

A regional evaluation of chromium tripicolinate supplementation of diets fed to reproducing sows^{1,2}

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ABSTRACT: A cooperative research study involving 353 litters was conducted at three stations to determine the effects of graded levels of supplemental Cr from chromium tripicolinate (CrPic) on reproductive performance of sows and preweaning performance of their pigs. Primiparous and multiparous sows were fed fortified corn–soybean meal diets with supplemental levels of 0, 200, 600, or 1,000 ppb Cr (as-fed basis). Each station used at least three of the supplemental Cr levels, with two of those levels being 0 and 200 ppb. Station effects were observed for sow gestation weight gain, lactation weight change, lactation feed intake, litter size at birth and weaning, and pig weight at birth and weaning ($P = 0.001$ to 0.087). Supplemental Cr increased the number of pigs born live per litter (9.49, 9.82, 10.94, and 10.07; quadratic, $P = 0.05$) and sow

lactation weight change (-0.2 , 0.8 , -4.1 , and -3.9 kg; linear, $P = 0.01$) but decreased individual birth weight of total pigs born (1.61, 1.57, 1.47, and 1.56 kg; quadratic, $P = 0.10$). Tissues were obtained from a subset of sows from one station after they had completed three parities on the study. The content of Cr in the adrenal gland (16.4, 20.0, 34.0, and 48.4 ppb), kidney (35.8, 56.4, 132.6, and 176.0 ppb), and liver (22.8, 37.4, 87.6, and 92.2 ppb) was increased linearly ($P = 0.001$ to 0.005) by increasing CrPic supplementation. The results suggest that the supplementation level that maximizes the biological response is above that currently allowed. Additionally, supplementation of Cr at 1,000 ppb (five times currently permitted supplementation levels) was not detrimental to sow performance, even when fed continuously for three parities. There may be merit to continued research to evaluate higher supplementation rates.

Key Words: Chromium, Reproduction, Sows

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Introduction

The earliest reports on the effects of organic Cr supplementation to swine were from Page et al. (1993), who reported dose-dependent increases in LM area and decreases in carcass backfat when Cr from chromium

tripicolinate (**CrPic**) was fed to growing-finishing pigs. In the first reported reproductive study (Lindemann et al., 1995a), gilts retained from a growing-finishing study and continued on supplementation of 200 ppb Cr from CrPic through two parities had increases in pigs born alive and weaned of approximately 2.1 pigs per litter. A subsequent study (Lindemann et al., 1995b) showed a numerical increase in pigs born live per litter (0.9) with Cr supplementation, but the response was not significant. It was noted in the initial study of Lindemann et al. (1995a) that the insulin:glucose ratio of midgestation females was lower, implying greater efficiency of insulin action.

There is substantial variation among swine in their response to glucose load and ability to control serum glucose concentrations (Bunding et al., 1956; Anderson and Elsley, 1970) and in the consequent effects on reproduction (George et al., 1978). Thus, the potential of Cr supplementation to improve reproductive performance

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Table 1. Distinctive features of stations involved in the study

Station ^a	Cr levels used, ppb	Females allotted	Genetic background of females ^b	Weaning age, d	Gestational housing
LSU	0, 200, 1,000	92	Purebred and crossbred (Y and Y × L)	19.4	Confinement pens
OSU	0, 200, 600	57	Purebred and crossbred (Y and Y × L)	21.4	Confinement pens
UK	0, 200, 600, 1,000	73	Crossbred (Y × L)	21.0	Confinement crates

^aStation designations are: LSU = Louisiana State University Agricultural Center, OSU = Oklahoma State University, and UK = University of Kentucky.

^bBreed designations are: Y = Yorkshire, and L = Landrace.

is of interest. And although the reported results of supplementation of sows with CrPic at the time of the initiation of this study were very promising, they were not definitive. A factor complicating obtaining definitive results in sow studies is the inherently large variation in normal reproductive measures; thus, a greater number of observations are essential to draw clear conclusions. Additionally, varied levels of supplementation had not been examined with sows as had been examined with growing pigs (Page et al., 1993; Lindemann et al., 1995a). Therefore, the objective of this study was to examine graded levels of CrPic supplementation to the diets of sows over consecutive parities at several research stations.

Materials and Methods

Supplementation of Cr from CrPic in the United States currently cannot exceed 200 ppb (Benz, 1996). Calculations of the amount of Cr supplied per unit of BW between growing pigs and reproducing animals fed the supplementation level of 200 ppb Cr show that reproducing animals receive between 2 to 3 µg of Cr/kg BW, whereas growing pigs receive 7 to 8 µg of Cr/kg BW. To supply an amount to the reproducing animal similar to that provided to the growing pig (per unit BW) would require a supplemental level of about 600 ppb Cr (computed range of 550 to 650 ppb) in the diet. Additionally, the levels of supplementation examined with growing pigs (Page et al., 1993; Lindemann et al., 1995a) were up to 1,000 ppb. Thus, supplemental Cr levels of 0, 200, 600, or 1,000 ppb from CrPic were chosen for evaluation. Specifically then, the purpose for the levels chosen were 200 ppb (that level allowed and marketed in the United States and used in previous research), 600 ppb (that level required to provide the same supplementation rate per unit BW as the growing pig), and 1,000 ppb (that level of fivefold current supplementation levels as had been used in the grower studies; this level allows assessment of any potential negative effects of supplementation). Participants on this project were required to use at least three of the four treatment levels, including the 0 and 200 ppb levels and at least one of the two higher levels of inclusion. The levels used at individual stations as well as some other distinctive features about each station are given in Table 1.

Gilts or sows that had not previously been supplemented with CrPic were allotted to treatment on the day of breeding with care given to balance the allotment relative to parity, weight, and genetic background. A common diet formulation was used (Table 2). Corn was used as the grain source and dehulled soybean meal as the protein source in both gestation and lactation diets. The diets were formulated to meet or exceed NRC (1988) requirement estimates for all nutrients. Dicalcium phosphate was the source of supplemental phosphorus. A common source of trace minerals (Prince Agri Products, Inc, Quincy, IL) and vitamins (Roche Vitamins Inc, Parsippany, NJ) was used at each station. An antibacterial agent was allowed in the lactation diet and in the prebreeding diet. Samples of diets at each mixing were collected by participating stations. The samples

Table 2. Composition of basal gestation and lactation diets (g/kg, as-fed basis)

Item	Gestation	Lactation
Ingredient		
Corn	836.75	734.25
Soybean meal (dehulled)	126.00	230.00
Dicalcium phosphate	21.00	19.00
Limestone	7.50	8.00
Salt	5.00	5.00
Vitamin premix ^a	1.00	1.00
Trace mineral premix ^b	0.75	0.75
Aureo-50 ^c	0.50	0.50
Choline chloride, 60% ^d	1.50	1.50
CHROMAX ^e	0.00	0.00
Calculated composition, %		
CP	13.22	17.40
Lysine	0.60	0.90
Ca	0.78	0.78
P	0.70	0.70

^aSupplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D₃, 1,000 IU; vitamin E, 75 IU; vitamin K, 2 mg; riboflavin, 7.5 mg; d-pantothenic acid, 24 mg; niacin, 30 mg; vitamin B₁₂, 30 µg; d-biotin, 350 µg; folic acid, 500 µg; thiamin, 1 mg; pyridoxine, 6 mg.

^bSupplied per kilogram of diet: Cu, 15 mg as copper sulfate; I, 1.28 mg as ethylenediamine dihydriodide; Fe, 100 mg as ferrous sulfate; Mn, 50 mg as manganous oxide; Se, 0.3 mg as sodium selenite; and Zn, 125 mg as zinc oxide.

^cSupplied 55 ppm chlortetracycline in the final diet.

^dSupplied 780 ppm choline in the final diet.

^eCHROMAX was the commercial product used. It contains chromium tripicolinate (CrPic) blended with limestone and it replaced limestone at the rate of 0.05% to supply 200 ppb Cr. Therefore, replacement rates of 0.05, 0.15, or 0.25% of the product for limestone were used for the 200-, 600- or 1,000-ppb treatments, respectively.

were composited by diet on a quarterly basis. After completion of the study, samples were sent to the study coordinator and further composited to yield a single gestation and lactation sample for each diet at each station. The samples were then sent to NP Analytical Services (St. Louis, MO), which had developed an HPLC assay method optimized for isolation and quantification of the specific compound, CrPic. The method used was developed to comply with U.S. FDA assay requirements.

During gestation, sows were fed 1.82 kg/d during the months of March to November, and 2.27 kg/d (as-fed intake) during the months of December to February for those stations that determined that sows needed greater feed intake during that time of year. Feed was provided on an ad libitum basis during lactation. Animals were on deworming and vaccination schedules particular to the individual stations. Newborn pigs were processed according to standard procedures at each station.

Data recorded were parity (actual parity as well as parity on this particular study); sow weights at breeding, d 110 of gestation, farrowing (within 24 h postpartum), d 21 postpartum, and weaning. Feed consumption of sows was recorded from farrowing to d 21 and from d 21 to weaning. Number and weights of pigs at birth (total and live), d 21, and weaning were recorded. After weaning, the number of days to first estrus was determined.

Tissue samples (adrenal gland, kidney, liver, and ovary) were obtained from five sows that had each completed three parities for each supplementation level on study at the University of Kentucky. Sows were electrically stunned and then killed by exsanguination. The tissues were removed, frozen, and stored at -12°C until shipped to the London Health Sciences Center (London, Ontario, Canada) for Cr analysis. Reference samples for egg powder, beef muscle, and tomato leaves from the National Institute of Standards and Technology (NIST, Gaithersburg, MD) were used to validate assay sensitivity for Cr. Tissue samples sent to the laboratory were opened with a nickel-plated scalpel and a triangular piece of tissue excised from the interior of the organ (to avoid Cr contamination of the tissue). These subsamples were dried at 103°C for approximately 30 min, solubilized in a HNO_3 matrix, and assayed on a Finnigan MAT Element 1 high-resolution inductively coupled plasma-mass spectrometer instrument (Mississauga, Ontario, Canada) in medium resolution (4800 Res) to eliminate Ar-C interference at a Cr mass 52. In addition to the NIST standards, aqueous hair standards from the Quebec Interlab Comparison Program were used for system validation.

The collected data were submitted on standardized forms to the study coordinator. Only females completing at least two parities and no more than three parities were considered in the data analysis. To further maintain balance across dietary treatment within the data set, those sows that began the study with a parity

Table 3. Assay values of diets supplemented with Cr, ppb (as-fed basis)

Phase	Expected Cr, ppb	Station ^{ab}	
		LSU	UK
Gestation	0	ND ^c	ND
Gestation	200	223	244
Gestation	600	—	604
Gestation	1,000	1,255	1,220
Lactation	0	ND	ND
Lactation	200	380	242
Lactation	600	—	568
Lactation	1,000	1,120	920

^aStation designations are: LSU = Louisiana State University Agricultural Center and UK = University of Kentucky.

^bThe samples from Oklahoma State University were lost due to storage failures.

^cND = not detected. The limit of detection was 50 ppb.

greater than three were removed. The data were subjected to ANOVA using the GLM procedures of SAS (SAS Inst., Inc., Cary, NC) as a randomized block design, with the sow or litter serving as the experimental unit. The model included terms for station, treatment, parity on study, and all possible interactions. Linear and quadratic contrast coefficients for unequally spaced treatments were computed and used to evaluate treatment effects. Because of the unbalanced contribution of data from each station and the fact that not all stations used all levels of Cr supplementation, least squares means are presented. Additionally, the SEM provided in each data table represent the pooled SD divided by the square root of the least number of observations for the means in that particular table.

Results and Discussion

Statistical analysis of the data resulted in no interactions of Cr level or station with parity of the sows, and only one interaction (for gestation weight gain) between Cr level and station. This interaction was most likely a function of the fact that some stations did not use all Cr levels. When the data were subdivided into stations with common treatment levels, the interaction was not present. For that reason, the interaction was not deemed to be a biological reality related to Cr supplementation itself and is not presented.

Diet assay results presented in Table 3 demonstrated that the incorporation of CrPic was close to intended supplementation levels. The results of sow and litter responses by station are provided in Table 4. As expected, there were many individual station effects. Missing values for some responses occurred because of station differences in data collection procedures.

The value of cooperative research studies for evaluation of reproductive responses is exhibited herein. Aaron and Hays (2001) stated that progress in sow nutrition and management research is hampered by the large variation among sows in the economically

Table 4. Least squares means of station effects on sow and litter performance (as-fed basis)

Item	Station ^a			SEM ^b	P-value
	LSU	OSU	UK		
No. of sows	167	70	116		
Sow responses					
Breeding wt, kg	187.1	209.6	169.6	2.6	0.001
Gestation gain, kg	33.8	48.7	38.0	2.1	0.001
Lactation wt change, kg	-4.9	-3.6	3.0	1.4	0.001
Lactation feed, kg/d	5.40	6.79	4.85	0.12	0.001
Days to estrus postweaning	5.67	5.31	5.26	0.27	0.355
Litter size					
Total born	10.85	12.21	10.19	0.38	0.001
Live born	9.68	10.80	9.77	0.36	0.038
Weaned	8.31	9.05	8.88	0.30	0.087
Individual pig wt, kg					
Total born ^b	1.52	—	1.60	0.03	0.020
Live born	1.55	1.57	1.61	0.03	0.254
Weaned	5.69	6.43	6.36	0.15	0.001

^aStation designations are: LSU = Louisiana State University Agricultural Center, OSU = Oklahoma State University, and UK = University of Kentucky. The mean age at weaning was 20.6 d.

^bBecause of different numbers of sows per station, the maximum pooled SEM is reported.

^cTotal born pig weights were not measured at OSU.

important reproductive traits. With normal variation, the number of replications needed to detect a 10% difference in litter size at birth with an 80% chance of detecting that difference and a 10% probability level is 112 sows per treatment. Although there were less than 100 litters on the higher levels of supplementation in the study reported herein, the numbers were adequate to detect differences in several responses.

The results of Cr supplementation on sow and litter performance are presented in Table 5. Chromium supplementation increased lactation weight loss (linear, $P = 0.01$) and days to return to estrus postweaning (linear, $P = 0.08$). The litter size response of the Cr

supplementation level was of a quadratic nature as hypothesized in the development of the supplementation levels chosen for the study. A quadratic effect for both total pigs born/litter ($P = 0.07$) and pigs born live per litter ($P = 0.05$) was observed. Associated with this increase in litter size was an expected small reduction in birth weight (quadratic, $P = 0.10$). The litter size response peaked at the supplementation of 600 ppb Cr, demonstrating merit to the concept that Cr needs may be related to body size. If actual Cr need is related to total body mass, further research related to the original question of whether reproducing animals may respond to higher levels of supplementation seems warranted.

Table 5. Least squares means of supplemental Cr effects on sow and litter performance (as-fed basis)^a

Item	Added Cr, ppb				SEM ^c	P-values ^b	
	0	200	600	1,000		L	Q
No. of sows	141	104	42	66			
Sow responses							
Breeding wt, kg	187.9	191.9	191.0	184.2	3.3	0.18	0.10
Gestation gain, kg	41.2	37.7	43.5	38.2	2.8	0.77	0.44
Lactation wt change, kg	-0.2	0.8	-4.1	-3.9	1.8	0.01	0.69
Lactation feed, kg/d	5.62	5.68	5.63	5.78	0.15	0.39	0.70
Days to estrus, d	4.9	5.7	5.2	5.9	0.35	0.08	0.91
Litter size							
Total born	10.34	11.13	11.76	11.11	0.49	0.12	0.06
Born live	9.49	9.82	10.94	10.07	0.46	0.08	0.05
Weaned	8.42	8.61	9.06	8.91	0.38	0.15	0.45
Individual pig weights							
Total born, kg	1.61	1.57	1.47	1.56	0.05	0.21	0.10
Born live, kg	1.63	1.58	1.52	1.58	0.04	0.25	0.12
Weaned, kg	6.16	6.08	6.04	6.36	0.19	0.33	0.28

^aThe mean age at weaning was 20.6 d.

^bThe P-values provided are for the linear (L) and quadratic (Q) effects of the four levels of supplementation.

^cBecause of different numbers of sows per treatment, the maximum pooled SEM is reported.

Table 6. Tissue assay values of sows fed diets supplemented with Cr, ppb (dry weight)^{a,b}

Item	Added Cr, ppb				Pooled SEM	P-values ^c	
	0	200	600	1,000		L	Q
Adrenal gland	16.4	20.0	34.0	48.4	4.9	0.001	0.726
Kidney	35.8	56.4	132.6	176.0	32.0	0.003	0.827
Liver	22.8	37.4	87.6	92.2	17.0	0.005	0.355
Ovary	11.2	30.8	48.8	32.0	13.5	0.257	0.132

^aEach mean represents five sows completing three parities on test with the exception of the liver mean which represents four sows. The sows used for these samples were from the University of Kentucky.

^bThe limit of detection for the assay was 0.2 ppb total Cr in solution.

^cThe P-values provided are for the linear (L) and quadratic (Q) effects of supplementation.

Additionally, although the results of sows supplemented with 1,000 ppb Cr gave no indication of additional benefit beyond that of 600 ppb, there should not be any concern about overt detrimental effects because the results were numerically greater than the results of sows supplemented with 200 ppb.

In addition to the study results of Lindemann et al. (1995a,b), Trottier and Wilson (1998), Hagen et al. (2000), and Lindemann et al. (2000) also present CrPic supplementation effects of increased litter size. The magnitude of responses has varied, perhaps affected by geographic differences, genetic differences, or other factors. The study of Hagen et al. (2000), which involved approximately 48,000 sows, had litter size responses (about 0.37 pigs per litter) similar to the 200 ppb level reported herein.

The ability of Cr supplementation to improve litter size is logical considering previous research related to the metabolic effects of Cr on glucose and insulin. Amoikon et al. (1995) addressed the issue of Cr supplementation and its effects on glucose metabolism with the classic methodologies of i.v. glucose tolerance tests (IVGTT) and insulin challenge tests (IVICT). In the variety of metabolic measurements made during the IVGTT and IVICT, glucose disappearance rate was increased ($P < 0.04$) and glucose half-life was decreased ($P < 0.04$) in pigs fed CrPic at 200 ppb, thereby demonstrating an improvement in tissue insulin sensitivity consistent with the improved insulin efficiency noted by Lindemann et al. (1995a) in the first reproductive study. Cox et al. (1987) demonstrated that insulin injection of gilts immediately preceding estrus increased ovulation rate. That research group has also noted that insulin injection to primiparous sows for 5 d after weaning increased follicular estradiol and progesterone levels (Whitley et al., 1998), but this response was not observed in studies when it was administered to prepubertal gilts (Matamoros et al., 1991). The same research group (Ramirez et al., 1997) demonstrated that the effects of that short term insulin administration after weaning and before breeding could still be seen at the next farrowing, where increases of up to one pig/litter were observed with some of the insulin treatments. They further demonstrated (Whitley et al., 2002) that the insulin administration effects for that 4- or 5-d pe-

riod could be dependent on the metabolic or nutritional state of the animal and could increase litter size by as much as two pigs. Thus, linkages between supplemental Cr, glucose, and insulin exist (Amoikon et al., 1995; Lindemann et al., 1995a). Further, effects of glucose on reproductive performance have been known (Bunding et al., 1956; George et al., 1978) and effects of insulin on reproductive performance recently demonstrated. Thus, the improvements in litter size associated with Cr supplementation in the current study are consistent with these findings. Although Cr may have other metabolic effects in pigs, the effects on insulin/glucose relationships and their consequent metabolic effects are undoubtedly a primary mechanism by which its effects on reproduction are exerted.

The tissue results for Cr are provided in Table 6. Linear increases in tissue Cr were observed for the adrenal gland ($P = 0.001$), kidney ($P = 0.003$), and liver ($P = 0.005$). The ovarian tissue response was quadratic ($P = 0.132$) with a 4-fold increase for sows supplemented with 600 ppb Cr. The magnitude of increase observed in the ovary at 600 ppb was about the same as that observed in the liver and kidney and greater than that of the adrenal gland, but the SEM (in relation to the mean tissue content) was greater with the ovary as a result of it being a less homogenous tissue from which to take a subsample for analysis. This dramatically affected the statistical significance for that individual tissue but the similarity of the response surface to that of the other tissues (where the response was clearly statistically significant) suggests that it, too, is a true biological response. When it is considered that the peak litter size response was obtained at 600 ppb Cr, the liver and ovarian Cr responses would seem to be consistent with that. The Cr content of the kidney clearly continues to increase with higher levels of supplementation. Because absorbed Cr is excreted through the kidney, we assume that increases in absorbed Cr beyond the body need would be reflected in urinary Cr; thus, the possibility exists that some of the "kidney" Cr may be from residual urine in the kidney sample.

In conclusion, chromium supplementation resulted in increased litter size in a dose-related manner up to 600 ppb Cr from CrPic. Further research related to the question of whether reproducing animals may respond to higher levels of supplementation seems warranted.

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