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Effects of cooling dry cows under heat load conditions on mammary gland enzymatic activity, intake of food and water, and performance during the dry period and after parturition

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ABSTRACT

Effect of evaporative cooling of pregnant dairy cows under heat load conditions during the dry and close-up period, on mammary gland enzymatic activity and intake of food and water, BCS, and milk performance after calving were measured in two consequent experiments. In experiment 1, 18 dry cows held in tie-stalls in a closed aerated barn under heat load conditions were used to measure the effect of evaporative cooling on the respiratory rate and body temperature, individual intake of food and water, enzymes expression level in mammary gland and adipose tissues, and BCS changes until calving. In experiment 2, two groups of 36 dry cows each, held in a commercial loose housing barn, were used to measure the effects of evaporative cooling under heat load conditions on calves' birth weight, colostrum quality and quantity, BCS changes and milk yield during 90 DIM. The non cooled (*NC*) cows responded to heat load by increasing their respiratory rate and daily water intake, while elevating their rectal temperature by 0.2–0.3 °C as compared with the cooled (*C*) cows. The external relief of heat load by the *C* cows in both experiments was expressed in increasing their voluntary DMI during the dry period as compared with the *NC* group. In experiment 2 the calves' birth weight of *C* cows was higher, and their colostrum quality and quantity were improved as compared with the *NC* group. Cooling also improved significantly BCS gain during the last 21 days until parturition, accompanied with higher cell proliferation process (based on enzymes expression at mRNA level) in the mammary gland of the *C* cows. Consequently, a significant increase in milk production by 5.3%, protein yield by 5.1%, ECM yield by 4.2% and FCM yield by 4.5%, was demonstrated in the *C* cows during 90 DIM as compared with the *NC* group.

1. Introduction

The importance of the dry period for better performance in the consequent lactation of high producing dairy cows is well-documented (Capuco et al., 1997; Hurley, 1989; Oliver and Sordillo, 1989). The dry period is essential since it comprises a phase of rapid fetal growth and endocrinal induction of lactation; mammary gland involution and subsequent development; structural changes related to development of rumen papillae and adaptation of the rumen flora to the more energetic ration fed after parturition. Proper management and nutrition during the dry period may decrease incidence of health problems and improve cow body condition, performance and reproduction during the next lactation (Drackley, 1999).

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A major problem of cow performance under heat load conditions is limited capability of heat exchange between the animal and the environment. Previous studies of heat stress effects and their relief in dairy cattle focused on the lactation period, when metabolic heat production is the largest (West, 2003; Collier et al., 1982b). The possibility that cow during dry and close up period might be particularly sensitive to heat stress was ignored, although it was suggested, that the endocrine system during this period is more sensitive to moderate heat stress than during lactation (Wolfenson et al., 1988).

Several studies reported pre-partum evaporative cooling increased milk production as compared to a non-cooled control group (Wolfenson et al., 1988; Urdaz et al., 2006). Unfortunately, a deep and comprehensive observation of the cooling effects under heat load conditions on dry cows mammary gland enzymatic activity, intake of food and water and BCS change, together with their performance at parturition and during next lactation have not been measured.

The general objective of this study was to measure the effects of evaporative cooling of cows during the dry and close-up period, under heat load conditions, on their respiratory rate and body temperature, individual intake of food and water, enzymatic activity (at mRNA level) in mammary and sub-cutaneous fat tissues, BCS changes until calving, calves' birth weight, colostrum quality and quantity, and milk performance after calving.

2. Materials and methods

2.1. Cows, diets, and sampling procedures

Analysis of performance data should base on large number of animals held in a herd in a commercial barn, while individual intake of food and water, as well as enzyme activity measurements, can be measured only when the cows are held in individual tie-stalls. Therefore, we separated this study into two experiments. In experiment 1, 18 dry cows held in individual tie-stalls in a closed barn under heat load conditions were used to measure the effect of evaporative cooling on the respiration rate, body temperature, individual intake of food and water, enzymes activity in mammary gland and adipose tissues, and BCS changes until calving.

In experiment 2, two groups of 36 dry cows each, held as part of the herd in a commercial loose housing barn, were used to measure the effects of evaporative cooling under heat load conditions on calves' birth weight, colostrum quality and quantity, BCS changes, and milk performance after calving.

2.1.1. Experiment 1

Eighteen multiparous Holstein dry cows (2.5 ± 0.10 lactations; yielding 10,200 + 60 kg milk in previous lactation) were housed during the trial in individual tie-stalls [140 × 180 cm on cement floor covered with a thick (30 mm) rubber carpet] located in a close aerated barn under ceiling and continuous lighting, in the Agricultural Research Organization (ARO) dairy farm, in Bet Dagan (central coastal plane of Israel). Experimental conditions were under the supervision of the Israeli Committee of Animal Welfare. The trial took part during summer 2007 (June–September). Cows were divided on dry off day into two groups of 9 cows each, similar in lactation number and performance on previous lactation. Nine cows were located in their individual tie-stalls in one side of the closed barn and received evaporative cooling (*C*) during the dry (5 weeks) and close-up (3 weeks) periods. The second group of 9 cows was located in the opposite side of the barn under heat stress conditions (*NC*). The parameters measured during the experiment included: rectal temperature, heart rate and respiration rate, measured in the morning (0800) and afternoon (1500) on a daily basis (Miron et al., 2008); DMI and water intake were measured in each tie-stall on a daily basis; body condition score (BCS) of cows was determined on a weekly basis (Ferguson et al., 1994). The mRNA levels of key enzymes in mammary and fat tissues explants were measured before parturition as described later.

After parturition, cows' health parameters including the presence of retained placenta, metritis, milk fever, ketosis and displaced abomasums were diagnosed (Markusfeld and Ezra, 1993) by a veterinarian. Calves' birth weight and the quantity of the first colostrums were also recorded. The quality of first colostrums was measured by using the densometric method (Morin et al., 2001). Milk and ECM production was also measured during the first 90 days of the coming lactation.

Environmental climate data in the barn including ambient temperature and humidity were monitored continuously during the experimental period using a weather monitoring station (RWMS-8, Rotem Computerized Controllers — Israel). The temperature humidity index (THI) was calculated according to Aharoni et al. (2003).

Evaporative cooling was repeated every hour; eighteen times per day from 0600 until 2400. Each cooling period lasted 40 min: 10 min of wetting cows using individual sprinklers (120 L/h) followed by 30 min exposure to fans promoting evaporation. The cooling system makes use of three high-velocity fans (air speed of 3 m/s). Each fan (20 in. diameter) was used to cool 3 cows of the *C* treatment.

Cows in both groups were fed the same conventional dry ration during the 5 weeks after dry off and the same special close-up ration during the following 3 weeks before estimated calving day. Composition of the two rations is given in Table 1. Fresh TMR was offered daily at 1000 h after the collection of orts, which were weighed, sampled and kept in a freezer (−20 °C) until analysis. TMRs were sampled daily and pooled on a weekly basis for each treatment. Dry matter intake (DMI) was determined based on oven drying a portion of the weekly TMR samples at 105 °C for 24 h. The rest of the weekly TMR samples were oven dried at 60 °C for 48 h, ground through a 1-mm screen, and used for chemical analyses. Drinking water was offered ad-lib, and measured by water meter on a daily basis for each individual cow.

Biopsies from each cow of each treatment were intended to obtained on day 10 before estimate calving date, (practically it occurred on days 11 and 17 pre-calving of the *C* and *NC* cows respectively), from the sub-cutaneous fat tissue in tail-hollows and from the mammary tissue. These biopsies were used for determining differences between cooling treatments in enzymatic expression level (at mRNA level) of acetyl CoA carboxylase (ACC); fatty acid synthetase (FAS); and hormone-sensitive lipase (HSL). FAS and ACC are key enzymes in lipogenesis de novo and HSL is responsible for the release of fatty acids from triacylglycerols within the adipose cells. These enzymes may be used as markers of the metabolic

Table 1
Ingredients and chemical composition of the TMR fed to dry and close-up cows in experiments 1 and 2.

	(% of DM)		(% of DM)	
	Experiment 1		Experiment 2	
Ingredients	Dry	Close-up	Dry	Close-up
Soybean meal (solvent extract)	8.8	13.5	11.9	17.5
Ground corn grain	11.9	18.4	10.3	21.8
Ground barley grain	6.7	10.5	0	0
Trace minerals and vitamin mixture [a]	0.9	0.9	0.9	1.3
Wheat hay	71.7	56.7	63.6	50.7
Wheat straw	0	0	13.3	8.7
Chemical composition	% of DM (±SE)		% of DM (±SE)	
DM	86.3 (0.54)	86.2 (0.51)	88.5 (0.53)	88.1 (0.50)
OM	91.0 (0.42)	92.0 (0.33)	93.1 (0.37)	93.8 (0.32)
CP	12.0 (0.30)	14.2 (0.30)	12.0 (0.31)	15.0 (0.32)
NDF	51.4 (2.7)	45.3 (3.0)	49.8 (2.9)	42.6 (3.1)
Roughage NDF	45.5	36.0	45.8	35.7
Total roughage	71.7	56.7	77.0	60.0
Calculated NE_L (MJ/kg DM)	6.03	6.49	5.86	6.36

Vit. A 1,000,000 IU; Vit. D_3, 200,000 IU; Vitamin E, 10,000 IU.
[a] The trace minerals and vitamin mixture contained (g/kg DM): Zn, 19; Fe, 0.44; Cu, 1.6; Mn, 2.1; I, 0.5; Co, 0.11; Se, 0.02.

process occurred in the mammary gland and in the fat tissue before calving (Bauman and Vernon, 1993; Vernon and Pond, 1997; Fielding and Frayn, 1998).

Biopsies taken from the upper portion of the mammary gland, was carried out under sedation with xylazine (Rompun, Bayer, Germany) at a dose rate of 0.05 mg/kg injected intravenously. Its site was selected to avoid prominent superficial blood vessels, at the anterior quarter, 2/3 down towards the teat. Biopsy was taken by the open surgical method. Following clipping and surgical preparation, a small wedge of tissue was removed using a number 11 blade. A depth of about 15–20 mm was attained. The skin was closed with staples, no antibiotic was used, and blood drain ceased within some minutes.

Biopsy from the tail-hollows was taken from the subcutaneous tissue at the level of the root of the tail. Following surgical preparation, a small cut was made in the skin of the fossa ventro-lateral to the tail base. Fat was pulled out using Allice tissue forceps and trimmed. Skin was closed with a staple.

Biopsies were rinsed in RNA-later solution until RNA isolation. Explants were prepared as previously described (Shamay et al., 1987). Total RNA was isolated from the mammary tissue explants by the acid guanidinium thiocyanate phenol–chloroform extraction method (Chomczynski and Sacchi, 1987). Upon isolation, RNA was frozen at −70 °C until analyzed by real time PCR (rt-PCR). Two micrograms of total RNA was reverse transcribed, and samples were analyzed by real-time PCR with the ABI Prism 7000 system (Applied Biosystems, Foster City, CA). Real-time PCR analysis was performed by the CT method (Applied Biosystems). as described previously by Feuermann et al. (2004). Bovine ribosomal 18S primers were used as normalizing controls.

2.1.2. Experiment 2

During summer 2007 (June–September), 72 adult cows (3.1 ± 0.10 lactations) were divided into 36 pairs at dry off day according to parity and milk yield during the previous lactation. Each pair was divided into one of the two treatments (*C* vs. *NC*) and allocated in adjacent yards in a shaded loose housing barn with free access to drinking water. The experiment took place in a commercial dairy farm (Hotam Dairy) in Moshav Timmorim (southern coastal plane of Israel).

Cows in both groups were fed the same conventional dry ration during the 5 first weeks after dry off, and the same close-up ration during 3 weeks before estimate calving day (Table 1). Rations were served daily at 1030 and fed for ad libitum intake.

The only difference between the two groups was that one group was cooled by an evaporative cooling system (*C* group) along the feeding line during dry and close-up periods, while the second group was held without cooling under heat load conditions (*NC* group).

During the dry period, evaporative cooling of the *C* cows was repeated 4 times per day, at 0800, 1200, 1600 and 1900 while head locking the cows along the feeding lane. Meantime, the *NC* cows were head locked without cooling. It should be noted that cows in both treatments were locked up for sum of 4 h per day using this time mostly for eating.

Each cooling period lasted 60 min and comprised 12 repeated cycles: 1 min of wetting the cows, followed by 4 min exposure to fans promoting evaporation. The cooling system used high-velocity fans (air speed of 3 m/s). The fans (20 in. diameter) were placed every 6 m and accompanied by 4 foggers supplying 21 L water/hour each.

Parameters measured: environmental temperature and humidity, rectal temperature, and respiration rate were measured in the morning (0900) and afternoon (1500) on a daily basis as described in Experiment 1. The group-fed DMI was measured on a daily basis. An expert, the same one throughout the study, measured BCS on a weekly basis.

After parturition, during lactation, all cows were housed and managed as one combined group under identical conditions including the same TMR and evaporative cooling system.

Cows' health parameters measured after parturition included the presence of retained placenta, metritis, ketosis, milk fever, and displaced abomasums events. Quantity and quality of the first colostrums and calves' birth weight, as well as reproduction parameters including number of resting days, lost days and open days, were also recorded.

Cows were milked 3 times daily at 0400, 1200 and 2000 h Milk yield of each cow was recorded daily by automatic meters (Afimilk SAE Israel). Milk samples were collected during three sequential milking on a weekly basis throughout the study. Each set of milk samples for each cow was stored at 4 °C in the presence of a preservative Bronopol (2-bromo-2-nitropropane-1,3-diol) tablet, until analyzed for the content of fat, true protein, lactose and urea by infrared analysis (Israeli Cattle Breeders Association Lab., Caesaria, Israel, using Milkoscan 4000, Foss Electric, Hillerod, Denmark).

Yield of energy corrected milk (ECM) was calculated according to the NRC (2001) equation: ECM (kg/day) = Milk yield (kg/day) * {(0.3887 * % milk fat) + (0.2356 * % milk protein − urea) + (0.1653 * % milk lactose)} / 3.1338 MJ/kg.

Table 2
Average ambient temperature, relative humidity and temperature humidity index (THI) in experiments 1 and 2 as measured in the morning (AM) and the afternoon (PM) during dry and close-up period.

	Experiment 1				Experiment 2			
	C	*NC*	SEM	*P*-value	*C*	*NC*	SEM	*P*-value
Ambient temperature, °C								
a.m.	24.4	24.7	0.13	NS [a]	27.6	28.0	0.14	NS
p.m.	29.0	29.3	0.11	NS	30.8	31.1	0.12	NS
Daily average [b]	26.7	27.0	0.10	NS	29.2	29.1	0.10	NS
Relative humidity, %								
a.m.	87.0	88.2	0.59	NS	73.5	74.6	0.60	NS
p.m.	67.9	67.7	0.58	NS	62.9	63.1	0.59	NS
Daily average [b]	77.9	78.0	0.46	NS	68.2	68.4	0.48	NS
THI								
a.m.	74.6	75.3	0.17	NS	78.2	79.0	0.16	NS
p.m.	79.9	80.0	0.23	NS	82.3	81.9	0.25	NS
Daily average [b]	77.4	77.9	0.20	NS	79.9	79.8	0.20	NS
Rectal temperature, °C								
a.m.	38.3	38.6	0.04	0.029	38.4	38.7	0.05	0.001
p.m.	38.4	38.7	0.05	0.007	38.5	38.8	0.07	0.001
Daily average [b]	38.4	38.6	0.03	0.007	38.5	38.8	0.05	0.001
Respiratory rate, min^{-1}								
a.m.	36.8	61.2	3.57	0.001	41.5	51.5	1.47	0.001
p.m.	43.0	72.8	4.39	0.001	45.4	57.4	2.19	0.001
Daily average [b]	39.8	66.7	3.93	0.001	43.5	54.5	1.83	0.001
Heart rate [a] (beat/min)	75.2	74.8	1.30	NS	NA [c]	NA		

[a] NS: Not significant, $P > 0.05$.
[b] Average of two daily tests at morning and afternoon.
[c] NA: Data is not available.

The yield of 4% FCM (fat corrected milk) was calculated using the Equation: 4% FCM (kg) = 0.4 × milk (kg) + 15.0 × milk fat (kg), NRC (2001).

2.2. Chemical analyses

Replicate samples of the weekly composites of each diet in experiment 2, and of the daily feeds and orts of the individual cows in experiment 1, were assayed in triplicate for DM content (drying at 105 °C for 24 h) and residual ash (4 h at 600 °C). Dietary samples were dried in an oven at 60 °C for 48 h, ground through a 1 mm sieve, and analyzed for crude protein (CP) content according to the Kjeldahl method (AOAC, 2001) and for the content of NDF (without sodium sulfite and with heat stable amylase, Van Soest et al., 1991). The Ankom apparatus (Ankom [220], Fairport, NY USA) was used for extracting and filtering the NDF. Net energy (NE_L) content of each TMR was calculated based on the NE_L content of the individual feeds (NRC, 2001).

2.3. Statistical analysis

Comparison between the two cooling treatments with respect to all the daily parameters examined in each of the two experiments was carried out according to the repeated measurement Proc-mixed model of SAS (SAS, 1996) and average condensed means for the daily averages for entire experimental period are presented in Tables 2–5. Tukey's test (SAS, 1996) was used for comparison between means.

3. Results and discussion

3.1. Experiment 1

Eighteen cows held in individual chambers in a closed barn were used in experiment 1. The daily average ambient temperature, relative humidity and the THI within the barn were similar for the two treatments (Table 2), since the evaporative cooling system was designed to cool directly the cows and not the environment. Ambient temperature was high (24.4–29.3 °C) during most of the day hours, with daily averages of 27 °C. Relative humidity (RH) was highest in the early morning hours and its daily average was 78%. Thus, THI was confined within a narrow range (75–80, Table 2) in the barn, which was typical of heat load conditions. Therefore we have used an external evaporative cooling method for direct cooling of the *C* group, while the *NC* group was kept under heat load conditions. Experiment 1 focused on measuring the effect of cooling on respiratory rate and rectal temperature; enzymatic activity (at mRNA level) related to processes of lipolysis and lipogenesis occurring during the pre-parturition period in fat and mammary gland tissues; and individual feed and water intake.

The THI elevation during day-time from 75 in the morning to 80 in the afternoon hardly affected cow's body temperature of the *NC* group (Table 2) since the cows responded to the environmental change by increasing their respiratory rate to get read of excess heat, as demonstrated previously in a study with milking cows (Miron et al., 2008).

The cooling system relieved the heat load of the *C* cows as reflected by an average decrease of 0.2 °C in their body temperature (38.6 °C vs. 38.4 °C, $P < 0.01$) and 40% in respiratory rate (66.7 vs. 39.8 respirations per minute, $P < 0.01$), as compared with the *NC* cows. No difference between treatments was found with respect to the average heart rate of the cows (Table 2) and this is in accord with a previous study in milking

Table 3
Voluntary DM, NDF and water intake of individual dry cows in experiment 1 and average DM and NDF intake of group-fed dry cows in experiment 2, under cooling vs. non-cooling conditions.

	Experiment 1				Experiment 2			
	C	*NC*	SEM	*P*-value	*C*	*NC*	SEM	*P*-value
Dry period								
DMI (kg/day)	10.6	9.30	0.29	0.029	12.2	11.3		
NDF intake (kg/day)	5.45	4.78	0.14	0.030	6.08	5.63		
Water intake (L/day)	53.0	80.0	6.12	0.038	NA [a]	NA		
Close-up period								
DMI (kg/day)	10.7	9.10	0.28	0.05	11.9	11.4		
NDF intake (kg/day)	4.85	4.12	0.20	0.05	5.07	4.86		
Water intake (L/day)	31.0	50.0	4.76	0.05	NA	NA		
BCS difference (scale 1–5/day)								
Dry to close-up	−0.03	−0.09	0.079	NS [b]	−0.07	0.01	0.046	NS
Close-up to calving	0.12	0.00	0.057	NS	0.01	−0.15	0.035	0.035

[a] NA: Data is not available.
[b] NS: Not significant, $P > 0.05$.

Table 4
Calves' birth weight, quality and quantity of 1st colostrum, post parturient disorder events and average yield of milk, ECM and FCM during 90 DIM of lactating cows held at the dry period under cooling vs. non cooling conditions in experiment 1.

	C	*NC*	SEM	*P*-value
Number of cows	9	9		
Pregnant days	279	273	1.6	NS [a]
Quantity of 1st colostrums, L	7.9	7.7	0.72	NS
Quality of 1st colostrum [b] IgG, g/L	73.6	66.3	6.45	NS
Calves birth weight, kg	43.6	41.1	1.8	NS
Post parturient disorder events	2	5		
Milk yield, kg/day	37.1	38.4	1.58	NS
ECM yield, kg/day	35.4	34.6	1.58	NS
4% FCM yield, kg/day	35.0	33.9	1.81	NS

[a] NS: Not significant, $P>0.05$.
[b] Densometric system.

cows (Miron et al., 2008). Similar effect of evaporative cooling on decreasing the body temperature of dry cows was also demonstrated in a previous study (Wolfenson et al., 1988) but changes in respiratory rate have not been measured previously in studies using evaporative cooling of dry cows.

The relief of heat load by external evaporative cooling was expressed in significantly higher ($P<0.05$) daily individual DMI of the *C* cows as compared with the *NC* group, during dry (10.6 vs. 9.3 kg/cow/day, respectively) and close-up (10.7 vs. 9.1 kg/cow/day, respectively) periods (Table 3). Similar effect of evaporative cooling on increasing DMI was also shown in previous studies employing milking cows (Umphrey et al., 2001), however there is lack of information in the literature about the effect of cooling on individual DMI of dry cows.

It was interesting to note that daily water consumed by the *C* cows was significantly lower than that of the *NC* group during the dry (53.0 vs.80.0 kg/cow/day, respectively) and the close-up period (31.0 vs. 50.0 kg/cow/day, respectively) (Table 3). This finding suggests that under heat load conditions without external evaporative cooling, the *NC* dry cows relief their excess body heat production by using two mechanisms: 1. increasing breathing\ panting (as shown in Table 2); and 2. Increasing water consumption (Table 3), excreted later in the urine and feces. These mechanisms enabled dry cows to maintain their body temperature below 39 °C (Table 2). It should be noted however, that on one hand, the high drinking of the *NC* cows might increase rate of digesta passage through their gastro-intestinal tract which might consequently reduce the digestibility of the diet, but on the other hand the reduced DMI in the *NC* cows might also cause a decrease in passage rate which would increase digestion.

Table 5
Calves' birth weight, quality and quantity of 1st colostrum, post parturient disorder events and average yield of milk, milk solids, ECM and FCM during 90 DIM of lactating cows held at the dry period under cooling vs. non cooling conditions in experiment 2.

	C	*NC*	SEM	*P*-value
Number of cows	36	36		
Pregnant days	278	274	0.8	0.04
Quantity of 1st colostrums, L	8.6	6.1	0.51	0.01
Quality of 1st colostrum [a], IgG, g/L	77.5	56.8	2.75	0.04
Calves birth weight, kg	43.6	40.8	0.68	0.04
Post parturient disorder events	8	9		
Milk yield, kg/day	41.4	39.3	0.13	0.03
Milk fat, %	3.37	3.41	0.05	NS [b]
Milk protein, %	2.97	2.99	0.02	NS
Milk lactose, %	4.77	4.82	0.02	NS
Milk urea—N, %	0.03	0.03	0.001	NS
Milk fat yield, kg/day	1.39	1.34	0.024	NS
Milk protein yield, kg/day	1.23	1.17	0.019	0.01
ECM yield, kg/day	37.2	35.4	0.13	0.02
4% FCM yield, kg/day	37.4	35.8	0.13	0.02

[a] Densometric system.
[b] NS: Not significant, $P>0.05$.

A trend of higher post parturient disorders was observed in *NC* cows as compared with the *C* cows (Table 4), and higher calves' birth weight and higher quantity and better quality of first colostrum were observed in the *C* cows, and these trends support the significant results obtained in experiment 2.

Non significant BCS changes between dry off to close up and close up to calving were observed in cows receiving the two treatments (Table 3). Also the production of milk, ECM and 4% FCM, during first 90 DIM was not significantly different between treatments (Table 4). These findings might be explained by the low number of cows (just 9 pairs) used in experiment 1. However, larger population as used in experiment 2 enabled to demonstrate significant differences between treatments with respect to BCS changes and milk performance (Table 5).

Enzyme expression (at mRNA level) in mammary tissue showed relatively higher HSL and lower ACC and FAS enzyme activity values in the *C* cows as compared with the *NC* cows (Fig. 1). This finding suggests an increase in mammary tissue metabolism associated with usage of fatty acids for proliferation of mammary epithelial cells (HSL activity) in the *C* cows (Yonezawa et al., 2008), accompanied with a decrease in the expression of enzymes (at the mRNA level) responsible for lypogenic activity in this tissue (ACC and FAS). It is suggested that the relative non-construction of the fat tissue in the developed mammary gland during the close-up period of *C* cows, reflected usage of energy from fatty acids for new epithelial cell synthesis in the developed mammary gland, which is important for milk production in the subsequent lactation. This explanation is based on previous studied with these enzymes showing their importance in the development of mammary gland tissue in lactating animals (Bauman and Vernon, 1993; Vernon and Pond, 1997; Fielding and Frayn, 1998).

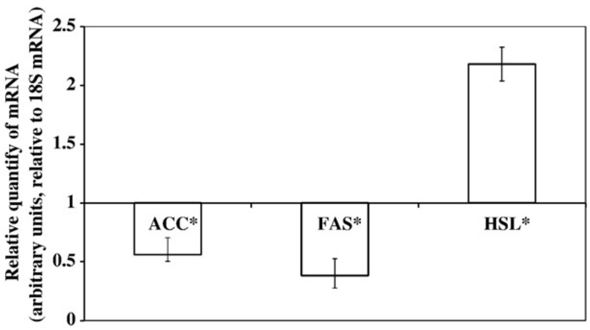

Fig. 1. Difference between treatment in expression values (at mRNA level) of the enzymes: acetyl CoA carboxylase (ACC); fatty acid synthetase (FAS); and hormone-sensitive lipase (HSL), in mammary tissue (non-differentiated on proliferation process) of cooled cows compared to non-cooled cows in experiment 1 ($* = p<0.05$).

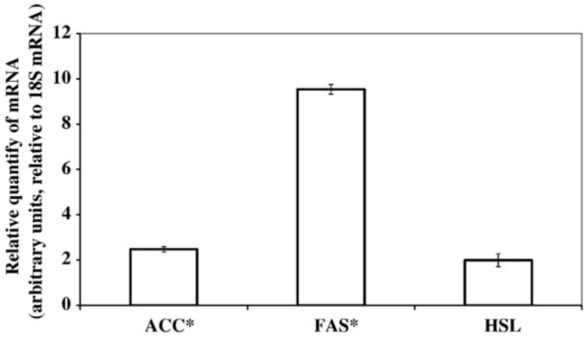

Fig. 2. Difference in expression values (at mRNA level) of the enzymes: acetyl CoA carboxylase (ACC); fatty acid synthetase (FAS); and hormone-sensitive lipase (HSL), in fat cells the of sub cutaneous fat tissue in tail-hollows of cooled cows compared to non-cooled cows in experiment 1 (* = $P<0.05$).

The enzymes expression (at mRNA level) in the subcutaneous fat tissue of *C* cows showed increase in their lypogenic enzyme expression (especially on FAS and ACC) meaning a massive construction of fat tissue reservoir (Fig. 2), and this finding is compatible with the BCS increase between close-up to calving as demonstrated in Table 3.

3.2. Experiment 2

The ambient temperature and THI found in the shaded loose housing barn during the trial are typical to high heat load conditions (78–82 THI, Table 2). Direct evaporative cooling of the *C* cows, reduced their rectal temperature by 0.3 °C (38.5 °C vs. 38.8 °C, $P<0.01$), and respiration rate by 20% (43.5 vs. 54.5 respirations per minute, $p<0.01$), as compared with the *NC* group (Table 2). This picture is similar to the trend found in experiment 1.

Average DMI of the *C* cows (group feeding) was higher than that of the *NC* group by 8.0%, (12.2 vs. 11.3 kg/cow/day, respectively) during the dry period, and by 4.4% during the close-up period (11.9 vs. 11.4 kg/cow/day, respectively) (Table 3). These findings based on group feeding, are in accord with the individual DMI data obtained in experiment 1.

Cooling improved significantly BCS gain during the close-up period (21 days prior calving until calving), (Table 3). Cooling of dry cows also improved the quantity (8.2 vs. 5.8 kg) and the quality of 1st colostrum (78.3 vs. 61.1 kg, Table 5), in accord with the findings of exp. 1.

A significant higher calves' birth weight of new born calves was demonstrated in the *C* cows as compared with the *NC* group (43.6 vs. 40.8 kg respectively, Table 5). This is probably related to the shorted pregnancy period of the *NC* cows as compared with the *C* group, since the gap of 4 days in the end of pregnancy provided better embryo development and higher calve's birth weight (Table 5). This finding is in accord with experiment 1 data (Table 4), and with previous studies which reported positive effect of heat stress relief during last trimester of pregnancy on calve's birth weight (Collier et al., 1982a; Wolfenson et al., 1988).

The larger sample size used in experiment 2, (36 pairs), enabled better measurement of performance data after parturition. Data in Table 5 show that cooling of the *C* cows resulted in a significant increase during the first 90 DIM in milk production by 5.3%, protein yield by 5.1%, ECM yield by 5.1% and FCM yield by 4.5%, as compared with the *NC* cows. This improvement in performance is in accord with previous observations of cooling dry cows (Wolfenson et al., 1988; Urdaz et al., 2006). It is suggested that the improvement in milk yield during 90 DIM is due to the better BCS and higher proliferation and development of the epithelial cells in the mammary gland of the *C* cows during the close-up period.

Cows' health and reproduction parameters in experiment 2 (Table 5), demonstrate that cooling cows during the dry and close-up periods didn't improve neither the number of incidences of post parturient disorders nor the reproduction parameters, including: number of resting days (period from calving to first insemination day), lost days (period from first to effective insemination) and open days (period from calving to effective insemination day) as compared with the *NC* group (73 vs. 75 days; 29 vs. 33 days; and 102 vs. 108 days, respectively).

Calculation of expenses vs. profit due to the use of evaporative cooling in experiment 2, shows that total cost of cooling a dry cow is equivalent to net income from 80 kg milk yield (including investment in cooling facilities, installation and operation costs). However, the *C* cows produced 190 kg milk more than the *NC* cows during the first 90 DIM, and since we have not measured DMI during 90 DIM, the net profit of 110 kg milk per cow achieved in this study should be corrected while considering the assumed higher DMI of the *C* cows.

4. Conclusions

This study shows that evaporative cooling during the entire dry period improved heat relief of pregnant cows, as demonstrated in their lower respiratory rate and body temperature, leading to improvement in their voluntary DMI, as compared with the *NC* cows. BCS of the cows was improved during the last 21 days before estimated calving, providing better energy resources for the adjacent calving and coming lactation. Consequently, the *C* cows were involved in fewer incidences of post parturient disorders, their calves' birth weight was higher, and their colostrum quality and quantity were improved. The improvement in BCS accompanied with an increase in mammary gland cell proliferation (HSL activity) of the *C* cows during the close up period, resulted in a significant increase of their milk and ECM production after calving.

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