CIRCULATING GHRELIN CONCENTRATIONS DURING THE TRANSITION PERIOD OF DAIRY CATTLE AND THE ASSOICATED RELATIONSHIP WITH MILK PRODUCTION

By

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Abstract:

The hormone ghrelin was discovered in 1999. It has a 27-28 amino acid sequence with a n-octanoylation at the third serine residue. Ghrelin is expressed in most tissues throughout the body and is primarily known for its GH releasing activities. Ghrelin also has a significant role in the regulation of feed intake and energy balance and may be a potent metabolic regulator of reproduction. During this experiment, plasma ghrelin concentrations were measured in dairy cattle (n=13) biweekly from 14 days prepartum to 60 days postpartum. Circulating ghrelin concentrations did not change with stage of lactation, but were affected by level of milk production. High producing and low producing cows had expected 305-day yields of 12,923 ± 217 and 10,332 ± 322 kilograms of milk, respectively ($P< 0.001$). The high producers had lower circulating ghrelin concentrations (45.1 ± 8.9 pg/mL) than low producers (73.3 ± 8.5 pg/mL). These results are different than anticipated but may, be explained by prioritized nutrient partitioning in low producing and high producing dairy cows.

Introduction

Ghrelin was first discovered in 1999 when it was purified from the stomachs of rats (Kojima et al., 1999). The name ghrelin is derived from a Proto-Indo-European word of 'ghre' which means grow and 'relin' because the hormone promotes growth hormone (GH) release (Lorenzi et al., 2009; Kojima et al., 1999).The active form of bovine ghrelin has a 27 amino acid sequence (28 amino acids in most other species) with an n-octanoylation at the third serine residue. It is this acetylation that activates the hormone secretagogue ligand. The non-acetylated form of ghrelin is considered inactive and is the primary form of ghrelin in the circulation. It does not promote the release of GH or other endocrine activities in rats. However, scientists are unsure if there is a specific receptor for this form of ghrelin, and if so, if it is biologically active (Litwack et al., 2008). Overall ghrelin has been shown to be relatively conserved, with an 82% homology between rats and human. It is responsible for inducing feeding, secretion of the hormone prolactin, the secretion of gastric juices and deposition of adipose tissue.

Ghrelin employs both autocrine and paracrine functions in order to regulate the body system (Barreiro and Tena-Sempere, 2004; Gottero et al., 2004). Ghrelin is primarily expressed in the X/A- like cells of the oxyntic gland in the stomach of monogastrics and the abomasum of ruminant animals (Date et al., 2000; Gottero et al., 2004; Litwack et al., 2008; Miura et al., 2004; Wretz-Lutz et al., 2006). There are lower levels expressed in the small intestine, the pancreas, lymphocytes, placenta, kidney, lung, brain, pituitary and gonads (Barreiro and Tena-Sempere, 2004).Ghrelin is called the "ultimate anabolic hormone" because it causes the body to consume and store energy (Litwack et al., 2008).

Ghrelin is the natural agonist for the growth hormone secreatogue receptor (GHS-R). There are two different GHS-R subtypes produced by alternative splicing with GHS-R1a being

the functionally active complete form of the receptor. GHS-R1a is a seven trasmembrane G-protein- coupled receptor that is responsible for signal transduction (Barreiro and Tena-Sempere, 2004; Litwack et al., 2008). GHS-R1b is the other receptor form. However, it lacks the transmembrane regions six and seven, reducing its binding affinity and signal transduction (Barreiro and Tena-Sempere, 2004). GHS-R1a is expressed in higher amounts when the organism is in a state of negative energy balance (Litwack et al., 2008). GHS-R1a is concentrated in the pituitary cells where ghrelin promotes GH releasing activities as well as oocytes, somatic follicular cells, hilus interstitial cells and all stages of luteal cells (Litwack et al., 2008; Lorenzi et al., 2009). An important point to note is that mice lacking the GHS-R1a suffer from neither a metabolic phenotypic nor a reproductive defect (Litwack et al., 2008; Lorenzi et al., 2009).

Through previous experiments, it has been shown that ghrelin may play a role in regulating energy balance (Bradford and Allen, 2008; Hayashida et al., 2001 Wren et al., 2000; Wretz-Lutz et al., 2006). One of its primary roles is to stimulate feed intake. Signaling by ghelin at the hypothalamus and the brainstem activate appetite stimulation genes, thus increasing feed intake. Research has shown that the amount of food per meal does not effect the expression of circulating ghrelin, but rather the number of meals is more important as a regulating factor because ghrelin levels are reduced after consuming food (Litwack et al., 2008; Wretz-Lutz et al., 2006).

Ghrelin also has a role in signaling the deposition of fat tissue by increasing food intake and reducing fat utilization (Bradford and Allen, 2008). There is now information that suggests that leptin acts in opposing fashion to ghrelin by signaling satiation (Dimaraki and Jaffe, 2006; Gottero et al., 2004; Nogueiras et al., 2008; Wren et al., 2000). This causes ghrelin to be

expressed at lower concentrations during states of positive energy balance and increased during negative energy balance (Dimaraki and Jaffe, 2006; Nogueiras et al., 2008). Because of this close relationship, it was also determined that both ghrelin and leptin may play roles in regulating reproduction.

The mechanisms responsible for altering ghrelin secretion remain unclear (Garcia et al., 2007; Gottero et al., 2004). Studies have shown that circulating concentrations depend on gender, developmental stages and reproductive hormones like estrogen. Other studies have postulated that blood glucose may have an integral role (Garcia et al., 2007). However, the extent that ghrelin secretion is dependent on these things is still yet to be determined (Garcia et al., 2007; Gottero et al., 2004).

Ghrelin's Role in Female Reproduction:

As previously mentioned, it has been suggested that ghrelin has a role in the control of reproduction. This is because of the effects of locally produced ghrelin as well as the gut derived hormone conferring systemic control of reproduction and the direct gonadal effects (Garcia et al., 2007; Litwack et al., 2008; Lorenzi et al., 2009; Wren et al., 2000).

In the female, gonadal expression of ghrelin is a function of the estrous cycle. Ghrelin expression is at its lowest at proestrus and highest during diestrus (Barreiro and Tena-Sempere, 2004). Proestrus is the period that immediately precedes estrus. This is when follicles form and estrogen secretion increases. Diestrus is the period of maximum luteal function with sustained secretion of progesterone from the corpus luteum (Senger, 2005). Ghrelin was not detected in follicles regardless of their developmental stage; however ghrelin expression was located in the ruptured follicle, the corpus luteum (CL; Barrerio and Tena- Sempere, 2004). The expression of

ghrelin is similar to the functionality of the CL because the expression of ghrelin is parallel to the production of progesterone, suggesting that ghrelin may have a role in its functionality. Progesterone is produced by the CL and maintains gestation (Senger, 2005). Ghrelin expression is higher during the beginning of gestation and also acts to maintain the pregnancy (Garcia et al., 2007; Forbes et al., 2009; Litwack et al., 2008; Lorenzi et al., 2009). The functional receptor, GHS-R1a is expressed in many reproductive tissues including oocytes, somatic follicular cells, hilus interstitial cells and luteal cells of all stages (Barreiro and Tena-Sempere, 2004; Litwack et al., 2008). The expression of GHS-R1a roughly paralleled the development of the follicle, although this relationship is not proven (Litwack et al., 2008).

Through previous studies it has been determined that ghrelin suppresses secretion of lutenizing hormone (LH; Barreiro and Tena-Sempere, 2004; Lorenzi et al., 2009). Ghrelin positively affected the secretion of follicle stimulating hormone (FSH) and LH at the pituitary level. However, hypothalamic ghrelin altered the release of gonadotropin- releasing hormone (GnRH) or lutenizing-hormone-releasing-hormone (LHRH), causing FSH response to be increased while LH was inhibited (Barreiro and Tena-Sempere, 2004; Lorenzi et al., 2009).

Ghrelin is also involved in the control of prolactin secretion, which stimulates milk production (Barreiro and Tena-Sempere, 2004; Gottero et al., 2004; Litwack et al., 2008). During lactation, females undergo a state of negative energy balance usually causing weight loss. Although, in rats ghrelin levels do not imitate a state of starvation as would be anticipated (Gottero et al., 2004; Litwack et al., 2008). Actually, ghrelin concentrations post parturition are lower than those of non-pregnant subjects (Litwack et al., 2008). Also, when ghrelin levels were increased, milk production also increased which then caused the weight of the offspring to also increase (Gottero et al., 2004; Litwack et al., 2008).

Ghrelin has a regulatory role in the forming embryo. Ghrelin acts as a chemical messenger for intercellular communication during multiple stages of embryo development from the blastocyst to the rate of apoptosis (Lorenzi et al., 2009). Ghrelin has been shown to negatively regulate cell viability and proliferation, thus inhibiting implantation when the female is experiencing a state of negative energy balance due to pregnancy and/or lactation (Litwack et al., 2008). This suggests that ghrelin can serve as a metabolic signal to the reproductive system by inhibiting implantation and development, thus protecting the female (Barreiro and Tena-Sempere, 2004; Garcia et al., 2007; Litwack et al., 2008).

Ghrelin is involved in embryo implantation through expression in the uterine endometrium. Implantation requires changes in the uterine environment to foster the development of the embryo. Research has shown that ghrelin has inhibited the implantation of mouse embryos. This might be explained by ghrelin acting as a key signal for energy insufficiency thus inhibiting pregnancy (Garcia et al., 2007). Ghrelin is expressed from the luminal and glandular epithelial cells of the secretory endometrium. The secretions produced by these cells are vital to embryo implantation, thus suggesting a mechanism by which ghrelin may be inhibiting implantation (Barreiro and Tena-Sempere, 2004; Garcia et al., 2007). Ghrelin is also expressed in human placenta during the first trimester in extravillious trophoblasts. Since these cells are involved in the placental invasion into the maternal uterus, this may be another pathway by which ghrelin interferes in embryo implantation (Garcia et al., 2007).

Ghrelin's expression levels vary throughout pregnancy. During the first trimester, ghrelin expression is greatly increased with peak ghrelin levels occurring during mid gestation (Litwack et al., 2008; Lorenzi et al., 2009). However, toward the end of pregnancy, ghrelin levels are greatly decreased compared to the levels of pregnant and non-pregnant subjects (Litwack et al.,

2008). This has been hypothesized to be a response to maternal energy intake. Ghrelin might prepare the uterus for parturition because of its uterorelaxant properties (Lorenzi et al., 2009). After parturition, ghrelin in rats is decreased below levels that are typically detected before pregnancy occurred (Litwack et al., 2008).

The hormone ghrelin has many roles in female reproduction. Ghrelin is expressed in many reproductive tissues and gonadal expression of ghrelin is highest during diestrus when the CL is functional. Ghrelin also has a role in regulating the secretion of the sexual hormones LH, FSH and prolactin. Virtually every role relates back to helping the female maintain a positive energy status.

Hypothesis

The primary hypothesis of this research experiment is that circulating ghrelin concentrations in dairy cattle are highest during early lactation due to a state of negative energy balance. In turn, these high ghrelin concentrations should be positively associated with milk production.

Objectives

1) To measure circulating ghrelin concentrations in plasma within the transitional period of Holstein dairy cattle.
2) To compare circulating ghrelin levels with the milk production of dairy cattle.

Materials and Methods

Animals.

The experiment was conducted on a commercial dairy farm in Coolidge, Arizona during the months of December through March Thirteen lactating multiparious Holstein dairy cows (n=13; days of gestation=270 ± 3.2 days) were housed in group pens and managed according to the standard operating procedures of the dairy. Animals were fed a TMR formulated for early lactation dairy cattle. All procedures were reviewed and approved by the University of Arizona Institutional Animal Care and Use Committee.

Blood Samples.

Blood samples were collected by coccygeal veinipuncture twice weekly beginning 2 weeks prior to parturition until 60 days postpartum. Samples were collected into an evacuated glass tube containing EDTA (BD Vacutainer; Franklin Lakes, NJ) and placed on ice until further processing. Plasma was immediately harvested via centrifugation at 2,300 x g for 20 minutes. Plasma samples were treated with 50µl of 1N HCl and 10µl of phenylmethylsulfonyl fluoride (C7H7FO2S; Enzo Lifesciences; Plymouth Meeting, PA) per ml of plasma, according to kit instructions for analysis of ghrelin by radioimmunoassay (RIA). Samples were frozen and stored at -20°C until further analysis.

Ghrelin Analysis.

Plasma ghrelin concentrations were measured by a validated commercially available RIA kit for the active form of ghrelin (Millipore Corp; Billerica, MA; Field et al., unpub.). The inter-assay CV averaged 4.52% over 2 kits and the intra-assay CV was 1.72% and 4.09%.

Statistical Analysis

Data was analyzed using the MIXED procedure of SAS (SAS Institute Inc.; Cary, NC). The compound symmetry, unstructured and autoregressive (1) covariance structures were tested and the most appropriate (lowest Akaike's information criterion, Akaike's information criterion

with correction, and Bayesian information criterion values) was used. Sample number was included in the model as a fixed variable and cow was included as a random effect. Cows were also divided into high production and low production groups for analyses. Results are reported as least squares means ± standard error of mean. Separation of means was conducted with the Tukey procedure of SAS. PROC CORR was used to detect correlations between all variables.

Results and Discussion

Previous studies have shown that ghrelin levels should decrease as food is being consumed. During the study, the feeding status of the Holstein cows was recorded (fed prior to or after blood sample collection) and later analyzed to see if circulating ghrelin concentrations were affected by feeding status. It was determined that there was no effect of feeding status, so it was excluded from the statistical model.

The relationship between ghrelin and milk production turned out to be the opposite of what we anticipated. The cows were split into high producing and low producing groups based on their individual expected milk production for their lactation. The average milk production for the high producing group and the low producing group was 12,923 ± 217 and 10,332 ± 322 kilograms of milk respectively ($P < 0.01$). Based on the information available in literature, we would anticipate that the high producing group would have higher circulating ghrelin concentrations compared to the low producing groups. However, the high producers had ghrelin levels of 45.1 ± 8.9 pg/mL while the lower producers had levels of 73.3 ± 8.5 pg/mL ($P < 0.05$). From this data a negative correlation between ghrelin and milk production was determined. This means that as the ghrelin levels decrease, milk production levels increase ($r=-0.35$; $P < 0.001$). However, this correlation could only explain 12% of the variation in milk production.

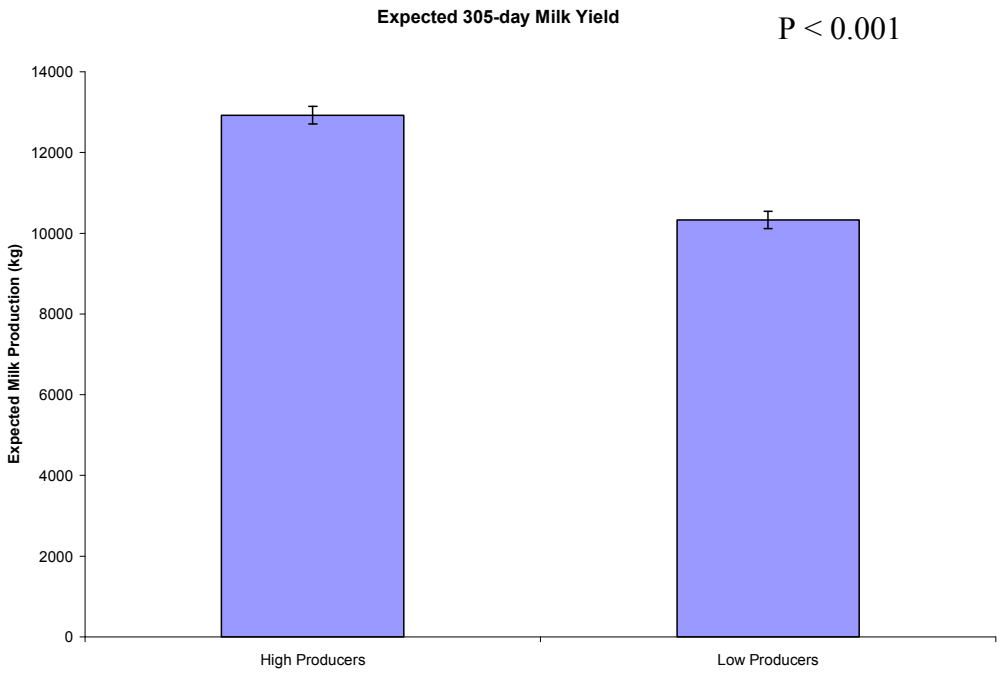

Figure 1: Milk production (kg) measured in least square means ± standard error for high producing cows and low producing cows (P<0.001).

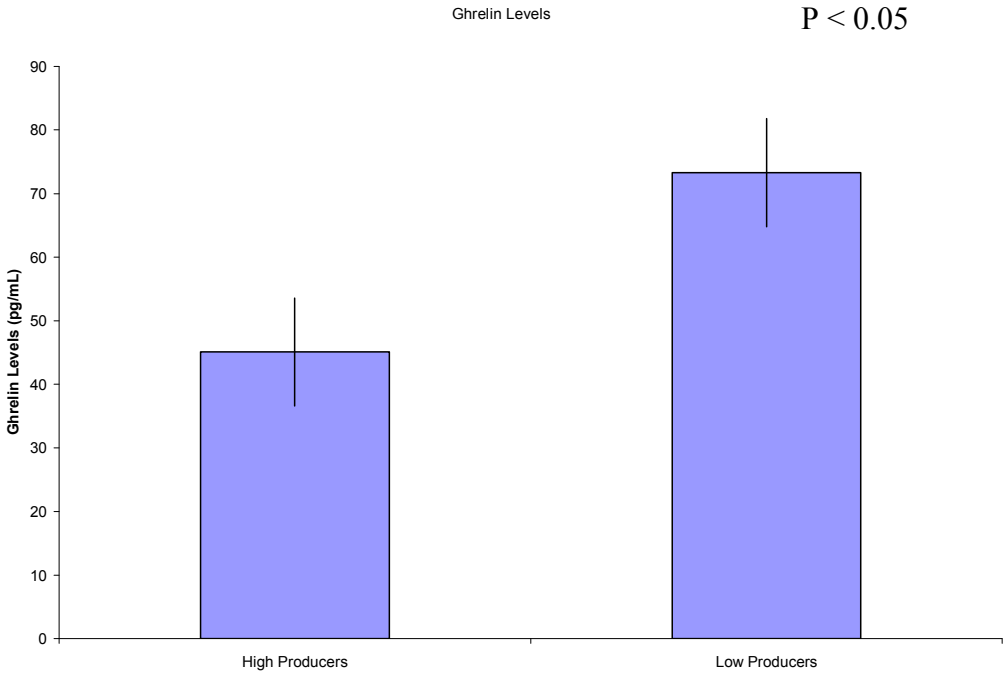

Figure 2: Plasma ghrelin concentrations (pg/mL) measured in least square means ± standard error for high producing and low producing cows. The ghrelin level for the high producing cows was 45.1 ± 8.9 pg/mL , and the ghrelin for the low producing cows was 73.3 ± 8.5 pg/mL ($P<0.05$).

These unexpected results might be attributed to the fact that milk is an energy demand on the cow. Higher ghrelin levels in the low producing cows could be a result of their body's attempt to conserve energy, thus decreasing milk production. Likewise, the lower ghrelin concentrations in the high producing cows would then allow those cows to partition more nutrients toward milk production.

Interestingly, there also was no effect of sample number, meaning that the concentration of ghrelin did not change over time with changes in stage of lactation. We expected plasma ghrelin levels to be highest either at calving or right after calving due to associated changes in feed intake and the initiation of lactogenesis. Thereafter, we expected ghrelin concentrations to decrease because the cow would no longer be in a state of negative energy balance. However, as

previously stated there was no change in circulating ghrelin concentrations based on sample number over time of lactation. While these results were unexpected, they are in agreement with lactational studies conducted in rats that were discussed earlier (Gottero et al., 2004; Litwack et al., 2008).

Summary

This experiment investigated changes in circulating ghrelin concentrations, during the transitional period in Holstein dairy cattle. We determined that the high producing cows (12,923 ± 217 kg of milk) had lower levels of plasma ghrelin(45.1 ± 8.9 pg/mL) compared to the low producing cows (10,332 ± 322 kg of milk; 73.3 ± 8.5 pg/mL of ghrelin), but the high producers produced more milk in comparison to the low producers. Although the results were the opposite of what we anticipated, they may be the result of prioritized nutrient partitioning in an attempt to maintain a favorable energy status.

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