Characterization of plasma metabolites at late gestation and lactation in early parity sows on production and post-weaning reproductive performance¹

Lea A. Rempel,² Jeffrey L. Vallet, and Dan J. Nonneman

USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933-0166

ABSTRACT: Lactation is a very energy demanding period for sows. The current study provides a better understanding of the biochemical response of first- ($n = 246$) or second-parity ($n = 127$) sows during late gestation through lactation and assesses relationships with piglet production and dam reproductive performance. Plasma samples were collected from first- or second-parity dams at late gestation (110 d gestation [d110G]), d 1 post-farrowing (d1PF), and weaning (WN) then analyzed for various stress and protein metabolism compounds, including; creatine, creatine phosphokinase (CPK) activity, creatinine, urea nitrogen, albumin, and lactate. Litter performance was measured as number of piglets nursed and piglet ADG. Post-weaning reproductive performance was assessed by measuring weaning-to-estrus interval (WEI) and subsequent ovulation rate collected at time of harvest. Plasma creatine and CPK activity increased ($P < 0.05$) between d110G and d1PF. Plasma creatinine decreased ($P < 0.05$) from d110G through WN in first-parity dams, but remained similar between d110G and d1PF before declining ($P < 0.05$) at WN in second-parity

dams. Plasma urea nitrogen increased ($P < 0.05$) over the course of the study and was negatively ($P < 0.05$) associated with piglet ADG at d110G and d1PF and with ovulation rate at d110G ($P < 0.05$). Similarly, plasma albumin increased ($P < 0.05$) in first-parity dams over the course of the study, whereas it plateaued ($P < 0.05$) at d1PF and remained similar ($P > 0.10$) through WN in second-parity dams. First-parity dams had less ($P < 0.05$) plasma lactate at d110G than at d1PF or WN. However, second-parity dams had increased ($P < 0.05$) plasma lactate at d110G and d1PF, then decreased ($P < 0.05$) levels at WN. Plasma lactate at WN was positively ($P < 0.05$) associated with WEI in first-parity dams, but negatively ($P < 0.05$) related to WEI at d1PF in second-parity dams. Plasma lactate levels at all time points were positively ($P < 0.05$) associated with ovulation rate in second-parity dams. The biochemical profile of these dams differed by parity and merits further investigations into these differences to identify methods to improve physiological response to lactation for improved animal welfare, production, and reproductive performance.

Key words: lactation, piglet performance, plasma metabolites, post-weaning reproduction, swine

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²Corresponding author: lea.rempel@ars.usda.gov

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INTRODUCTION

Parturition and lactation are physically stressful and energy-demanding events. Modifications in plasma metabolites may reflect reproductive tissue repair, and also catabolism of the sow's body reserves in response to inadequate energy intake during lactation and intense piglet suckling. Several metabolites are known to change sharply

in response to stressful events. Serum creatine phosphokinase (CPK) activity was elevated 1 d post-partum in women, and Cesarean-delivery subjects had a greater response than muciparous deliveries (Abramov et al., 1996). Plasma lactate concentrations in sows were elevated 1 d post-farrowing and was attributed to nesting activity and increased muscle contractions from events surrounding parturition (Hansen et al., 2012). Stressful events can alter other plasma markers of protein metabolism, such as; albumin, globulin, urea, and total protein, which were changed following transportation of cattle and it was suggested that changes were a result of muscle damage (Tarrant, 1990; Buckham Sporer et al., 2008).

The objective of the current study was to establish metabolite activity of candidate markers at different times surrounding pre- and post-parturition (i.e., late gestation, post-farrowing, and weaning) in young sows and relate these components to piglet production, specifically lactation load (i.e., litter size nursed and piglet gain), and subsequent post-weaning reproductive performance (i.e., wean-to-estrus interval [WEI] and ovulation rate). The current study indicates metabolite profile differences exist between first- and second-parity dams from late gestation through weaning. Individual plasma metabolites correlated marginally to piglet production or reproductive performance of dams. Future studies using a global profiling approach, such as metabolomics, will identify plasma markers that predict a dam's performance potential, thereby improving welfare and production parameters.

MATERIALS AND METHODS

All experimental procedures and techniques were reviewed and approved by the U.S. Meat Animal Research Center Animal Care and Use Committee. Procedures for handling animals complied with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) and met the USDA guidelines for the care and use of animals (USDA-ARS, 1990).

Animal Population and Sampling

A four-breed composite line (Maternal Landrace × High-lean Landrace × Duroc × Yorkshire; LDY) maintained at USMARC for at least seven generations was used for the collection of data in this project and have been previously described (Rempel et al., 2011; Rempel et al., 2015). Data were collected in 2009 during late gestation through post-weaning

from either first ($n = 257$) or second ($n = 127$) parity females; no repeated measures were collected. Dams were excluded from the analyses if they were incapable of successfully completing lactation ($n = 11$). Of these, 10 first-parity females were removed from the study for one of the following reasons: death of sow post-farrowing ($n = 4$), unfit to nurse ($n = 4$), or destruction of litter ($n = 2$). Another first-parity sow was removed from the study as a litter of 7-d old piglets were transferred following the death of their dam. Crossfostering of piglets occurred within 48 h of farrowing when necessary: if a dam had more than 13 live piglets, if functional teat number was insufficient to support number of offspring, or if the dam had health issues that may have been detrimental to the piglets.

Females entered the farrowing facility at 110 d gestation (**d110G**) at which time they were weighed and started on a standard 18% CP lactation corn-soybean meal based diet that met NRC requirements for lactating sows (Table 1; NRC, 2012). Sows were not fed on day of farrowing, fed 1.8 kg of the lactation diet per day and incrementally adjusted daily to achieve the equivalent of 1.8 kg + 0.45 kg per piglet nursed by 7 d lactation and continuing through weaning. Blood samples were collected by jugular venipuncture in combination with K-EDTA for plasma acquisition at d110G, d1 post-farrowing (**d1PF**), and weaning (**WN**). Plasma was aliquoted and stored at -80°C until analyses.

Table 1. Composition of as-fed lactation and breeding (post-weaning) diets

Item	Lactation	Breeding
Ingredients, % as is		
Ground corn	70.1	82.7
Soybean meal	25.0	13.7
Dicalcium phosphate	1.5	1.5
Limestone	0.95	0.9
Soybean oil	1.0	-
Salt	0.5	0.5
Vitamin premix	0.2	0.2
Trace mineral	0.2	0.2
Choline	0.2	0.2
Lysine HCl	0.1	-
Tylan-40	0.13	-
Phytase 600	0.09	0.09
Calculated analyses, DM basis ^a		
ME, MJ/kg	14.00	13.77
CP, g/kg	177.9	133.7
TID lysine, g/kg	9.0	5.4
Ca, g/kg	8.3	7.8
Available P, g/kg	3.31	3.2

^aCalculated analysis based on standard feed tables (NRC, 2012).

Following weaning, sows were group penned ($n = 20$), fed $2.3 \text{ kg head}^{-1} \text{ d}^{-1}$ of a 13% CP corn-soybean meal based diet that met the NRC requirements for breeding and early gestating sows (Table 1; NRC, 2012). Estrous activity was monitored daily by animal technicians with fence-line boar contact. WEI was measured as the duration of days following weaning until first day of detected estrus. Females that expressed estrus following 15 d post-weaning or no expression of estrus were removed from WEI regression analyses (i.e., 14 first-parity females and 5-second parity females failed to express estrus and two first-parity females and 1-second parity female expressed estrus after 15 d post-WN). Ovulation rate (OR) was determined as the number of *corpora lutea* at time of slaughter, average of 14 d (range; 6 to 35 d), following the first- or second-parity post-WN estrus.

Plasma Metabolites

All samples were measured in duplicate for each metabolite analyzed. Creatine was assayed from perchloric acid deproteinized plasma extracts using a modified method (Harris et al., 1974; Kennaugh et al., 1997). Briefly, NADH oxidation was photometrically measured at 340 nm in the presence of creatine, creatine kinase, and lactate dehydrogenase (Sigma, St. Louis, MO). Concentration of creatine in the sow was established by comparing data to a standard curve ranging from 0 to 200 μM . Total CPK activity was measured from plasma as the production of NADH in the presence of creatine phosphate, hexokinase, and 6-phosphogluconate at 340 nm using a two-part reagent system (Pointe Scientific, Canton, MI). Plasma concentrations of creatinine, urea N (PUN), and albumin were measured using a Technicon Autoanalyzer System (Technicon Autoanalyzer Systems, Tarrytown, NY). Creatinine levels were measured using a colorimetric method as described in the Technicon Method SF4-0011FH4 (Technicon Industrial Systems., 1974). The method for measuring PUN was adapted from the Technicon AutoAnalyzer II Industrial Method #339-01 (Technicon Instruments Corp., 1977). Concentrations of plasma albumin were established using a previously described bromocresol green procedure (Dumas et al., 1997). Direct measurement of immobilized L-lactate from the plasma was performed using the YSI 2700 SELECT Biochemistry Analyzer (Yellow Springs, OH) and a lactate oxidase specific enzyme membrane.

Statistical Analyses

General production statistics were calculated using GLM of SAS (SAS Institute, Cary, NC) to derive LSMEANS $\pm$ SE. The model used to identify farrowing statistics (i.e., gestation length, lactation length, total number born, number born alive; NBA, number of live piglets nursed following cross-fostering; NUM_NUR, and number of piglets weaned) included parity of dam (Parity; 1 or 2) and farrowing group (Farrowing Group; 1 to 5). All dams were unique therefore there were no repeated measures within the dataset.

Metabolite data were analyzed using the MIXED procedure of SAS (SAS Institute). The model for determining differences in metabolites (i.e., creatine, CPK activity, creatinine, albumin, urea, and lactate) included discrete effects defined as day of measurement (day; d110G, d1PF, and WN), parity of dam (1 or 2), farrowing group (1 to 5), and the covariate NBA, along with the random effect of sire family ($n = 12$). Since all dams used were unique, either first parity or second parity, there was no need to adjust the model for repeated measures. All data were collected in 2009, therefore, year was also excluded from the model.

Regression analyses were performed to determine the relationship of each metabolite and piglet performance (NUM_NUR and ADG) or reproductive performance (WEI and OR) within parity. The initial model used for the analysis of each metabolite included $Y_{ijklm} = \mu + \text{Metabolite}_i + \text{Parity}_k + \text{Metabolite}_i * \text{Parity}_k + \text{Farrowing Group}_l + \text{Sire Family}_m$. Sire family was identified as a random effect. Number of piglet deaths during lactation (d1 post-farrowing through weaning) was included as a covariate within the model for NUM_NUR. Correlation analyses (SAS Institute) were performed to verify that number born alive and NUM_NUR were similar ($P < 0.0001$; parity 1 = 0.73 and parity 2 = 0.74). Initial modeling tested the interaction for the parity slope difference with metabolite and if slopes were not different then the interaction was dropped and main effects of metabolite and parity were tested in the final regression model and least squares means for parity were also determined using the appropriate model (i.e., with or without interaction).

RESULTS AND DISCUSSION

In the current study, various plasma metabolites were measured from either first- or second-parity dams at late gestation through weaning. The

physiological state of these animals, transitioning through parturition followed by the demands of lactation, was constantly under some level of stress. The measured metabolites were identified for their characteristic relationship to either stress or tissue catabolism. Differences were evident between parities, indicating that first- and second-parity females had unique responses to the stress of parturition and lactation. General production statistics were as expected between first- and second-parity dams. First-parity dams had a slightly longer ($P < 0.05$) gestation period in contrast to second-parity dams (115.9 ± 0.19 d and 115.0 ± 0.25 d, respectively). Likewise, first-parity dams had; fewer total number of pigs born (10.1 ± 0.32 piglets vs. 10.9 ± 0.42 piglets, respective to parity; $P < 0.05$), fewer pigs born alive (9.5 ± 0.32 piglets vs. 10.2 ± 0.41 piglets, respective to parity; $P < 0.05$), and smaller litter sizes following cross-fostering (NUM_NUR; 9.7 ± 0.20 piglets vs. 10.4 ± 0.26 piglets, respective to parity; $P < 0.05$) in comparison to second-parity dams. A total of 30 litters, from the 373 litters that successfully completed lactation (i.e., first parity, $n = 239$ and second parity, $n = 127$), had four or more piglets crossfostered (removed or added) within 48 h of farrowing, while 248 litters had none to ± 1 piglet crossfostered. However, LSMeans for NUM_NUR was similar to NBA by parity and as previously stated, NUM_NUR and NBA were positively correlated with each other. As our interest was to investigate lactation load, we utilized NUM_NUR instead of NBA to evaluate regression analyses with all values including late gestation values. Number of piglets weaned tended ($P < 0.10$) to be less from first-parity litters in comparison to second-parity litters (8.7 ± 0.19 piglets and 8.9 ± 0.19 piglets, respectively). Lactation length was similar between first- and second-parity females (17.8 ± 0.18 d vs. 17.5 ± 0.23 d, respectively).

Body condition differences for these dams were previously reported (Rempel et al., 2015). Briefly, first- and second-parity dams continued to lose body weight and 10th rib backfat thickness from late gestation through post-weaning. However, 10th rib loin eye area increased between weaning and post-weaning measurements, with second-parity dams substantially outgaining first-parity dams (increase of 2.8 cm^2 vs. 0.6 cm^2 , respectively; Rempel et al., 2015). Although first-parity dams had smaller litters to care for, their associated body condition losses indicate a greater metabolic demand persisted throughout lactation and post-weaning. However, the physiological recovery of the loin eye muscle in second-parity dams and, to a much lesser extent, in

the first-parity dams may be partially explained by the metabolite measures within the current study.

The creatine pathway has long been associated with high-energy metabolism of cells and tissues, in which creatine serves as a phosphate carrier for the production of ATP from ADP (Wyss and Kaddurah-Daouk, 2000). In the current study, a parity by time interaction ($P < 0.05$) was detected for plasma concentrations of creatine (Table 2). First-parity females had decreased ($P < 0.05$) plasma concentrations of creatine at d110G in comparison to d1PF as well as WN. Second-parity dams also had decreased ($P < 0.05$) plasma concentrations of creatine at d110G in comparison to d1PF, however at WN, plasma concentrations of creatine were similar to d110G values.

Plasma concentrations of creatinine were different for parity by time ($P < 0.05$; Table 2). Plasma concentrations of creatinine were greatest ($P < 0.05$) at d110G and were subsequently less at day 1 PF and least ($P < 0.05$) at WN within first-parity dams, levels at weaning were similar at d110G and d1PF for second-parity animals. Furthermore second-parity animals had greater ($P < 0.05$) plasma concentrations of creatinine than first-parity dams at d110G and d1PF. Plasma concentrations of creatinine at WN were less ($P < 0.05$) than either d110G or d1PF values within the respective parity. Our study indicated that the profiles of creatine and creatinine levels in first-parity dams were different from second-parity dams and likely was a response to greater protein catabolism of skeletal muscle in the younger dams. Endurance race horses have similar profiles as the first-parity dams in the current study, in which creatine levels increased while creatinine levels did not change following long-distance exercise and was a response of muscle catabolism (Le Moyec et al., 2014). Others have reported that creatine directly suppressed insulin binding (Rocic et al., 1991) and decreased glycation of albumin (Rocic et al., 1995). Mice with a creatine deficiency (i.e., knockout of the *S-adenosyl-L-methionine:N-guanodinoacetate methyltransferase* gene; the final enzyme required for the production of creatine) experienced a non-leptin-mediated reduction in body weight (Schmidt et al., 2004). And, reduction of creatine in rat hypothalamus resulted in decreased feed intake and body weight with increased *Fos* expression, suggesting creatine concentrations in the brain contribute to regulating appetite and weight (Galbraith et al., 2006). It is likely that in the current study, elevated creatine and reduced creatinine levels in the younger dams, not only reflects catabolism of skeletal muscle to

Table 2. Metabolite measurements from sows during late gestation through weaning

Item, SI unit	Across parities (<i>n</i> = 384)	Parity 1 (<i>n</i> = 257)	Parity 2 (<i>n</i> = 127)
Creatine, $\mu\text{mol/liter}$		786.5 $\pm$ 27.98	805.4 $\pm$ 35.86
Day 110 gestation	750.0 $\pm$ 33.48	710.6 $\pm$ 34.70 ^a	789.4 $\pm$ 46.37 ^a
Day 1 post-farrowing	870.3 $\pm$ 33.51	843.7 $\pm$ 34.75 ^b	896.9 $\pm$ 46.37 ^b
Weaning	767.5 $\pm$ 33.76	805.2 $\pm$ 35.28 ^b	729.8 $\pm$ 46.48 ^a
Creatinine, $\mu\text{mol/liter}$		311.3 $\pm$ 4.93	341.9 $\pm$ 6.36
Day 110 gestation	338.9 $\pm$ 5.25	322.1 $\pm$ 5.28 ^{a,x}	355.6 $\pm$ 6.91 ^{a,y}
Day 1 post-farrowing	337.6 $\pm$ 5.26	314.3 $\pm$ 5.28 ^{b,x}	360.8 $\pm$ 6.92 ^{a,y}
Weaning	303.3 $\pm$ 5.27	297.4 $\pm$ 5.32 ^c	309.3 $\pm$ 6.92 ^b
CPK activity, U/liter		317.8 $\pm$ 20.88	283.7 $\pm$ 26.76
Day 110 gestation	204.7 $\pm$ 25.01	224.2 $\pm$ 25.89 ^a	185.2 $\pm$ 34.66 ^a
Day 1 post-farrowing	516.0 $\pm$ 24.96	565.7 $\pm$ 25.88 ^{b,x}	466.3 $\pm$ 34.50 ^{b,y}
Weaning	181.5 $\pm$ 25.19	163.4 $\pm$ 26.32 ^c	199.7 $\pm$ 34.66 ^a
Urea Nitrogen, mmol/liter		3.5 $\pm$ 0.08	3.4 $\pm$ 0.10
Day 110 gestation	2.7 $\pm$ 0.09 ^a	2.8 $\pm$ 0.09	2.6 $\pm$ 0.12
Day 1 post-farrowing	3.3 $\pm$ 0.09 ^b	3.3 $\pm$ 0.09	3.3 $\pm$ 0.12
Weaning	4.4 $\pm$ 0.09 ^c	4.4 $\pm$ 0.09	4.4 $\pm$ 0.12
Albumin, g/liter		34.9 $\pm$ 0.27	35.4 $\pm$ 0.35
Day 110 gestation	33.5 $\pm$ 0.30	32.6 $\pm$ 0.30 ^{a,x}	34.5 $\pm$ 0.40 ^{a,y}
Day 1 post-farrowing	35.1 $\pm$ 0.30	34.5 $\pm$ 0.30 ^{b,x}	35.7 $\pm$ 0.40 ^{b,y}
Weaning	36.8 $\pm$ 0.30	37.5 $\pm$ 0.30 ^{c,x}	36.1 $\pm$ 0.40 ^{b,y}
L-Lactate, mmol/liter		1.7 $\pm$ 0.09	1.9 $\pm$ 0.10
Day 110 gestation	1.7 $\pm$ 0.08	1.3 $\pm$ 0.09 ^{a,x}	2.0 $\pm$ 0.11 ^{a,y}
Day 1 post-farrowing	2.0 $\pm$ 0.08	1.9 $\pm$ 0.09 ^b	2.1 $\pm$ 0.12 ^a
Weaning	1.7 $\pm$ 0.09	1.9 $\pm$ 0.09 ^{b,x}	1.5 $\pm$ 0.12 ^{b,y}

^{abc}Columns with different superscript differ by time within a parity ($P < 0.05$).

^{xy}Rows with different superscript differ by parity ($P < 0.05$).

support energetic demands and continued growth of the dam herself, but may also serve as a catalyst to stimulate feed intake.

CPK activity was different ($P < 0.05$; Table 2) for the interaction between parity and time. Levels of CPK activity at d110G were similar across parities. Values were greatest ($P < 0.05$) at d1PF for both parities, and were greater ($P < 0.05$) in first-parity vs. second-parity dams. CPK activity levels in first-parity dams at WN were reduced ($P < 0.05$) in comparison to d110G or d1PF values. Second-parity dams had similar CPK activity at WN to first-parity dams, but second-parity WN values were not different from d110G measurements of second-parity dams. The abrupt change in plasma concentrations of CPK was not surprising, as sharply increased levels of CPK activity following parturition has been reported in women (Abramov et al., 1996; Conner et al., 2006) and primi- and multi-parous sows (Nogueira et al., 2000). The dramatic increase in CPK activity observed in the current study was likely the result of uterine ischemia and reperfusion injury due to parturition since the activity levels at weaning were similar to or less than late gestation values.

Regression analyses were employed to investigate potential relationships between plasma metabolites and production (piglet ADG and NUM_NUR; Table 3) and reproduction (WEI and OR; Table 4) traits. Plasma concentrations of creatinine at d110G and WN were positively ($P \leq 0.05$) associated with NUM_NUR in both first- and second-parity dams. Plasma concentrations of creatinine at d110G and d1PF were inversely associated with ADG ($P < 0.05$). Creatinine, a by-product of creatine, which is typically discarded in the urine, reflects the minimum quantity of creatine needed from endogenous production or dietary intake (Brosnan and Brosnan, 2007). Regression analyses of creatinine within the current study, suggest increasing creatine provided to the dams, even as early as late gestation, to support a large litter or to provide a greater gain by suckling piglets. Plasma enzyme activity levels for CPK was negatively associated with NUM_NUR in first-parity dams at d110G ($P < 0.10$) and again at WN ($P < 0.05$). There is a continual need for creatine and its ability to generate metabolic energy through CPK activity within growing animals (Brosnan and Brosnan, 2010), similar to the first-parity dams in the current study. The inverse relationship between CPK

Table 3. Regression coefficients for metabolites from sows on number of piglets nursed and piglet ADG

Trait	Number of piglets nursed		Piglet ADG (g)	
	Parity 1	Parity 2	Parity 1	Parity 2
Creatine				
d110G	-0.0003 ± 0.00027	0.0002 ± 0.00030	-0.009 ± 0.0060	-0.002 ± 0.0065
d1PF	-0.0003 ± 0.00022^x	0.0003 ± 0.00022^y	-0.00005 ± 0.0049	0.001 ± 0.0047
WN	-0.0003 ± 0.00020	0.00007 ± 0.00028	0.0005 ± 0.0044	0.009 ± 0.0060
Creatinine				
d110G	$0.003 \pm 0.0016^{b,x}$	$0.004 \pm 0.0015^{a,y}$	$-0.12 \pm 0.036^{a,x}$	$-0.08 \pm 0.033^{a,y}$
d1PF	-0.001 ± 0.0016	-0.00001 ± 0.00153	$-0.11 \pm 0.025^{a,x}$	$-0.07 \pm 0.033^{a,y}$
WN	$0.005 \pm 0.0017^{a,x}$	$0.006 \pm 0.0018^{a,y}$	-0.05 ± 0.039	-0.02 ± 0.039
CPK activity				
d110G	-0.0005 ± 0.00033^b	-0.0002 ± 0.00063	-0.005 ± 0.0072	0.008 ± 0.0139
d1PF	-0.0003 ± 0.00023	0.0001 ± 0.00031	0.0009 ± 0.0049	0.008 ± 0.0067
WN	-0.001 ± 0.0006^a	-0.0002 ± 0.00065	$0.024 \pm 0.0128^{b,x}$	$0.033 \pm 0.0140^{a,y}$
Urea nitrogen				
d110G	-0.08 ± 0.114	0.06 ± 0.135	$-7.82 \pm 2.511^{a,x}$	$-5.12 \pm 2.961^{b,y}$
d1PF	$-0.20 \pm 0.073^{a,x}$	$-0.13 \pm 0.075^{b,y}$	$-7.87 \pm 1.546^{a,x}$	$-4.20 \pm 1.580^{a,y}$
WN	0.09 ± 0.093	0.16 ± 0.096^b	-1.55 ± 2.014	0.15 ± 2.106
Albumin				
d110G	0.04 ± 0.033^x	$0.05 \pm 0.031^{b,y}$	0.68 ± 0.739	0.91 ± 0.706
d1PF	-0.03 ± 0.031	-0.02 ± 0.030	0.57 ± 0.668^x	0.85 ± 0.674^y
WN	-0.02 ± 0.027	-0.01 ± 0.026	0.56 ± 0.581^x	0.82 ± 0.577^y
L-Lactate				
d110G	-0.04 ± 0.120	0.07 ± 0.086	-0.50 ± 2.644	2.41 ± 1.902
d1PF	-0.09 ± 0.087	0.06 ± 0.101	0.38 ± 1.901	3.26 ± 2.17
WN	-0.05 ± 0.097	0.15 ± 0.134	0.14 ± 2.107	4.78 ± 2.880^b

^aRegression coefficient significant at ($P < 0.05$).

^bRegression coefficient significant at ($P < 0.10$).

^{x,y}Regression coefficient with different superscript differ by parity ($P < 0.05$).

activity at late gestation and number of suckling piglets suggests that young, growing, first-parity dams are needing more metabolic energy before lactation in order to support suckling piglets. Likewise we hypothesize that first-parity females continue to be in need of energy at weaning as evidenced by continued body weight loss (previously reported; Rempel et al., 2015). CPK activity at WN tended to be positively ($P \leq 0.07$) associated with ADG. Increased CPK activity in dams indicates increased cellular energy, which can contribute to milk production and quality. Therefore, piglets receiving ample energy from milk will also have improved daily gains.

Plasma concentrations of creatine at d110G, d1PF, and WN in second-parity dams were inversely ($P < 0.05$) associated with WEI (Table 4). WEI in first-parity dams was positively ($P < 0.05$) associated with plasma concentrations of creatinine at d110G and again at WN. CPK activity in second-parity dams was inversely ($P < 0.05$) associated with WEI at all time points (Table 4). Plasma concentrations of creatine and creatinine were inversely ($P \leq 0.05$) related with OR in first-parity

females at all times measured (Table 4). Plasma concentrations of creatine at WN in second-parity dams was positively ($P = 0.058$) associated with OR (Table 4). Plasma CPK activity at d110G, d1PF, and WN was positively ($P < 0.05$) associated with OR in second-parity dams (Table 4). Regression analyses indicated significance between members of the creatine pathway and reproductive parameters, and the values suggest limited biological relevance. However, we cannot discount a possibility that these metabolites may be contributing to an overall effect, directly or indirectly. For instance, rats treated with exogenous estrogen had increased CPK production within ovarian tissues (Malnick et al., 1983). Furthermore, CPK (brain isoform) was upregulated in human cumulus cells removed from oocytes that generated good-quality embryos following IVF in comparison to cumulus cells associated with poor-quality embryos (Lee et al., 2010). The relationship we observed in the current study between CPK and ovulation rate may indicate a role for CPK to assist with timing of ovulatory events thereby subtly influencing the rate of ovulation. Also, we know that WEI is a complex

Table 4. Regression coefficients for metabolites from sows on WEI and ovulation rate

Trait	WEI (d)		Ovulation rate	
	Parity 1	Parity 2	Parity 1	Parity 2
Creatine				
d110G	0.0004 ± 0.0003 ^x	-0.0009 ± 0.00034 ^{a,y}	-0.002 ± 0.0010 ^{a,x}	-0.001 ± 0.0009 ^y
d1PF	-0.0001 ± 0.00026 ^x	-0.0007 ± 0.00025 ^{a,y}	-0.002 ± 0.0007 ^{a,x}	0.0005 ± 0.00056 ^y
WN	0.0007 ± 0.00023 ^x	-0.0007 ± 0.00033 ^{a,y}	-0.002 ± 0.0005 ^{a,x}	0.001 ± 0.0008 ^{b,y}
Creatinine				
d110G	0.004 ± 0.0018 ^{a,x}	0.001 ± 0.0017 ^y	-0.01 ± 0.004 ^{a,x}	-0.003 ± 0.0039 ^y
d1PF	0.001 ± 0.0018 ^x	-0.001 ± 0.0017 ^y	-0.009 ± 0.0044 ^{b,x}	-0.001 ± 0.0041 ^y
WN	0.005 ± 0.0020 ^{a,x}	0.003 ± 0.0020 ^y	-0.009 ± 0.0047 ^{b,x}	-0.001 ± 0.0046 ^y
CPK activity				
d110G	-0.0003 ± 0.00037 ^x	-0.002 ± 0.0007 ^{a,y}	-0.001 ± 0.0008 ^x	0.005 ± 0.0017 ^{a,y}
d1PF	0.00008 ± 0.00026 ^x	-0.001 ± 0.0003 ^{a,y}	0.0001 ± 0.00059 ^x	0.003 ± 0.0008 ^{a,y}
WN	-0.0003 ± 0.00064 ^x	-0.002 ± 0.0007 ^{a,y}	-0.0003 ± 0.0016 ^x	0.004 ± 0.0017 ^{a,y}
Urea nitrogen				
d110G	-0.01 ± 0.130 ^x	-0.31 ± 0.153 ^{a,y}	-1.40 ± 0.313 ^{a,x}	-0.79 ± 0.363 ^{a,y}
d1PF	0.20 ± 0.081 ^{a,x}	-0.05 ± 0.083 ^y	-0.55 ± 0.209 ^{a,x}	0.15 ± 0.243 ^y
WN	0.17 ± 0.105 ^x	-0.02 ± 0.113 ^y	-0.33 ± 0.241 ^x	0.15 ± 0.256 ^y
Albumin				
d110G	-0.008 ± 0.0381 ^x	-0.031 ± 0.0363 ^y	-0.036 ± 0.0910 ^x	0.031 ± 0.0866 ^y
d1PF	-0.07 ± 0.034 ^{a,x}	-0.10 ± 0.035 ^{a,y}	0.033 ± 0.0803 ^x	0.101 ± 0.0808 ^y
WN	-0.04 ± 0.030 ^x	-0.06 ± 0.030 ^{a,y}	0.093 ± 0.0693 ^x	0.156 ± 0.0686 ^{a,y}
L-Lactate				
d110G	0.17 ± 0.136 ^x	-0.12 ± 0.098 ^y	-0.296 ± 0.3193 ^x	0.562 ± 0.2238 ^{a,y}
d1PF	0.06 ± 0.096 ^x	-0.28 ± 0.112 ^{a,y}	-0.287 ± 0.2177 ^x	0.973 ± 0.2494 ^{a,y}
WN	0.21 ± 0.106 ^{a,x}	-0.09 ± 0.146 ^y	-0.104 ± 0.2510 ^x	0.779 ± 0.3484 ^{a,y}

^aRegression coefficient significant at ($P < 0.05$).

^bRegression coefficient significant at ($P < 0.10$).

^{x,y}Regression coefficient with different superscript differ by parity ($P < 0.05$).

trait, and components of the creatine pathway may be contributing to a global metabolic effect, which may be more biologically relevant than assessing the current panel of individual metabolites.

Concentrations of plasma urea nitrogen (PUN) were significant ($P < 0.05$; Table 2) for time only. Concentrations of PUN were least ($P < 0.05$) at d110G, intermediate ($P < 0.05$) at d1PF, and greatest ($P < 0.05$) at WN. Plasma concentrations of urea nitrogen steadily increased from late gestation through weaning in the current study and were not distinguishable by parity. Previous reports for serum concentrations of urea nitrogen in primi- and multi-parous sows were similar to values reported here (Friendship et al., 1984; Verheyen et al., 2007). Breakdown of skeletal muscle in lactating sows to provide amino acids for energy leads to deamination and urea synthesis in the liver, therefore blood urea nitrogen can be used as an index of amino acid degradation (Reese et al., 1982; Nelssen et al., 1985b). Furthermore, serum urea nitrogen concentrations were inversely correlated to energy intake in primiparous sows (Nelssen et al., 1985a). Mosnier and others (Mosnier et al., 2010) reported

an increase in PUN in lactating sows and positive correlation to increased daily feed intake, therefore it was proposed that increased urea nitrogen levels were due in part to increased metabolism of dietary amino acids. Since the sows in the current study were fed a fixed amount during late gestation and adjusted amounts during lactation based upon the number of piglets nursed, the increase in PUN over the course of the study was likely due to skeletal muscle breakdown, as sows were observed to have reduced backfat and bodyweight as well (Rempel et al., 2015).

Plasma concentrations of urea nitrogen at d1PF tended to be or were negatively associated ($P \leq 0.093$) with NUM_NUR, while PUN at WN in second-parity dams tended to be positively ($P = 0.091$) related to NUM_NUR. PUN levels in first-parity dams at d1PF had a positive ($P < 0.05$) relationship with WEI, while d110G levels in second-parity dams were negatively ($P < 0.05$) associated with WEI. Piglet ADG was also associated with various plasma metabolite levels in first- and second-parity dams (Table 3). The most dramatic effect, was tendency for an inverse relationship (P

= 0.084) of plasma concentrations of urea nitrogen at d110G and d1PF in both first- and second-parity dams with ADG. And a negative ($P < 0.05$) relationship between PUN and OR was observed at d110G in first- and second-parity dams and continued at d1PF in first-parity dams ($P < 0.05$). These data suggest that reductions in protein deamination, as early as late gestation, and throughout lactation would serve to improve piglet performance and consequentially improve post-weaning ovulation rate.

Plasma concentrations of albumin were significant ($P < 0.05$) for an interaction between parity and time (Table 2). First-parity females had increasing ($P < 0.05$) plasma concentrations of albumin from d110G to WN. However, plasma concentrations of albumin at each measured time was less ($P < 0.05$) in first-parity dams than second-parity dams. Plasma concentrations of albumin in second-parity dams was least ($P < 0.05$) at d110G and similar at d1PF and WN. Production of albumin occurs in the liver and decreased concentrations of albumin are indicative of protein deficiencies (Jahoor et al., 1996). Within the current study, albeit slightly different between parities, plasma concentrations of albumin increased or remained similar over the course of the study suggesting that availability of protein was adequate. Because there was an increase in plasma concentrations of urea nitrogen between late gestation and weaning, it is plausible that the available protein was derived from muscle catabolism to support lactation. From our previous work (Rempel et al., 2015), we observed a greater reduction in 10th rib loin eye area at weaning, further supporting the availability of protein through muscle catabolism.

Plasma concentrations of albumin at d110G tended to be positively ($P = 0.089$) associated with NUM_NUR in second-parity dams (Table 3). Plasma concentrations of albumin at d1PF ($P < 0.05$) in first- and second-parity dams and at WN in second-parity dams ($P < 0.05$) were also inversely related to WEI (Table 4). Plasma concentrations of albumin at WN in second-parity dams was positively ($P < 0.05$) related to OR (Table 4). Since plasma concentrations of albumin had a slight negative relationship with WEI at post-farrowing in all dams and again at weaning in second-parity dams, this suggests that protein availability, as measured by albumin, had a favorable effect upon reproductive performance. Likewise, second-parity dams had a subtle positive relationship between ovulation rate and plasma concentrations of albumin at weaning. Pigs lack a sex-hormone binding globulin, but a

low affinity sex-hormone binding property exists with albumin (Cook et al., 1977). Albumin may be serving as a reservoir for steroid hormones in the transitioning sow preparing her for the subsequent reproductive cycle. Therefore, the dams with increased albumin may have a greater steroid hormone reserve eliciting an improved opportunity for reproductive performance.

Plasma concentrations of lactate were significant ($P < 0.05$) for an interaction between parity and time (Table 2). First-parity dams had lower ($P < 0.05$) plasma concentrations of lactate at d110G in comparison to d1PF and WN, which were not different. Second-parity females had an inverse pattern of plasma concentrations of lactate in comparison to first-parity females with d110G and d1PF similar and plasma concentrations of lactate being least ($P < 0.05$) at WN. As a result, circulating levels of lactate in first-parity dams was less ($P < 0.05$) at d110G and greater ($P < 0.05$) at WN in comparison to second-parity dams. Of great intrigue is the complimentary profile of plasma concentrations of creatine and lactate observed in the current study. Specifically, at weaning, plasma concentrations of creatine and lactate remained similar to d1 post-farrow values in first-parity dams, whereas second-parity dams had reduced levels of creatine and lactate at weaning in comparison to d1 post-farrow values. Plasma lactate in pigs has been previously shown to increase in response to stress (Darrah et al., 1981) and in sows during peri-parturient nesting activity (Hansen et al., 2012). Parturition in first-parity dams is a naïve event, therefore a greater stress response could be expected. In a pig trauma model, severe trauma (i.e., femur fracture, 60% hemorrhage, and mild hypothermia) induced an increase in blood lactate and CPK within 30 min and persisted through 16 h post-trauma (Wong et al., 2015). In the current study, the elevated lactate levels in first-parity dams continued through weaning, suggesting an extended response. As previously mentioned, creatine becomes phosphorylated to assist with the production of ATP for energy (Wyss and Kaddurah-Daouk, 2000). Cellular lactate production increases in response to proton release from skeletal muscle ATP break down, impeding acidosis by preventing pyruvate accumulation (Robergs et al., 2004). Therefore, increased lactate may be functioning to compensate for the elevated creatine that likely is producing ATP for cellular energetics. Another plausible effect of increased plasma lactate may be to stimulate insulin resistance as a response to body weight and backfat loss in the younger females. Increased lactate in rats has been shown

to suppress insulin-stimulated glycolysis and insulin-stimulated glucose uptake by decreasing insulin receptor-associated PI 3-kinase activity, specifically GLUT4 activity (Choi et al., 2002). Feeding a sugar-rich lactation diet to primiparous sows 8 d before weaning increased plasma glucose and insulin concentrations without affecting body weight in comparison to primiparous sows fed a fat-rich or control lactation diet (Chen et al., 2013b) and also tended to shorten the WEI in dams with extreme body condition loss (Chen et al., 2013a; Chen et al., 2016).

Second-parity dams tended to have a positive relationship between plasma concentrations of lactate at WN and ADG ($P = 0.098$; Table 3). Plasma concentrations of lactate at WN in first-parity dams was positively ($P < 0.05$) associated with WEI, however levels at d1PF in second-parity dams was inversely ($P < 0.05$) related to WEI (Table 4). Plasma concentrations of lactate at all time points were positively ($P < 0.05$) associated with OR in second-parity dams (Table 4). Since we observed a greater level of lactate at weaning in first-parity dams in comparison to second-parity dams, it is theorized that lactate may be influencing insulin and glucose activity to support recovery of the dam, over reproductive function. Whereas the second-parity dams had a negative relationship between lactate and WEI and a positive relationship with ovulation rate demonstrating the mature females are more competent to handle the physical demands of lactation without limitations on their subsequent reproductive performance.

In conclusion, the biochemical behavior of first-parity dams is unique from second-parity dams. The differences in plasma profiles were likely a response based upon physical maturity and the naïve status of the first-time dam. From the complementary body condition study (Rempel et al., 2015), we know that second-parity dams recovered more quickly following weaning. The differences in plasma metabolites may be functioning at a variety of planes. For example, plasma lactate and creatine may be working together to mitigate acidosis during skeletal muscle reperfusion and repair due to catabolism, but may simultaneously be stimulating feed intake and regulating insulin activity to support recovery from weight loss and muscle catabolism. Therefore it may be of great interest to supplement late lactation and post-wean females with exogenous creatine monohydrate to enhance recovery from lactation demands. Previous efforts using exogenous creatine supplementation in pre-parturient sows to assist with farrowing, had

no effect on farrowing interval, stillbirth weights, or preweaning mortality rates in comparison to control-fed dams (Vallet et al., 2013). However, a more complete profile is warranted and can be ascertained using more comprehensive approaches such as metabolomics. A greater understanding of these and a global profile of metabolites, known and unknown, will provide information for development of feed supplementation programs, which likely will include various metabolites or their derivatives, during lactation and post-weaning that will improve dam recovery, thereby likely improving longevity and production performance of dams.

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