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Plane of Nutrition Affects Plasma Ghrelin Concentrations in Neonatal Calves

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Summary and Implications
Investigating different planes of nutrition on appetite-related hormones could provide knowledge into the role of these hormones on growth performance in neonatal calves. The objective of the current study was to investigate the effects of feeding rates on ghrelin in plasma from preruminant calves. Treatments (n = 8 per treatment) were designed to achieve three targeted daily rates of gain (No Growth = 0.0 kg, Low Growth = 0.55 kg, or High Growth = 1.2 kg) in live weight over a 7-wk period. All calves were fed a 30% crude protein, 20% fat, all-milk protein milk replacer reconstituted to 14% dry matter. Daily growth rates for No, Low, and High Growth calves were different (P<0.001) throughout the experimental period and averaged 0.11 ± 0.02 kg, 0.58 ± 0.02 and 1.16 ± 0.04 kg, respectively. Fasting ghrelin active concentration was higher (P < 0.0001) in the No Growth calves over the 7-wk period in comparison to the Low and High growth calves. Circulating concentrations of ghrelin in neonates fed different planes are similar to responses of adult humans to feed intake. These results indicate an inverse relationship of ghrelin active concentration with respect to plane of nutrition and growth rate in neonates.

Introduction
Since the discovery and isolation of ghrelin, many studies have considered the function of ghrelin in the body. Ghrelin stimulates growth hormone release independently of growth hormone releasing hormone. Additionally, leptin activity is controlled by ghrelin. Through its action upon the neuropeptide Y/Y1 receptor pathway, ghrelin antagonizes leptin. Leptin promotes satiety and ghrelin stimulates nutrient intake thereby regulating the action of each other.

Ghrelin is synthesized in the arcuate nuclei and oxyntic glands of the stomach. Stomach ghrelin is thought to be involved in physiological effects and possibly stimulates secretion of growth hormone. Some of the physiological effects of ghrelin include hyperglycemia in humans, adiposity in rodents, increased gastric acid secretion in rats, and increased gastric motility in rats.

Rats fed a high carbohydrate diet have higher plasma ghrelin than do rats fed a low carbohydrate diet. In a recent study healthy non-obese women were fed either a high fat or high carbohydrate meal. The high carbohydrate meal caused the greatest increase in plasma ghrelin. The high carbohydrate diet suppresses the hunger sensation more than the high fat diet. Ghrelin concentration, however, increases with weight loss of humans on a low fat, high carbohydrate diet. Diet-induced obesity, however, is not related directly to ghrelin concentration in juvenile rats prone to obesity. High fat diets decrease adiposity without increasing appetite. The conflicting results from studies involving ghrelin and diet composition leave the relationship between ghrelin and diet composition unclear.

Mechanisms by which ghrelin causes adiposity are poorly understood. Plasma and cerebrospinal fluid ghrelin concentrations in obese humans are lower than those of normal weight individuals. This observation is contrary to the findings showing increased adiposity in rodents injected with ghrelin. In 2002, English and colleagues demonstrated that re-feeding after fasting does not decrease ghrelin concentrations in obese human patients. In normal weight humans, fasting ghrelin concentrations decrease after feeding.

Ghrelin has a negative association with ad libitum feed intake. However, they evaluated fasting (average) ghrelin which may not reflect the rise in ghrelin concentration before a meal. Furthermore, many studies use a total ghrelin assay rather than the active ghrelin assay and may explain the disparity in the literature regarding relationships between ghrelin, diet composition, and obesity.

The objective of the study was to investigate the effects of three different feeding rates to achieve three targeted growth rates on ghrelin concentration in plasma. We hypothesized that calves at the lowest growth rate will have the highest plasma ghrelin concentration and calves at the highest growth rate will have lowest plasma ghrelin concentration.

Materials and Methods
Twenty-four Holstein bull calves were acquired from a single Wisconsin dairy herd over a 2-wk period. All were given 3.9 L of colostrum within 6 h of birth. Before the trial, calves were fed twice daily 0.3 kg of a 20% CP, 20% fat milk replacer (Instant Nursing Formula: Dairy Herd & Beef Calf Milk Replacer; Land O’ Lakes, Inc., Shoreview, MN) reconstituted to 15% of dry matter.

On the first Monday after arrival (average age 9.1 ± 2.4 d; wk 0 of the experiment), calves were weighed and assigned randomly to 1 of 3 treatment groups (8 calves per treatment) designed to achieve three targeted daily rates of
gain (No Growth = 0.0 kg, Low Growth = 0.55 kg, or High Growth = 1.2 kg) in live weight. The NRC Nutrient Requirements of Dairy Cattle calf model computer program (NRC, 2001) was used to estimate the milk replacer intakes needed to achieve target growth rates. Calves were housed in elevated pens in a temperature-controlled barn with a 12 hour light/dark cycle. All calves were fed a 30% crude protein, 20% fat, all-milk protein milk replacer (Land O’ Lakes, Inc.) reconstituted to 14% dry matter. The diet was formulated to ensure that protein would not be a limiting nutrient. Calves were weighed weekly and the amount of milk replacer fed was adjusted at these times to allow for changes in body weight. Because vitamin concentrations in the milk replacer were based on the dry matter intake of High Growth calves, No Growth and Low Growth calves were supplemented once weekly to compensate for decreased milk replacer consumption. Supplements were calculated to ensure that all calves received similar amounts of vitamins A, D, and E. Calves were bucket-fed twice a day (0700 and 1800 h) and offered water ad libitum. No starter grain was offered. The amounts of milk replacer offered and refused were recorded at each feeding. All calves were vaccinated on wk 3 with Mycobacterium bovis, strain bacillus Calmette-Guerin (BCG) and weeks 3 and 5 with ovalbumin (OVA).

Blood was collected weekly by jugular venipuncture in the morning before feeding. Plasma was collected and stored at -20°C until analysis. Plasma ghrelin (active and total) were analyzed by using radioimmunoassay (Linco Scientific). All data were analyzed by using the GLM procedure in SAS (SAS Institute). Animal procedures were approved by the Animal Care and Use Committee of the National Animal Disease Center.

Results and Discussion
Calves had significantly different growth rates in all three treatments groups (Figure 1). The body weight of calves were not different before administration of dietary treatments and averaged 45.0 ± 0.7, 46.0 ± 2.1, and 46.1 ± 1.3 kg for No, Low and High Growth treatments, respectively (Figure 1). Mean body weight of the calves from the 3 treatment groups differed (P < 0.05) by week 1 and remained so for the duration of the study. Growth rates for No (0.11 kg/d), Low (0.58 kg/d), and High Growth (1.16 kg/d) calves differed (P < 0.0001) throughout the experimental period (Figure 1). Growth rates during the period before vaccination (wk 0 to 3) differed (P < 0.05) and averaged 0.06 kg/d for the No Growth, 0.53 kg/d for the Low Growth, and 1.16 kg/d for the High Growth groups.

Calves in the No Growth group had higher concentrations of active ghrelin than did calves in the Low and High growth groups over the course of the entire study (Figure 2). Calves in the Low and High growth groups did have different ghrelin active concentrations, however, the difference between the Low and High Growth groups ghrelin active concentration was not as pronounced after week 3 (Figure 2).

On weeks 4 and 6 of the study, there was a decrease in ghrelin active concentration in all groups. This decrease is one week following each vaccination. We hypothesize that cytokines, such as interferons and interleukins, may be regulating appetite through the suppression of ghrelin active concentration.

There was no significant difference in ghrelin total concentrations among all three treatment groups over the seven-week period (Figure 3). The ratio of ghrelin active concentration to ghrelin total concentration was higher in the No Growth calves than the ratio of ghrelin active concentration to ghrelin total concentration in Low and High Growth calves (Figure 4).

The decrease in ghrelin active concentration after week 3 in the Low Growth group is most likely an adaptive response to energy intake. The calves in the Low Growth group were adapting to the amount of energy provided. Calves in the No and Low growth groups had higher ghrelin active than did the calves in the High Growth group. The total amount of ghrelin (active and desacyl ghrelin) secreted is the same in response to energy intake. Altering the ratio of active ghrelin to desacyl ghrelin is a possible route in which calves regulate the physiological effects of active ghrelin in response to energy intake.

Circulating concentrations of ghrelin in neonates fed different planes are similar to responses of adult humans to feed intake. The immune response of neonatal calves is similar to infant humans. Neonate calves are an appropriate model for studying the relationship between hormones related to appetite control and immune system function.

Future research is needed to further investigate the effects of plane of nutrition on the physiological effects of ghrelin.

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Figure 1. Calf Growth Curve.
Mean ± SEM  Significant p<0.05
Growth rate was significantly different (p<0.0001) among all three treatments.

Figure 2. Effect of Plane of Nutrition on Ghrelin Active Concentration.
Treatment effect over 7 weeks:
No Growth vs Low Growth: p = <0.0001
Low Growth vs High Growth: p = 0.0036
No Growth vs High Growth: p = <0.0001
Significant p < 0.05
Mean ± SEM
Figure 3. Effect of Plane of Nutrition on Ghrelin Total Concentration.
Treatment effect over 7 weeks:
No Growth vs Low Growth: p = 0.9385
Low Growth vs High Growth: p = 0.7466
No Growth vs High Growth: p = 0.6890
Significant p < 0.05
Mean ± SEM

Figure 4. Effect of Plane of Nutrition on Ghrelin Active to Ghrelin Total Ratio.
Treatment effect over 7 weeks:
No Growth vs Low Growth: p = <0.0001
Low Growth vs High Growth: p = 0.1428
No Growth vs High Growth: p = <0.0001
Significant p < 0.05
Mean ± SEM