General and Comparative Endocrinology 235 (2016) 64–69

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Seasonal and parity effects on ghrelin levels throughout the estrous cycle in dairy cows

Hen Honig [a], Lior Ofer [b], Michal Elbaz [b], Moshe Kaim [b], Dima Shinder [c], Eran Gershon [b,*]

[a] Institute of Animal Sciences, Agricultural Research Organization, PO Box 6, Bet-Dagan 50250, Israel
[b] Department of Ruminant Science, Agricultural Research Organization, PO Box 6, Bet Dagan 50250, Israel
[c] Poultry and Aquaculture Sci. Department, Agricultural Research Organization, PO Box 6, Bet Dagan 50250, Israel

ARTICLE INFO

Article history:
Received 28 December 2015
Revised 23 May 2016
Accepted 7 June 2016
Available online 8 June 2016

Keywords:
Ghrelin
Heat stress
Estrous cycle
Parity
Dairy cow fertility

ABSTRACT

In dairy cows, heat stress depresses appetite, leading to decreased food intake, a negative energy balance, and modifies ghrelin levels. Ghrelin is a gut–brain peptide with two major forms: acylated, with an O-n-octanoylated serine in position 3, and nonacylated. To date, the effect of heat stress and estrous cycle on ghrelin secretion in dairy cows has not been studied. We characterized ghrelin secretion during the estrous cycle in each, the winter and the summer seasons. We further examined the effects of parity on ghrelin secretion. Blood was collected from 10 primiparous or multiparous Israeli-Holstein dairy cows throughout the estrous cycle, in both, the hot and cold seasons. The levels of acylated and total ghrelin were measured in the blood samples. We found that both acylated and total ghrelin levels during heat stress were lower than their respective levels in the winter in both, primiparous and multiparous cows. No differences in acylated and total ghrelin levels were found between primiparous and multiparous cows in both seasons. We further found that in multiparous but not primiparous cows acylated ghrelin secretion oscillated during the estrous cycle in both seasons. Its levels peaked on the last days of the first follicular wave and on the days before and during ovulation. Interestingly, we found that elevated acylated ghrelin levels correlated with conception success and increased total ghrelin levels were associated with successful conception from first insemination. Our data is the first to demonstrate seasonal variation in ghrelin secretion. This study provides evidence for the yet unfamiliar link between heat stress, ghrelin and fertility. Increased circulating acylated ghrelin may contribute to improved fertility in dairy cows. It further raises the possibility of a link between ghrelin levels and successful inseminations. Further research is required to determine the effects of ghrelin on dairy cow performance.

1. Introduction

Israeli herd book data shows a drop of 20–25% in conception rates between the winter and summer seasons in the years 1994–2008. This interesting observation may be related to heat stress exposure. Another outcome of heat stress in dairy cows is depressed appetite and a decrease in food intake (Chagas et al., 2007). In many mammals, including dairy cows, these effects are followed by a negative energy balance leading to increased ghrelin levels (Bradford and Allen, 2008; Muccioli et al., 2011).

Ghrelin is a gut–brain peptide with two major forms: acylated with an O-n-octanoylated serine in position 3 and a nonacylated form, known as des-acyl ghrelin (Al Massadi et al., 2011). In ruminants, the ghrelin-producing cells are found in the abomasum (Hayashida et al., 2001). Although ghrelin is most well known as a gut-derived hormone that acts to stimulate appetite, it also has effects to stimulate GH secretion (Iqbal et al., 2006). In fact, the discovery of ghrelin was achieved through screening for ligands which activated the receptor for GH releasing peptide (Kojima et al., 1999). Only later studies demonstrated that ghrelin is a circulating orexigen, potently stimulating feeding (Tschop et al., 2000; Wren et al., 2000) in many species, including sheep (Grouselle et al., 2008). These orexigenic effect of ghrelin is achieved by regulating the ARP neurons in the hypothalamus (Langhans et al., 2009; Reichenbach and Andrews, 2012). In addition to appetite control regulation and GH secretion, ghrelin takes part in many other physiological processes, including stress (Chuang et al., 2011; Diz-Chaves, 2011), sleep and memory (Steiger et al., 2011), regulating insulin secretion (Broglio et al., 2001) and glucose uptake (Gershon and Vale, 2014).

* Corresponding author at: Department of Ruminant Science, The Volcani Center, Bet-Dagan 50250, Israel.
E-mail address: eran.gershon1@mail.huji.ac.il (E. Gershon).

http://dx.doi.org/10.1016/j.ygcen.2016.06.006
0016-6480/

Another major physiological process affected by ghrelin is fertility (Rak-Mardyla, 2013). Previous studies in many farm animals demonstrated that ghrelin actions on reproduction performances are within a concentration spectrum (Young et al., 2001; Zhang et al., 2007; Du et al., 2010; Dashtizad et al., 2010; Wang et al., 2013). However, less is known of ghrelin and seasonal effect in the cow, especially the dairy cow, in which a major fertility issue has emerged in conjunction with selection for increased milk production. Ghrelin plays a pivotal role in many essential processes, including food intake, growth and fertility; however, little is known about its secretion levels and pattern during the estrous cycle and in response to heat stress in dairy cows. In this study, we characterized ghrelin secretion during the estrous cycle in dairy cows in the hot and cold seasons.

2. Materials and methods

2.1. Animals

All experiments were approved by the Volcani Center Animal Care Committee. The experiments were conducted at the cowshed in Kibutz Shoval, Israel, during winter (December 2014 to January 2015) and summer (July to August, 2015). During each season, 21 Israeli-Holstein dairy cows (10 primiparous and 11 multiparous in their second to fourth lactation) were used. The cows were housed in covered loose pens with an outside yard.

Cows were milked 3 times a day (0500, 1200, 2030 h) and weighed automatically after each milking with a walk-in electronic scale (S.A.E. Afikim, Kibbutz Afikim, Israel). Milk yields were recorded electronically at each milking. Cows were fed a typical Israeli lactating-cow ration made up of 1.75 Mcal NE_L, 16.5% CP, and 31.5% crude NDF.

Cows' estrous cycles were synchronized by injection of two doses of PGF2α analog (GONAbreed®, gonadorelin acetate, 100 mcg/ml, Parnell Manufacturing PTY Ltd., Australia) administered 12 days apart. Ovulation occurred 36 h after the second injection.

2.2. Blood collection and handling

Blood samples were collected from the tail vein every other day until day 18 of the cycle and then every day until ovulation. Blood was collected after the third milking. Whole blood was centrifuged at 10,000 rpm for 10 min and serum was collected. 0.05 N HCl and 0.5 mg PMSF (Sigma, Rehovot, Israel) were added to the serum immediately following its collection. Samples were stored at −80 °C.

2.3. Hormonal measurement

Acylated and total ghrelin concentrations were measured in blood samples using the Millipore active and total ghrelin RIA kit (GHRA-88HK and GHRT-89HK, respectively, Millipore Corp., Billerica, MA) according to the manufacturer's guidelines. These assay kits were previously validated for use with bovine plasma (Wertz-Lutz et al., 2006; Foote et al., 2014). The inter-assay CVs for the hormonal assays were 7.7% for the active ghrelin assay and 8.4% for the total ghrelin assay. The intra-assay CVs for the hormonal assays were 6.8% for the active ghrelin assay and 7.9% for the total ghrelin assay.

2.4. temperature-humidity indices (THI) Measurements

Ambient temperature (AT) and relative humidity (RH) were recorded every 3 h by the Israel Meteorological Service (Lahav, Israel). Maximum RH and minimum AT were determined at 0200 h, and minimum RH and maximum AT were determined at 1400 h. Accordingly, the minimum and maximum temperature-humidity indices (THI) were determined at 0200 and 1400 h, respectively. The following equation was used for THI calculation (Bohmanova et al., 2007):

$$THI = (1.8 \times T_{db} + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26)$$

where T_{db} is dry-bulb temperature.

Table 1
Mean (±SEM) DIM, lactation number, age, milk yield, weight, percentage of milk fat, protein and lactose in primiparous and multiparous cows in the summer and winter seasons.

Factor variable	Summer		Winter	
	Primiparous	Multiparous	Primiparous	Multiparous
Number of cows	10	11	10	11
DIM	71 ± 2.66	74 ± 1.98	87.56 ± 6.2	78.4 ± 4.57
Lactation number	1 ± 0	2.4 ± 0.18	1 ± 0	3.6 ± 0.34
Age (days)	850 ± 39.77	1373 ± 97.78	834 ± 10.46	1834 ± 149.5
Milk yield (kg)	35.72 ± 1.65	43.45 ± 1.1*	37.72 ± 1.77	48.85 ± 2.01*
Milk fat (%)	3.49 ± 0.15	2.86 ± 0.13	3.25 ± 0.12	3.5 ± 0.18
Milk protein (%)	3.1 ± 0.03	2.97 ± 0.05	3.06 ± 0.07	2.8 ± 0.08

* $p < 0.05$.

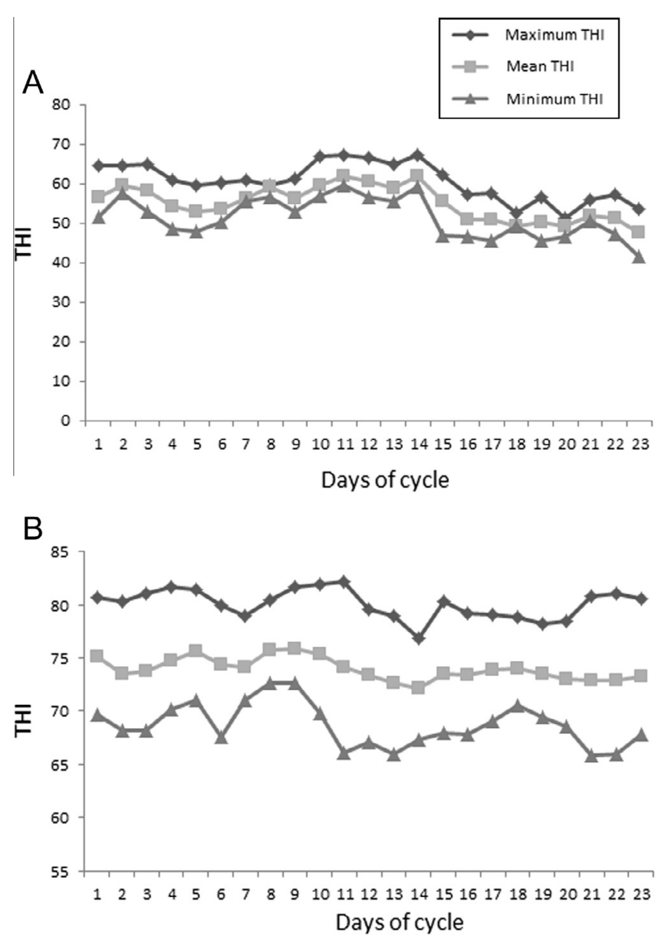

Fig. 1. (A) Daily temperature-humidity indexes (THI) during the study period at winter. (B) Daily temperature-humidity indexes (THI) during the study period at summer.