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Dietary L-carnitine increases plasma leptin concentrations of gestating sows fed one meal per day

J.C. Woodworth[1], J.E. Minton*, M.D. Tokach, J.L. Nelssen, R.D. Goodband, S.S. Dritz[2], S.I. Koo[3], K.Q. Owen[1]

Department of Animal Sciences and Industry, Kansas State University, 253 Weber Hall, Manhattan, KS 66506-0201, USA

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Abstract

Thirty-four sows (parity = 1.8; BW = 206 kg) were used to determine the influence of L-carnitine and/or chromium tripicolinate on plasma leptin concentrations of gestating sows fed one meal daily. Treatments were arranged in a 2 × 2 factorial with main effects of carnitine (0 or 50 ppm) and chromium (0 or 200 ppb). Diets were fed for approximately 167 days (through one gestation, the following lactation, the interval from weaning to estrus, and 28 days into the following gestation) prior to blood collection. Leptin concentration was determined in plasma that was collected at feeding, every 15 min for the first 3 h after feeding, and at 6, 9, 15, 20, and 24 h after feeding. Sows fed diets containing carnitine had greater ($P < 0.02$) overall mean plasma leptin concentrations and greater ($P < 0.05$) leptin concentrations at 2.25, 3, 6, 15, 20, and 24 h after feeding compared to sows fed either the control diet or the diet containing chromium. Leptin concentrations of sows fed diets containing carnitine also were greater ($P < 0.05$) than control sows at 2.5 and 2.75 h postprandial and greater than ($P < 0.05$) sows fed diets with both carnitine and chromium at 6 h after feeding. Chromium had no effect ($P > 0.10$) on plasma leptin concentration. These results suggest that dietary carnitine, but not chromium, increases circulating leptin in gestating sows fed one meal per day. These results may help to explain the improvements in reproductive function previously observed from feeding sows diets containing carnitine.

Keywords: Sow; Carnitine; Chromium; Leptin

* Corresponding author. Tel.: +1-785-532-1238; fax: +1-785-532-7059.
E-mail address: eminton@ksu.edu (J.E. Minton).
[1] Present address: Lonza, Inc., Fair Lawn, NJ 07410, USA.
[2] Present address: Food Animal Health and Management Center, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506-5705, USA.
[3] Present address: Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269-4017, USA.

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1. Introduction

Carnitine is a vitamin-like compound that is needed for optimum utilization of fatty acids. Dietary carnitine has been shown to improve reproductive function in sows [1,2]. Chromium, as a component of the glucose tolerance factor, is essential in maintaining insulin sensitivity and glucose tolerance. Dietary chromium has also been shown to improve reproductive function in sows [3,4]. Recently, Real [5] observed an additive effect on farrowing rate and total number of pigs born alive when both carnitine and chromium where fed to sows for two parities.

Leptin is a protein that is released from the adipose tissue and has been shown to regulate energy balance [6]. In a review, Houseknecht et al. [7] reported that leptin also is released from tissues other than adipose, and that leptin plays an important role in food intake regulation, energy metabolism, hematopoiesis, and reproduction. The fact that carnitine and chromium are important for energy metabolism suggests that they could interact with leptin. In addition, the improvements in reproductive performance previously observed from feeding carnitine or chromium might be reflective of an influence on circulating leptin concentrations. The effect of dietary carnitine or chromium on circulating leptin concentrations in gestating sows fed one meal per day has not previously been determined. Therefore, this experiment was designed to determine the effects of L-carnitine and/or chromium tripicolinate on circulating leptin concentrations of gestating sows fed one meal per day.

2. Materials and methods

2.1. General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment. Gestating sows ($n = 34$; BW = 206 kg; parity = 1.8; PIC C-22) were randomly allotted to four dietary treatments based on parity and weight at initial breeding. Sows were fed the experimental diets starting at initial breeding, through gestation and the following lactation; weaning to breeding interval; and approximately 28 days into the subsequent gestation, when blood for the current study was collected. Experimental treatments were arranged in a 2 × 2 factorial with main effects of carnitine (0 or 50 ppm) and chromium (0 or 200 ppb). Both the L-carnitine (Carniking®) and chromium tripicolinate (Chromapure®) were obtained from Lonza Inc., Fair Lawn, NJ. Diets were fed in gestation as one daily meal, similar to commercial production. Sows were fed 2.04 kg of the gestation diet from breeding until day 100 of gestation, then 2.95 kg per day until they farrowed. A lactation diet was fed ad libitum from farrowing until weaning. After weaning, sows were returned to the 2.04 kg daily of the gestation diet fed in a single meal to provide 6700 kcal ME per day. Diets were corn-soybean meal-based and were formulated to contain 0.65% lysine, 0.90% Ca, and 0.80% P; and 1.00% lysine, 0.90% Ca, and 0.80% P in gestation and lactation, respectively. Sows were always allowed ad libitum access to water.

At approximately day 28 after the second breeding, or approximately 167 days after dietary treatments began, blood was collected from each sow at feeding; then once every 15 min for the first 3 h after feeding; and at 6, 9, 15, 20, and 24 h after feeding. Approximately

5 days prior to blood collection, sows were removed from the gestation barn and transported to a nearby surgery room for installation of venous catheters. Sows were anesthetized intravenously with sodium thiopental (25 mg/kg) prior to surgery and the surgical plane of anesthesia was maintained by inhalation of halothane (2–5%). Indwelling cephalic vein catheters (100 cm long; 1.02 mm i.d. and 2.16 mm o.d.; Helix Medical Inc., Carpinteria, CA) were inserted using techniques modified slightly from those described previously [8].

Plasma was analyzed to determine leptin concentration using a swine specific immunoradiometric assay (DSL-82100; Diagnostic Systems Laboratories Inc., Webster, TX). We tested the assay for dilutional parallelism and quantitative recovery of added leptin from porcine samples. The assay protocol called for a sample volume of 10 μl. Porcine leptin was quantitatively recovered at additional sample volumes of 15, 20, and 25 μl. The ratio of porcine leptin measured in the assay (when corrected for volume assayed) to leptin expected averaged 111% in three samples. Over a range of 0.5–25 ng/ml added to three samples of porcine sera, the ratio of leptin measured in the assay to the concentration expected averaged 84.9%. The assay was sensitive to 0.5 ng/ml (lowest concentration in the standard curve), and had intra- and interassay CVs that averaged 3.5 and 13.7%, respectively.

2.2. Statistical analysis

Data were analyzed as a randomized complete block design with repeated measures over time using the MIXED procedure of SAS (SAS Inst., Cary, NC) and sow was considered the experimental unit. The experimental model included all two-way interactions and main effects of carnitine and chromium. Covariates of sow weight and parity at blood sampling were used. Least square means were used to compare treatment means within time.

Originally 44 sows were sampled. Only sows that had completed the 167 days feeding period and that were pregnant at catheterization were included. Of the original 44 sows that met these criteria, nine sows exhibited undetectable leptin concentrations at almost all sampling times. Because the number of sows with undetectable leptin was distributed similarly across treatments (one sow from the carnitine and chromium combination treatment; two from the chromium treatment; and three sows each from the control and carnitine treatments), these sows were removed from the analysis rather than assign them entire profiles of leptin concentration at the assay sensitivity. Additionally, one sow that was fed the chromium treatment was removed as an outlier because her mean leptin concentration was greater than two standard deviations above the treatment mean. Thus, the data set contained a total of 34 sows. Furthermore, 10 sows had some samples in their profile that were undetectable (average of 4.6 undetectable samples of a total of 18 samples/sow). Data from these sows was included in the analyses, with each undetectable sample represented as a missing data point. Of these 10 sows with missing data points, four were fed the control diet, two carnitine, three chromium, and one was fed the treatment with both carnitine and chromium. Complete leptin profiles were obtained for four sows fed the control diet, six sows fed the carnitine and the chromium treatments, and eight sows fed the combination carnitine and chromium treatment. Therefore, the total number of sows (with and without missing data points) that were included in the analysis (and therefore represented in Figs. 1 and 2) was eight sows fed the control diet, eight sows fed the diet containing carnitine, nine sows fed the diet containing chromium and nine sows fed the diet containing both carnitine and chromium.

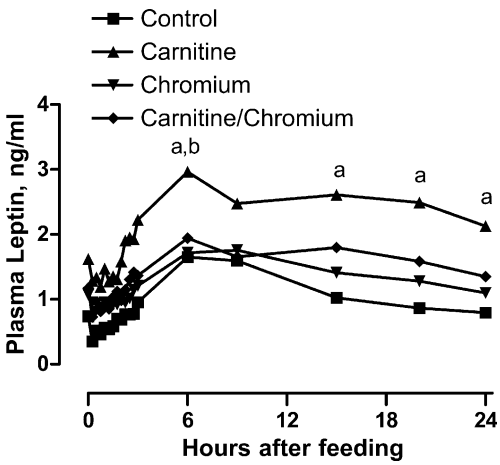

Fig. 1. Twenty-four hour profile of plasma leptin in sows fed a single meal daily of diets containing carnitine and/or chromium. Control diets contained neither supplemental carnitine nor chromium. Profiles represent mean concentrations of eight (Control and Carnitine) or nine (Chromium and Carnitine/Chromium) sows per treatment. The standard error of the mean for each data point is 0.47 ng/ml. [a]Carnitine > Control or Chromium; $P < 0.05$. [b]Carnitine > Carnitine/Chromium; $P < 0.05$.

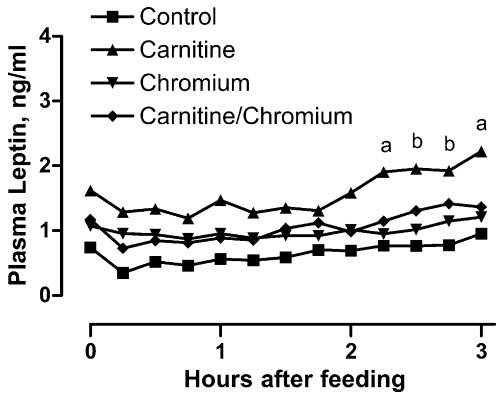

Fig. 2. Acute effect of feeding on plasma leptin in sows fed a single meal daily of diets containing carnitine and/or chromium. Control diets contained neither supplemental carnitine nor chromium. Data in the figure represent an expanded view of the first 3 h after feeding represented in Fig. 1. [a]Carnitine > Control or Chromium; $P < 0.05$. [b]Carnitine > Control; $P < 0.05$.

3. Results

Sow body weights were taken as an indicator of body condition. At the time of catheter insertion, body weights averaged between approximately 201 and 213 kg and did not differ significantly between any of the dietary treatments (Table 1).

Sows fed diets containing carnitine had greater ($P < 0.02$; Table 1) mean leptin concentrations compared to sows fed diets without carnitine. The leptin concentrations were greater

Table 1
Influence of carnitine and/or chromium on sow body weight and mean leptin concentration[a]

Item	Carnitine (ppm)				S.E.M.	Probability (P less than)		
	0	50	0	50		Carnitine	Chromium	C × C
	Chromium (ppb)							
	0	0	200	200				
Number of sows	8	8	9	9				
Sow weight (kg)	212.9	202.6	208.2	201.0	6.5	0.17	0.62	0.80
Leptin mean (ng/ml)[b]	0.80	1.84	1.12	1.22	0.38	0.02	0.56	0.06

[a] Values represent a total of 34 sows (average parity = 1.8).
[b] Values represent the mean of samples collected at feeding, once every 15 min for the first 3 h after feeding, and at 6, 9, 15, 20, and 24 h after feeding.

($P < 0.05$; Figs. 1 and 2) for sows fed diets with carnitine at 2.25, 3, 6, 15, 20, and 24 h after feeding compared to sows fed either the control diet or the diet containing chromium. Sows fed diets containing carnitine also had greater ($P < 0.05$) leptin concentrations compared to control sows at 2.5 and 2.75 h after the meal and compared to the sows fed diets with both carnitine and chromium at 6 h after the meal. The increased leptin concentrations of sows fed diets with carnitine appeared to be an increase in the baseline concentration of leptin that may have occurred because of the long supplementation period prior to sample collection. Chromium elicited no influence ($P > 0.10$) on plasma leptin concentrations. However, there was a tendency for a carnitine by chromium interaction ($P < 0.06$) for circulating leptin concentration.

4. Discussion

The absolute concentrations we report for circulating leptin are generally lower than those reported for gestating gilts [9] or lactating sows [10–12]. However, in all of those reports, the same multi-species leptin assay was used, while the assay employed in the current study utilized a swine recombinant standard and antibodies developed against the same porcine leptin preparation as the standard in the assay. Differences in assays used across studies could account for some of the variation in absolute leptin concentrations in sows and gilts, but variables such as daily energy intake [13] and sow body condition [10] undoubtedly contribute as well. In general, the post-feeding increase in circulating leptin observed in the current study was maximal in all treatments at about 6 h postfeeding. This feeding-induced increase in leptin has been reported previously for lactating sows fed three times daily [11].

Our inability to detect circulating leptin from entire blood profiles of a subset of sows, and occasional samples that could not be measured within the profiles of a few additional sows deserves comment. First, we did not measure backfat in these sows and, therefore, cannot associate the unmeasurable leptin with sows having the thinnest body condition. However, as noted earlier, these sows were fed a single meal daily according to contemporary swine

industry standards. Based upon our casual observations at feeding, we estimate these meals were consumed within the first 10–15 min following feeding. This left the animals without feed for most of the more than 23 h remaining until the next feeding. Based upon profiles of blood glucose, insulin, and insulin-like growth factor-I [14], we estimated the animals to be in an anabolic state for approximately 2 h after feeding, and in a catabolic state for the remaining 22 h of the 24-h bleeding period. Assuming that this is an accurate portrayal of the metabolic status of the sows as a whole, high levels of a circulating hormone that would suppress appetite, such as leptin, certainly would not be expected. Thus, perhaps it is not surprising that leptin concentrations were generally low in the majority of sows that could be measured and were unmeasurable in some additional animals.

This is the first experiment to demonstrate that carnitine influenced the concentration of circulating leptin in swine. Hongu and Sachan [15] conducted the only other experiment to determine the influence of carnitine on leptin concentration. They fed rats a control diet or a diet supplemented with 0.1, 5, and 11.5 g/kg of caffeine, carnitine, and choline, respectively, and observed that rats fed the supplemented diet had lower serum leptin compared to rats fed the diet without supplementation. Because of limitations in their experimental design, Hongu and Sachan [15] could not determine if the reduction in serum leptin occurred because of the actions of one of the supplements or the combination of all three. To our knowledge, no other experiments have been conducted to determine the effects of carnitine or chromium on plasma leptin in animal or human experimental models.

It is widely known that leptin is secreted by the adipose tissue and acts as a satiety signal to regulate energy metabolism [16,17]. However, the fact that leptin has also been shown to be secreted by the placenta [18], stomach [19], and skeletal muscle [20], and the fact that leptin receptors have been identified on most tissues [7] would suggest that leptin plays a greater role in the body than just regulating energy metabolism. Of importance to the sow, leptin is suggested to play a significant role in normal reproductive performance because of its actions on the hypothalamus–pituitary–gonadal axis [21] as well as the discovery of leptin receptors on the ovaries [22] and testes [23].

Previous experiments have shown that reproductive performance is improved when sows are fed diets containing carnitine [1,2]. However, the reason that carnitine improves reproductive performance has not been determined. Woodworth [14] suggested that the improvements in reproductive performance from feeding sows carnitine could be reflective of the improved energy utilization of these sows compared to those fed diets without carnitine. Our observation that sows fed carnitine exhibited elevated plasma leptin would suggest that energy metabolism was affected. In addition, the observation that elevated circulating leptin was associated with improved reproductive performance in other species [24] would suggest that the improvements in reproductive performance exhibited by sows fed diets with carnitine may be linked to the increased circulating leptin concentrations.

Real [5] observed that when sows were fed diets containing carnitine for two parities, the interval from weaning to estrus was shortened, the number of sows in estrus by day 7 tended to improve in the first parity; and first service farrowing rate tended to improve in the second parity compared to sows fed diets not containing carnitine. Additionally, although not analyzed, Real [5] noted that more sows fed diets containing carnitine completed both parities (were not removed from the experiment) compared to sows fed diets not containing carnitine. In that study, the main reasons sows were removed from the experiment were

failure to return to estrus by 18 days after weaning, failure to conceive, and failure to maintain pregnancy. In commercial production, it is common for sows to exhibit a negative energy balance during lactation, and thus they must rely on mobilization of body energy reserves for milk production. This period of significant negative energy balance may lead to an inability to return to estrus or maintain subsequent pregnancy. This is supported by the observation that adequate nutrition and weight maintenance is needed for normal reproductive cycling and function, and that significant weight loss (negative energy balance) may lead to reproductive failure [25,26]. Our observation that carnitine increased the leptin concentration in plasma of sows may be the reason that fewer sows were removed because of reproductive failure in the study conducted by Real [5]. It is possible that the elevated leptin was associated metabolically with positive energetic signals, and therefore allowed more sows to return to estrus and/or maintain pregnancy when fed diets with carnitine. Thus, carnitine, perhaps associated with leptin, may improve the reproductive longevity of sows.

In a separate analysis, Woodworth [14] observed for the first time in swine, that dietary carnitine reduced the circulating concentrations of insulin and glucose immediately after feeding, suggesting an enhanced glucose tolerance. In other experiments, leptin has been shown to improve glucose utilization and insulin sensitivity in vitro [27,28] and in vivo [29,30]. Therefore, our finding that dietary carnitine increased circulating leptin concentrations would support the previous findings of lower circulating glucose and insulin concentrations also associated with dietary carnitine.

Woodworth [14] also observed that sows fed diets with carnitine tended to have higher plasma IGF-1 and IGF binding protein-3, in addition to the higher leptin reported herein. These findings are supported by research that serum concentrations of IGF-1, IGF binding protein-3, and leptin are highly correlated in lean human subjects [31].

There was a tendency for a carnitine by chromium for circulating leptin concentration, and the biological significance of this tendency is not clear from out data. However, this trend for an interaction can most likely be explained by the fact that the difference in average circulating leptin in the sows fed the diet containing both carnitine and chromium compared to that in sows fed chromium alone was of lesser magnitude than the increment in leptin observed in sows fed the diet containing carnitine compared to plasma leptin in sows fed the diet containing neither carnitine or chromium.

These results suggest that feeding L-carnitine, but not chromium tripicolinate elevates the concentration of circulating leptin in gestating sows fed one meal per day. The elevated leptin concentrations suggest that feeding carnitine will influence the biochemical pathways involved in energy metabolism. Likewise, the improvements in reproductive performance previously observed from feeding sows diets with carnitine may be reflective of the elevated circulating leptin exhibited by these sows.

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