

## **Cooling Management Effects on Dry Matter Intake, Metabolic Hormones Levels and welfare Parameters in Dairy Cows during Heat Stress**

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### **SUPPLEMENTARY FILE**

#### **Dry matter intake (DMI) measurement**

DMI was measured every day by weighing feed delivery minus orts used as gold standard for DMI measurement based on summarizing of daily visit's size monitored by the computer. All cows were held in a common shaded well-aerated corral as a single group and had free access to water and individual feeder. Each cow was individually fed daily between 0900 to 1000 h the same TMR for the whole experiment period. At morning milking, additional quota of 10% TMR was added to empty feeders to ensure ad libitum intake (5% orts). Total mixed ration samples were taken daily from the individual feeders immediately after feeding and orts were collected from each feeder before next morning feeding.

Cows vaginal temperature measurement In the synchronized cows, vaginal temperature was recorded as a means to record core body temperature. ibutton temperature recording loggers (Thermochron iButton, Maxim Integrated Products, Inc., CA, USA) were attached to blank intravaginal drug release devices (CIDR's) and inserted in to the cows vagina during the second and fourth weeks of the experiment, for the duration of approximately 3 days each time. The CIDR can not be inserted for more than 3 days, since it might cause vaginal infection.

#### **Temperature-humidity indexes (THI) measurements**

Ambient temperature (AT) and relative humidity (RH) were recorded every 10 min by the Israel Meteorological Service (Bet Dagan, Israel). The following equation was used for THI calculation {Bohmanova, 2007 #117}:  $THI = (1.8 \times T_{db} + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26)$ . Where  $T_{db}$  is dry-bulb temperature.

The mean AT and RH during the study period were  $28.39 \pm 2.4^\circ\text{C}$  and  $67.55 \pm 9.7\%$ , respectively. Minimum, maximum and mean THI during the study period were 73.93, 82.25, and  $78.42 \pm 25.2$ , respectively (Sup. Fig. S1).

#### **Hormonal measurements**

Acetylated and total ghrelin concentrations were measured in the 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 {Foote, 2014 #114; Wertz-Lutz, 2006 #41}. The inter-assay CVs for the hormonal assays were 7.7% for the active ghrelin assay and 8.4% for the total ghrelin assay.

Leptin concentrations were measured in the blood samples using the Millipore leptin RIA kit (XL-85K, Millipore Corp., Billerica, MA) according to the manufacturer's guidelines. This assay kit was previously validated for use with bovine plasma {Meikle, 2013 #133}.

### Digestibility measurements

10 feces samples collected from each synchronized cow, during three and a half days in the third week of the experiment, were analyzed for digestibility similarly to the method presented at {Adin, 2009 #118}.

### Rumination, Activity and lying time measurements

Rumination time was monitored with collar-mounted tags (HR-Tags; SCR Engineers Ltd., Hadarim, Netanya, Israel) {Schirmann, 2009 #119}. Rumination data were recorded by a special microphone that detected chewing actions by analyzing vocal signals. Data were stored in 2-h blocks and uploaded 3 times daily at the milking parlor. Activity (steps/h) and lying time (min) was monitored with a tag sensor (Pedometer Plus; S.A.E. Afikim, Israel) that was fitted to the rear leg of each cow and the data were accumulated and transmitted to management software (AfiFarm; S.A.E. Afikim, Israel) each time the cows passed an antenna located in the milking parlor {Higginson, 2009 #134}.

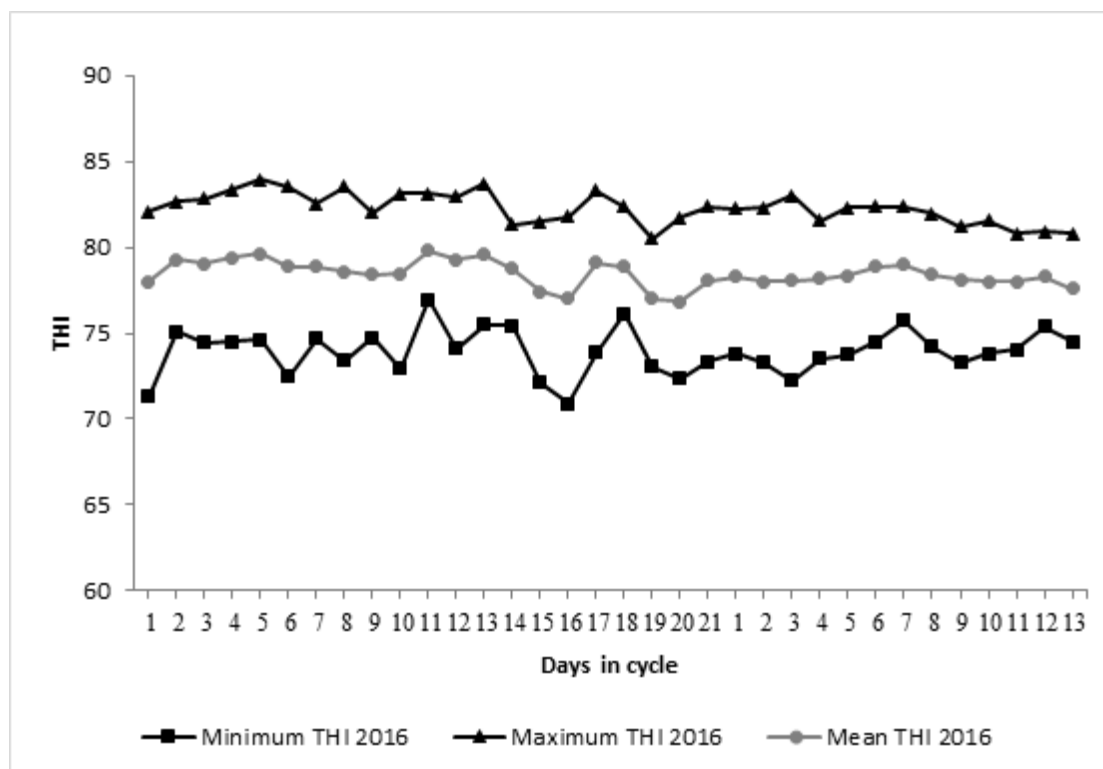


Figure S1. THI. Daily temperature-humidity index (THI) during the study period.

Round	Date	8-9.8.16			10-11.8.16		
I	Total h measured	36h			36h		
	Coolings	Mean	SEM	Pv	Mean	SEM	Pv
	8CS	2.39	0.88	0.031	4.44	1.35	0.018
	5CS	6.81			10		
II	Date	22-23.8.16			24-25.8.16		
	Total h measured	36h			36h		
	Coolings	Mean	SEM	Pv	Mean	SEM	Pv
	8CS	3.78	0.81	0.014	5.6	1.33	0.033
	5CS	7.17			10.5		

**Table S1.** Mean accumulative hours of vaginal temperature that exceeded 39.4°C, measured at synchronized multiparous cows submitted to 8 cooling sessions per day (8CS) or 5 cooling sessions per day (5CS).