or MET administration within the G90-110 window that confers and maximizes these benefits remain to be established.

Administering MET *per os* led to a transient increase in serum PRL for 45 min after dosing, followed by a decrease to baseline within 6 h, where this transient response lasted from G90 to G104. When DOM was administered using a similar regimen [6], PRL levels were elevated for up to six days (G97) when they were measured 12 h after the evening administration of DOM, although the kinetics of PRL release over 12 h were not determined. When DOM was administered I.M. to pigs there was sustained elevation of PRL for up to 20 days [14]. Brouwers *et al*. [20] reported that in human subjects MET had a more prolonged ability to induce hyperprolactinemia than DOM, likely due to differences in its ability to cross the blood brain barrier.

In a previous study VanKlompenberg *et al.* [6] found that DOM-induced gestational hyperprolactinemia led to persistent gene expression changes within the mammary epithelium throughout the first lactation. One potential explanation for that phenotype was an altered epithelial epigenome, which prompted us to examine herein whether the hyperprolactinemia-induced lactation benefit during the first parity might also carry into the second lactation. Our findings here were inconsistent; MET sows from one experimental block had significantly higher milk yield (21%) than CON sows on day 21 of their second lactation, whereas those in the other block had 8% lower milk

298 production. Milk protein expression [21] and alveologenesis [22] are epigenetically regulated via altered
299 methylation in the mammary glands during pregnancy and lactation, where hypomethylation of the
300 STAT5A gene is conserved following pregnancy and lactation [23]. In cows, the STAT5-binding
301 enhancer that activates αS1-casein gene expression is also hypomethylated during lactation, although
302 whether this persists after lactation is unknown [24,25]. The transcriptome of the mammary glands
303 during pregnancy and lactation in sows has been profiled, albeit not followed into subsequent lactations
304 [26]. An additional consideration is that there may have been a genetic basis for the differences observed
305 between the experimental blocks into the second lactation given that different sow lines were used, in
306 keeping with the widespread recognition of genetic differences in milk production and composition
307 across breeds [27]. Our findings of a potential trans-parity effect of hyperprolactinemia during the first
308 gestation, while inconclusive, warrant further investigation.

309 Our findings have potentially-significant practical implications given that sow milk yield can be
310 a major limitation for piglet growth and pork production [1, 2]. Sows that experience postpartum
311 dysgalactia can cost producers up to 500 dollars per sow annually, and piglets from those sows wean at
312 lower body weights [28]. Starting the second week of lactation, insufficient milk yield begins to limit
313 piglet growth rate which can subsequently delay the time for progeny to reach market weight [29]. In
314 this study, MET-treated sows weaned larger piglets that grew faster (0.93 kg/d MET vs. 0.84 kg/d for
315 CON piglets), albeit the difference was not significant. Others have shown that piglets weighing
316 between 6.8 and 8.2 kg at weaning reach market weight an average of 10 days faster than those weaning
317 at between 4.1 and 5.5 kg [30,31]. Given that the swine industry relies on efficient and inexpensive ways
318 to produce pigs [32], any intervention that can reduce these costs while producing the same product
319 stands to provide significant economic benefit.

320

321 *5. Conclusions*

In conclusion, twice-daily administration of MET from G90-110 led to post-dosing stimulation of PRL release until at least G104. The route and timing of administering dopamine antagonists such as MET appear to be critical for enhancing milk yield, alveolar lumen differentiation, and subsequent piglet performance. Our findings lend support to the concept that the growth rate of piglets can be improved by a prepartum therapeutic strategy targeted to transiently increase PRL secretion, and set the stage for further, large-scale studies.

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335
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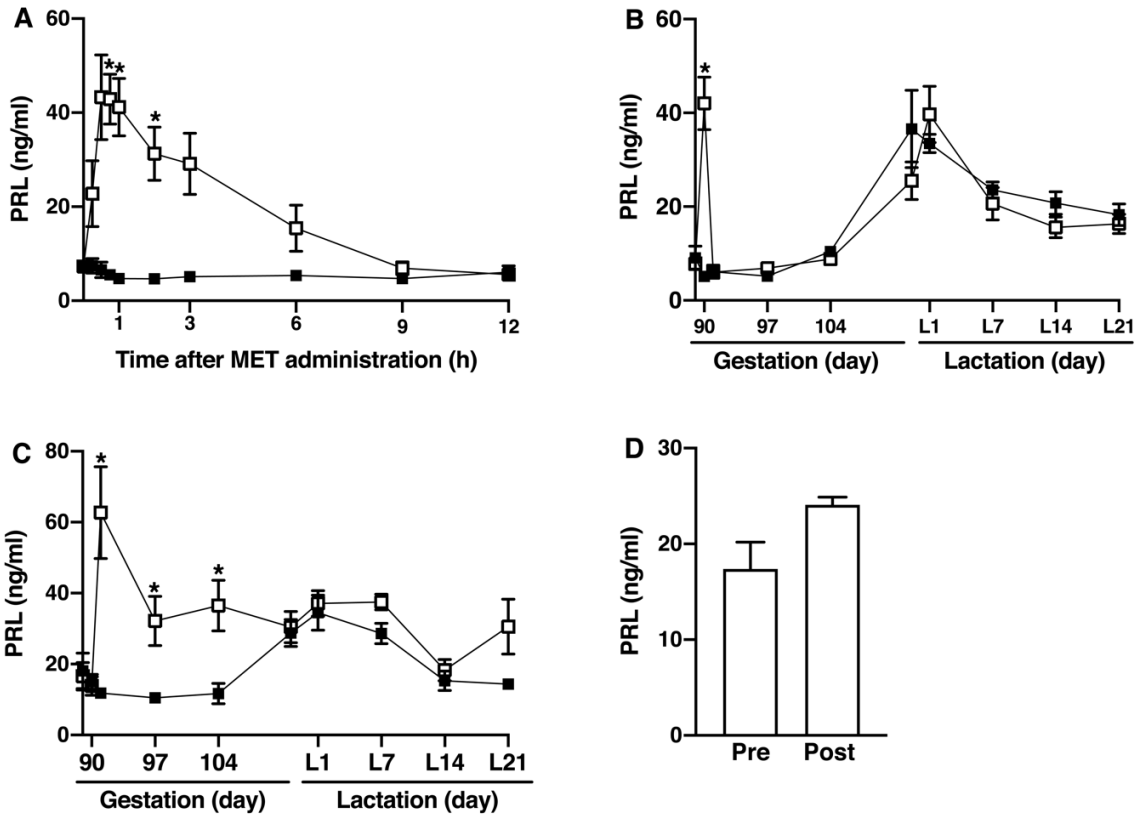
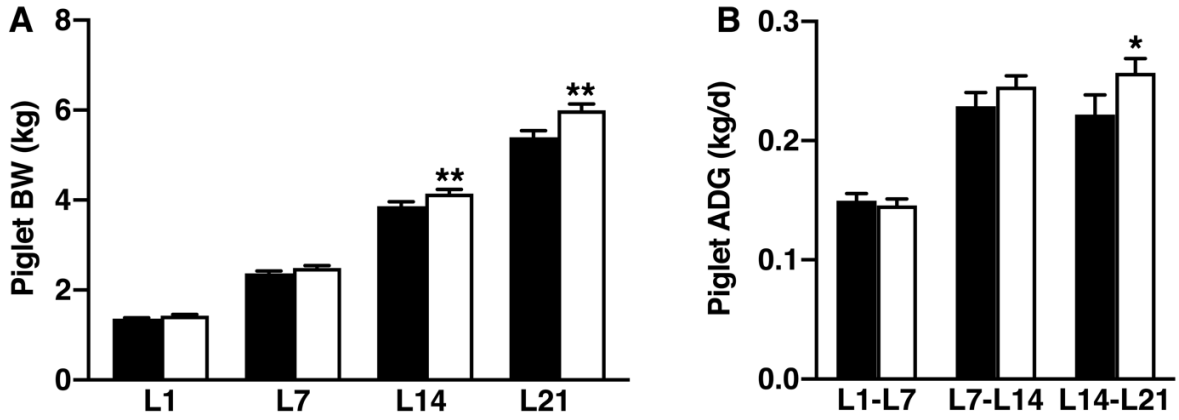
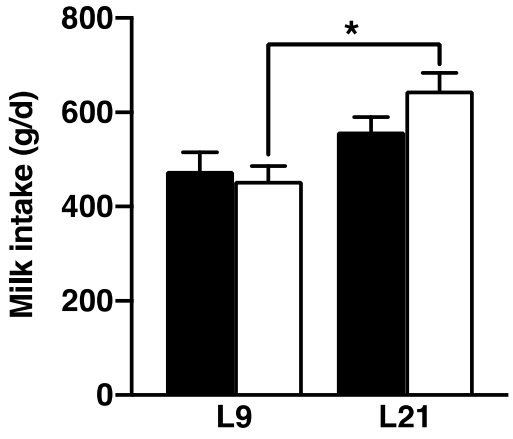
8. *Figures*


A
PRL (ng/ml)
Time after MET administration (h)
B
PRL (ng/ml)
Gestation (day)
Lactation (day)
C
PRL (ng/ml)
Gestation (day)
Lactation (day)
D
PRL (ng/ml)
Pre
Post


A
Piglet BW (kg)
8
6
4
2
0
**
**
L1
L7
L14
L21
B
Piglet ADG (kg/d)
0.3
0.2
0.1
0.0
*
L1-L7
L7-L14
L14-L21

425
426



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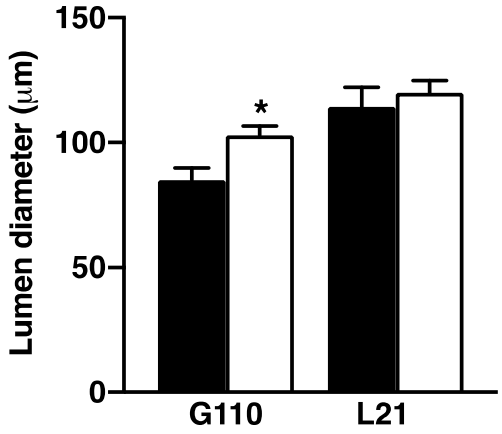

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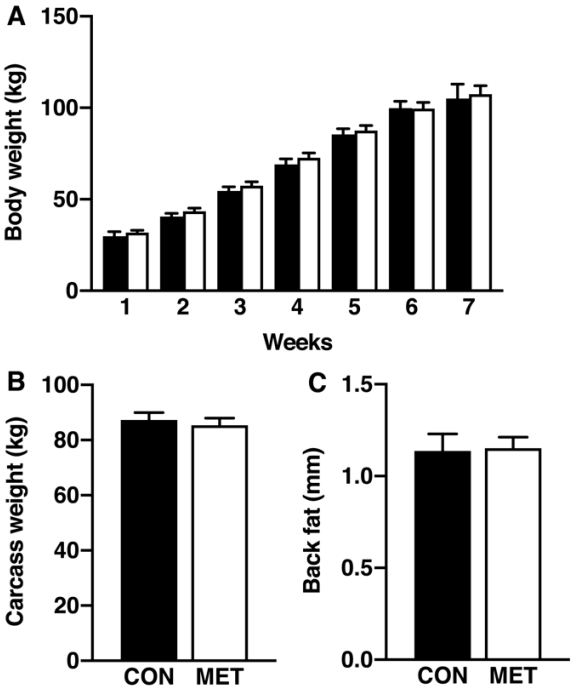
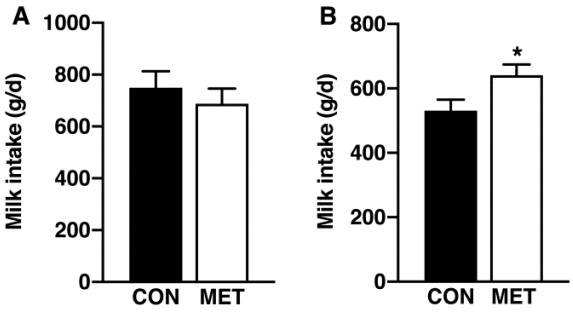

A
Body weight (kg)
150
100
50
0
1
2
3
4
5
6
7
Weeks
B
Carcass weight (kg)
100
80
60
40
20
0
CON
MET
C
Back fat (mm)
1.5
1.0
0.5
0.0
CON
MET

431
432


A
1000
800
600
400
200
0
Milk intake (g/d)
CON
MET
B
800
600
400
200
0
Milk intake (g/d)
*
CON
MET

433
434

9. Figure legends

Figure 1. Prolactin (PRL) concentrations in gilts treated with vehicle (CON; closed boxes) or metoclopramide (MET; open boxes) from gestation days 90-110 (G90-110). Figures A and B are from fall 2017, C and D are from fall 2018. **A,** Plasma was collected to measure PRL on the first day of treatment at day G90. Plasma was obtained at -15 min before MET dosing, then at 0, +15, 30, 45 mins, 1h, 2h, and then every 3h for up to 12h. (CON n = 8 gilts,1 missing data point; MET n = 7 gilts, no missing data points). **B**, PRL was measured in plasma collected at 0800h from G89 until day 21 of lactation (L21) immediately prior to the morning dosing with MET. (CON n = 8 gilts, 5 of which had 4 missing data points; MET n = 8 gilts, 7 of which had 3 missing data points). **C**, Circulating PRL was measured in plasma collected 45-min after the evening dosing with MET on G89, 90, 91, 97, and weekly thereafter until day L21. (CON n = 5 gilts, 3 of which had 1 missing data point3; MET n = 5 gilts, 3 of which had 1 missing data point). **D**, Plasma PRL levels 15 min before (Pre) and 45 min after (Post) MET dosing on day G104. (n = 2 gilts, no missing data points). All data are means ± SEM. * $P <$ 0.05.

Figure 2. Bodyweight change for piglets throughout lactation. **A,** piglet bodyweight on lactation (L) days 1-21 (CON L1 n=129, L7 n=126, L14 n=121, L21 n=120 piglets from n = 12 litters, MET L1 n=119, L7 n=115, L14 n=107, L21 n=102 piglets from n = 11 litters) and **B,** average daily gain (ADG; CON n = 97 piglets from n = 9 litters, MET n = 105 piglets from n = 10 litters). Piglets were from CON (closed bars) or MET (open bars)-treated sows. All data are means SEM. **$P <$ 0.001, *$P <$ 0.05. CON, vehicle-treated. MET, metoclopramide-treated.

Figure 3. Milk intake by piglets on lactation (L) days 9-21. Milk intake was estimated using the deuterium oxide dilution method in control (CON, L9 n = 26, L21 n = 25 piglets from n = 10 litters, closed bars) and metoclopramide (MET, L9 n = 25, L21 n = 26 piglets from n = 11 litters, open bars) groups. All data are means ± SEM. Treatment x time $P < 0.05$. MET L7-9 versus L19-21, $P < 0.05$.

Figure 4. Diameter of mammary alveolar lumenae during gestation and lactation. Diameter was determined from tissue biopsied on gestation day 110 (G110) and lactation day 21 (L21) from control (CON, G110 n = 10, L21 n = 11, closed bars) and metoclopramide (MET, G110 n = 13, L21 n = 11, open bars) sows. All data are means ± SEM. Treatment $P < 0.05$, MET G110 versus CON G110, $P <$ 0.05.

Figure 5. Grower body weight, carcass weight, and backfat in control piglets (CON, closed bars) and metoclopramide piglets (MET, open bars). **A**, Body weight was obtained bi-monthly (CON, weeks 1-5 n = 7, week 6 n = 6, week 7 n = 3 piglets; MET, weeks 1-5 n = 6, week 6 n = 4, week 7 n = 3). **B**, chilled carcass weight was obtained 24 h post-slaughter (CON, n = 10; MET, n = 15 piglets), and **C,** backfat was determined at slaughter (CON, n = 10; MET, n = 15 piglets). All data are means ± SEM.

Figure 6. Milk intake by piglets on lactation days (L) 19-21 of the second lactation for control (CON, closed bars) and metoclopramide (MET, open bars) sows . **A,** Sows previously administered CON (n = 15 piglets from 5 litters) or MET (n = 11 piglets from 4 litters) in the fall of 2017 and **B,** Sows previously administered CON (n = 9 piglets from 5 litters) or MET (n = 11 piglets from 5 litters) in the fall of 2018. All data are means ± SEM. Treatment $P < 0.05$.