Journal of Dairy Research

cambridge.org/dar

Research Article

Cite this article: Kleinjan-Elazary A *et al* (2020). Cooling management effects on dry matter intake, metabolic hormones levels and welfare parameters in dairy cows during heat stress. *Journal of Dairy Research* **87**, 64–69. https://doi.org/10.1017/S0022029919001055

Received: 14 April 2019
Revised: 31 October 2019
Accepted: 9 December 2019

Keywords:
Cooling management; DMI; Ghrelin; heat stress; Leptin

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Cooling management effects on dry matter intake, metabolic hormones levels and welfare parameters in dairy cows during heat stress

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Abstract

This research paper addresses the hypothesis that intensive cooling management during the summer improves the secretion of metabolic hormones in dairy cows. To test this hypothesis, we characterized the effect of different cooling managements on the different ghrelin isoforms and leptin secretion of 20 Israeli-Holstein dairy cows during 5 weeks during heat stress. The cows were divided into two groups: one was exposed to 5 cooling sessions per day (5 CS) and the other to 8 cooling sessions per day (8 CS). Blood was collected and leptin and ghrelin isoforms level were radioimmunoassayed. Analysis of the interaction between coolings and the week of the experiment showed that the 8 CS group consumed more food and produced more milk, although neither difference was statistically significant. In addition, the 8 CS group exhibited higher blood levels of acyl-ghrelin and leptin as compared to the 5 CS group. Conversely, the blood levels of total ghrelin were lower in the cows exposed to 8 CS as compared to cows from the 5 CS treatment. Furthermore, a significant correlation was found only between total ghrelin levels and the weeks, but not with other parameters examined. We further compared digestibility as well as stress parameters between the groups. We found that the 8 CS group cows ruminated and lay down more hours during a day and simultaneously had better activity time. No significant difference was detected between groups in milk yield and digestibility parameters. Our results suggest that intensive cooling management during the hot season influences the levels of metabolic hormones in the circulation and helps to mitigate the detrimental effect of heat stress on dairy cow welfare and production.

It is well established that heat stress has an adverse effect on dairy cows that manifests itself through behavioral change and decline in performance (West, 2003; Honig *et al*., 2012). Lactating cows are particularly susceptible to heat stress, due to the high metabolic heat production associated with increased milk production (West, 2003). The Israeli herd data book (2015) shows a drop of approximately 20% in conception rates between winter and summer, which indicates a major pitfall in the successful continuation and profitability of the dairy cattle herd. In order to relieve heat stress dairy farmers implement various management tactics such as environment modification, mainly by various means of cooling (Flamenbaum *et al*., 1986; Her *et al*., 1988; Flamenbaum and Galon, 2010). In Israel, the widely used cooling system is based on sessions comprised of direct watering of the cows, followed by forced air ventilation.

During heat load cows exhibit reduced feed intake in order to reduce metabolic heat (West, 2003). In many mammals, including dairy cows, reduced feed intake is followed by a negative energy balance which leads to reduced leptin secretion (Liefers *et al*., 2003) and increased ghrelin levels (Bradford and Allen, 2008; Muccioli *et al*., 2011). Leptin is predominantly synthesized in adipose tissue and is a protein hormone with important effects on eating behavior, energy expenditure, and body weight (Budak *et al*., 2006; de la Hoya, 2015). Leptin serves as a signal of body energy status to the brain, whereas it acts on the hypothalamus to inhibit food intake (Budak *et al*., 2006; de la Hoya, 2015).

Ghrelin is a gut—brain peptide composed of 27 amino acid in ruminants (Dickin *et al*., 2004). In cattle it is synthesized by abomasal and ruminal tissues (Hayashida *et al*., 2001; Gentry, 2003). Ghrelin has two known isoforms, des-acyl ghrelin and acylated ghrelin that binds to the growth hormone secretagogue receptor (GHS-R1A) (Kojima *et al*., 1999; van der Lely *et al*., 2004; Fernandez-Fernandez *et al*., 2006). Acyl ghrelin is known to be secreted

https://doi.org/10.1017/S0022029919001055 Published online by Cambridge University Press

in an oscillatory manner, concentrations increase prior to scheduled meals and in response to fasting while feeding suppresses its secretion (Hayashida *et al*., 2001; Miura *et al*., 2004; Wertz-Lutz *et al*., 2006). Interestingly, ad libitum feeding in ruminants diminishes this oscillation (Sugino *et al*., 2002; Borner *et al*., 2013). These observations suggest that acyl ghrelin release is affected by serum modification of nutritional factors and function as circulating signal for energy insufficiency. Previously, we found that acyl ghrelin levels measured during heat stress in summer were lower than those in the winter (Honig *et al*., 2016). Des-acyl ghrelin circulates at much greater concentrations than acylated ghrelin (Hosoda *et al*., 2000; ThidarMyint *et al*., 2006), and seems to be less studied. Its effects on food intake and metabolism are less characterized.

Little is known about ghrelin isoforms and leptin levels under conditions of heat stress in dairy cows. In this study, we demonstrate that increasing the number of cooling sessions from 5 a day to extensive cooling management comprised of 8 cooling sessions a day, during heat stress in the summer, significantly elevated acyl ghrelin and leptin levels whereas total ghrelin levels decreased in dairy cows.

Materials and methods

Cows and treatments

All experiments were approved by the Agricultural Research Organisation Animal Care Committee. The experiments were conducted at the Agricultural Research Organisation experimental farm at Bet Dagan, Israel, during the summer (August). 40 Israeli-Holstein dairy cows were housed in covered loose pens with an adjacent outdoor yard, that were equipped with a real-time electronic individual feeding system. Cows were fed a typical Israeli lactating-cow ration made up of 1.78 Mcal NE_L, 16.5% CP, and 29.8% NDF. The cows were allocated to two treatment groups subjected to different cooling schedules carried out in the holding area of the milking parlor. One group was subjected to the ordinary cooling management preformed in Bet Dagan dairy farm comprised of 5 cooling sessions per day (5 CS). Cooling was carried out at 0415, 0945, 1215, 1645, and 1945 h. The second group was subjected to extensive cooling comprised of 8 cooling sessions per day (8 CS). In this case cooling was carried out at 0415, 0645, 0945, 1245, 1445, 1645, 1945, and 2230 h. Both groups were subjected to a cooling session before each milking. During the day, between milkings, the 5 CS cows were brought twice and the 8 CS cows were brought 5 times for further cooling sessions. Each cooling session lasted 45 min, comprised of repeated cycles of 60 s of showering and 4.0 min of only forced ventilation.

Cows were milked three times a day (0500, 1300, 2030 h). Milk yields were recorded electronically at each milking and cow weight was recorded automatically after each milking, with a walk-in electronic scale (S.A.E. Afikim, Kibbutz Afikim, Israel).

During the experiment period the cows dry matter intake (DMI), metabolic hormones blood levels, vaginal temperature, digestibility and rumination, activity and lying time were measured as described in the online Supplementary File.

Blood collection and handling

Blood samples were collected from 20 multiparous cows (10 cows from each group), every 2 to 3 d until the end of the experiment. The collection occurred at the time of food distribution approximately at 1030 h, and plasma was extracted after centrifuging at 10 000 rpm for 10 min. 1 N HCl and 0.5 mg PMSF (Sigma, Rehovot, Israel) dissolved in iso-propanol were immediately added to plasma samples designated for acyl ghrelin measures. Samples were stored at −80°C until further analysis until determinations of acyl and total ghrelin as well as leptin blood levels as described in the online Supplementary File.

Table 1. Mean, SEM and *P*-value of days in milking, dry matter intake (DMI), milk yield, energy corrected milk (ECM), content of milk fat, protein and lactose of all cows under different cooling managements

Factor variable	5 CS	8 CS	SEM
Days in milking	121.5	112	0.9
DMI (kg/d)	27.8	28.3	0.13
Milk yield (kg/d)	44.5	45.4	0.26
ECM (kg/d)	40.3*	41.3	0.2
Milk fat (%)	3.42	3.43	0.01
Milk protein (%)	3.17	3.18	0.005
Milk lactose (%)	4.89	4.92	0.004
Total ghrelin (pg/ml)	357.12**	305.85	8.99
Acyl ghrelin (pg/ml)	55.64**	67.59	2.19
Leptin (ng/ml)	16.7***	22.83	0.49

8 cooling sessions per day (8 CS), 5 cooling sessions per day (5 CS). * $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$.

Statistical analysis

The data were analyzed by two-way analysis of variance (ANOVA) with Cooling [5 or 8 sessions], periods [week of treatment], and their interaction as main fixed effects and with repeated-measures in one factor (period, also called mixed-model ANOVA). Each cow served as its own control because the hormone parameters for each cow were measured at all time periods. The Tukey—Kramer honest significant difference test was used to test the separation of the means, in comparing the periods within each cooling regime. These statistical analyses were conducted with JMP software (SAS Institute). All results are presented as mean ± standard error of the mean.

Results

Characterization of the cows at the two cooling management groups

The descriptive statistics of days in milking, dry matter intake (DMI), lactation number, age, milk yield, and weight, content of milk fat, protein and lactose between the two different cooling methods are presented in Table 1. No between-group differences in days in milking, DMI, milk yield, milk fat, protein and lactose contents were observed, but ECM was significantly higher in the cows exposed to 8 CS as compared to the cows from the 5 CS group ($P < 0.05$; Table 1).

Measuring the vaginal temperature of the cows at the two cooling management groups

First we calculated the THI index of the environment in order to approve the heat conditions in the area (online Supplementary

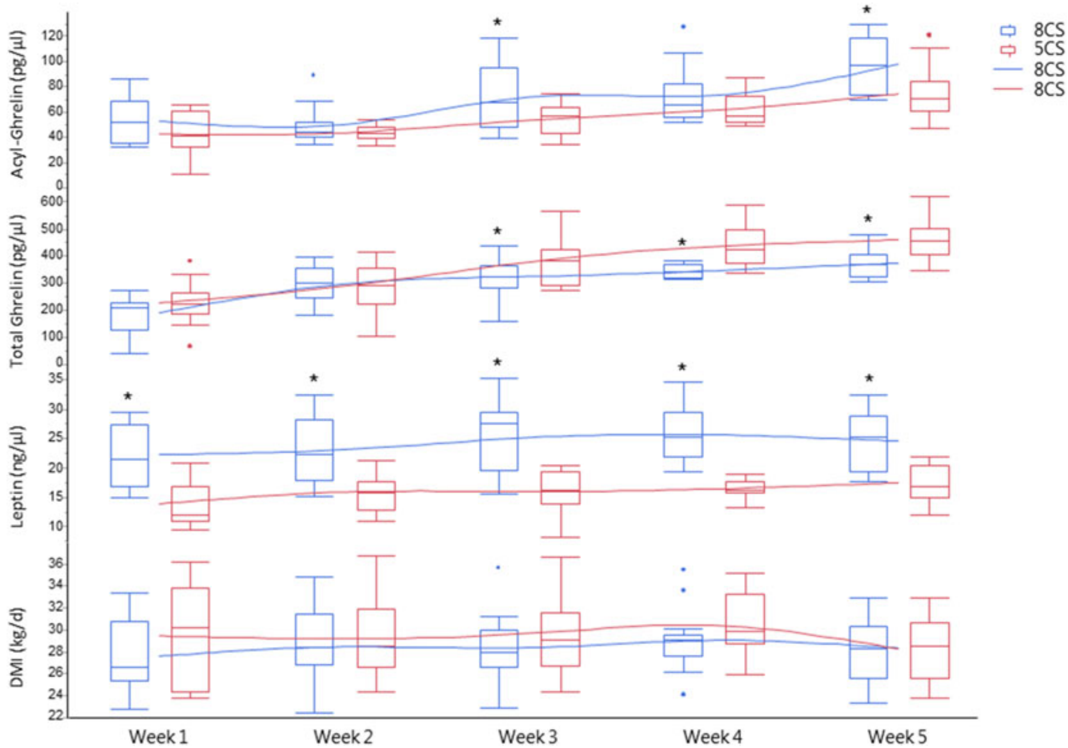

Fig. 1. Metabolic hormones levels and DMI throughout the study period in 5 CS vs. 8 CS. Mean ± SEM of acyl ghrelin, Total ghrelin, Leptin levels and DMI through the study period of multiparous cows submitted to 8 cooling sessions per day (8 CS) and multiparous cows submitted to 5 cooling sessions per day (5 CS). *$P < 0.05$ between 8 CS and 5 CS treatments.

File Fig. S1). Next, we measured the vaginal temperature of the cows in order to examine the effectiveness of the 8 cooling sessions as compared to the 5 CS. The temperature was measured during the second and fourth weeks of the experiment, for the duration of approximately 3 d each time. The cumulative number of hours that the body temperature of cows in the 5 CS group was above 39.4°C heat-stress threshold (Burfeind *et al.*, 2012) was significantly higher as compared to the 8 CS group (online Supplementary File Table S1) at all measurements.

Measuring the DMI of the cows at the two cooling management groups

We compared DMI in cows exposed to 5 or 8 cooling sessions per day and found that the DMI in the group of 8 CS was elevated every week of the experiment, while in the group of the 5 CS only one elevation in the DMI intake was observed between week 2 and 3 (Fig. 1 and Table 2). In the following weeks no changes in the DMI was observed (Fig. 1 and Table 2). No significant difference was detected between the DMI of the 8 CS group and the DMI measured in the 5 CS treated cows (Table 2). In addition, no correlation was found between the DMI and the weeks (Table 2).

Acyl ghrelin

We compared blood levels of acetylated ghrelin during the weeks of the experiment in cows exposed to 5 or 8 cooling sessions per day. In both groups there is a significant rise in the hormone levels (Fig. 1 and Table 2). At the last 3 weeks of the experiment acyl ghrelin levels were significantly higher in 8 CS group compared to the cows in the 5 CS group, which was also reflected in the overall means (Table 1). No correlation was found between the DMI and the weeks (Table 2).

Total ghrelin

We further compared blood levels of total ghrelin in cows exposed to 5 or 8 cooling sessions per day. We found that the total ghrelin levels increased in both group throughout the experiment (Fig. 1) with significant higher levels of total ghrelin in 5 CS group (Fig. 1 and Table 2). Overall, total ghrelin levels were significantly higher in 5 CS group compared to the cows in the 8 CS group (Table 1). Furthermore, a significant correlation was found between the DMI and the weeks (Table 2).

Leptin

Leptin levels were significantly higher in 8 CS group during the whole experiment as compared to the 5 CS group (Fig. 1 and Tables 1 and 2). Leptin levels were significantly elevated each week in both treatment groups (Fig. 1 and Tables 2). No correlation was found between the DMI and the weeks (Table 2).

Welfare parameters of multiparous cows subjected to 8 CS and 5 CS

The welfare parameters rumination, lying time and activity are presented in Table 3. In all these parameters there was a significant difference between groups. The 8 CS group cows ruminated and lay down more time during a day and simultaneously had better activity time. Furthermore, the DM digestibility percentage was numerically higher in the 8 CS group as compared to the 5 CS cows ($P = 0.07$, Table 3). Finally, no between-group differences in

https://doi.org/10.1017/S0022029919001055 Published online by Cambridge University Press

Table 2. Least Square Mean ± Standard error of acyl ghrelin, Total ghrelin, Leptin levels and DMI at interaction between coolings and week of the experiment, of all cows under different cooling managements

Acyl-Ghrelin	5 CS	8 CS	Prob week	
Week 1	43.24 ± 4.70	53.35 ± 4.52	Prob $F < 0.0001$	Prob Treat × Week
Week 2	43.89 ± 4.72	49.26 ± 4.88		
Week 3	57.11 ± 5.04 b	72.04 ± 4.70 a		
Week 4	63.20 ± 4.85	72.86 ± 4.70		
Week 5	76.57 ± 4.85 b	98.58 ± 4.52 a		
Prob Treat		Prob $F = 0.0088$		NS
Total Ghrelin			Prob $F < 0.0001$	Prob Treat × Week
Week 1	226.57 ± 15.46	185.80 ± 17.98		
Week 2	286.87 ± 15.46	296.28 ± 17.98		
Week 3	378.34 ± 15.46	321.61 ± 16.53		
Week 4	435.08 ± 15.46 C, D, E	355.11 ± 18.18 A, B		
Week 5	468.73 ± 15.46 B, C, D	372.03 ± 17.22 A		
Prob Treat		Prob $F = 0.0369$		Prob $F = 0.0373$
Leptin				
Week 1	13.73 ± 0.63 b	22.36 ± 0.63 a	Prob $F < 0.0001$	Prob Treat × Week
Week 2	15.86 ± 0.63 b	23.16 ± 0.63 a		
Week 3	15.87 ± 0.63 b	25.28 ± 0.63 a		
Week 4	16.36 ± 0.63 b	25.72 ± 0.63 a		
Week 5	17.26 ± 0.65 b	24.63 ± 0.63 a		
Prob Treat		Prob $F = 0.0018$		NS
DMI				
Week 1	29.48 ± 0.49	27.35 ± 0.53	Prob $F = 0.0132$	Prob Treat × Week
Week 2	29.24 ± 0.49	28.48 ± 0.49		
Week 3	30.00 ± 0.51	28.33 ± 0.49		
Week 4	30.81 ± 0.51	29.03 ± 0.49		
Week 5	28.30 ± 0.49	28.33 ± 0.49		
Prob Treat	NS			NS

** Levels not connected by same letter (A, B, C, D,E) are significantly different between interactions within a week.

Table 3. Mean, SEM and *P*-value of welfare parameters: rumination, lying time, activity and DM digestibility, of the synchronized multiparous cows under different cooling managements

Factor variable	5 CS	8 CS	SEM
Rumination (min/d)	421**	528	3.3
Lying time (min/d)	548**	595	2.9
Activity (steps/h)	127**	149	1.23
DM Digestibility (%)	0.669	0.678	0.0028

8 cooling sessions per day (8 CS), 5 cooling sessions per day (5 CS). ** $P < 0.001$.

digestibility were observed (0.671 ± 0.004 at 5 CS compare to 0.674 ± 0.003 pv 0.25).

Discussion

The regulation of nutritional state occurs at the hypothalamus. Such a complex regulation requires the activity of more than one regulator. One such regulator with a pivotal role in the regulation of food intake is ghrelin. There are two known isoforms of ghrelin, des-acyl ghrelin and acylated ghrelin (Gutierrez *et al*., 2008; Yang *et al*., 2008). In our study, both groups were in ad libitum feeding during heat load conditions. Although the cows were not in scheduled or restricted food conditions, we speculate that as result of scheduled fresh food distribution in the morning, especially after milking and cooling, acyl ghrelin level in the time around food distribution would rise in a resemble manner to scheduled meal. Thus the blood samples were collected during the cows morning meal time. We found that in synchronized multiparous cows from the 8 CS group acyl ghrelin concentration was higher. Since acyl ghrelin is associated with food intake, we examined the DMI. In our study, the 8 CS group consumed significantly more food. This result is in agreement with other studies that showed an association between high levels of acyl ghrelin and increased food consumption (Wertz-Lutz *et al*., 2006; Foote *et al*., 2014).

In similarity to the results presented here, our previous study (Honig *et al*., 2016) that compared levels of acyl ghrelin in the

https://doi.org/10.1017/S0022029919001055 Published online by Cambridge University Press

different seasons demonstrated that acyl ghrelin levels in winter were higher compared to summer. Combining this data with the results presented here, allows us to infer that 8 CS promotes higher physiological levels of acyl ghrelin and improved coping ability for the deleterious heat stress effects. The combined results of our studies further support the notion that intensive cooling management mimics at least in part the winter condition and can relieve heat stress during the hot season.

Compare to acyl ghrelin, des-acyl ghrelin seems to be less studied and its effects on food intake and metabolism are less characterized. Primary studies in cattle reported a lower rumen pH in heat stressed dairy cows (Collier *et al.*, 1982). Interestingly, in our study, coincidentally to higher acyl ghrelin levels, 8 CS had lower levels of total ghrelin, which indicates lower levels of des-acyl ghrelin. In that respect, we surmise that as a consequence of low rumination time, due to heat stress conditions, less saliva would buffer the PH in the rumen and it may relate to the levels of des-acyl ghrelin in the circulation.

In our study we demonstrate that 5 CS group spent more time in heat stress conditions and it is evident that they spent less time ruminating, their des-acyl ghrelin level were higher and they consumed less food. Therefore, it can be speculated that there is a negative link between levels of des-acyl ghrelin and food consumption, in resemblance to studies shown in rodents (Asakawa *et al.*, 2005; Chen *et al.*, 2005). Further studies should be conducted in order to understand whether the lower levels of total ghrelin in 8 CS is due to higher acylation rate toward acyl ghrelin or due to reduction in the production of des-acyl ghrelin itself, or both.

Previous studies suggested leptin as a candidate hormone that operates in collaboration with ghrelin. Leptin is an important mediator in eating behavior, energy expenditure, and body weight. Our results demonstrate that in synchronized multiparous cows from 8 CS group, leptin concentration was about 1.4 times higher compare to 5 CS, suggesting that the 8 CS cows were in better nutritional state. This notion is further supported by the acyl-ghrelin levels measured in this study and described above.

Heat stress further disrupts the productivity and welfare of dairy cows. At heat load state cows reduce DMI, rumination time, activity and lying time together with longer standing time and panting (Silanikove, 2000; De Rensis and Scaramuzzi, 2003; West, 2003; Berman, 2006; Allen *et al.*, 2015; Moretti *et al.*, 2017). In overview it seems that the extra cooling sessions in the 8 CS group positively affected the welfare of the cows. In the 8 CS group the accumulated time that the vaginal temperature measured exceeded a critical temperature of 39.4°C was shorter, the cows spend more time lying down, ruminating, walking around the pen and consumed more food.

Supporting our results, a previous study also compared five to eight cooling sessions, and found similar differences between the two cooling regimes for rumination time, lying time and ECM yield (Honig *et al.*, 2012). However, these authors reported higher milk yield and DMI of cows receiving the 8 coolings regime, whereas in the current study, no significant differences were found in milk production and DMI between the two groups. This difference in results between the two studies may be due to the different facilities used: large fans were added into the barn used in the present experiment and the duration of Honig's study was longer and under higher THI conditions.

In conclusion, we have shown that adding three extra cooling sessions during the day helps to mitigate the detrimental effect of heat stress on ghrelin and leptin levels, hormones with paramount importance to food intake and metabolism. Adding extra cooling also improves welfare and production. From this study it can be speculated that 8 CS cooling can assist in reducing the negative effects of heat stress on lactating dairy cows, without harming the level of existing production and even improving it to some extent, taking into account the welfare of the cow.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029919001055

Acknowledgements. The authors would like to thank the team at the Volcani Center's experimental dairy farm (Bet Dagan, Israel) for their assistance with animal care. This research was financially supported by the Israeli Milk Board.

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