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Effects of an injectable mineral supplement on physiological responses and milk production of heat-stressed Holstein cows

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Abstract Summer heat stress in northwest Mexico compromises the physiological thermoregulation capacity and productive performance of lactating Holstein cows, and supplementation of minerals appears to reduce the adverse impact of heat stress in cattle. The objective herein was to evaluate the effects of an injectable mineral supplement containing phosphorus, selenium, potassium, magnesium, and copper on physiological responses, milk production, and milk composition of Holstein cows exposed to heat stress. Sixteen cows were blocked by parity and assigned to one of two treatments (n = 8) using a randomized complete block design: 1) control cows and 2) mineral-treated cows. All cows were exposed to environmental heat stress conditions (i.e., temperature-humidity index = 79.4 ± 4.3 units). No study variable was affected ($P \geq 0.20$) by the treatment x sampling day interaction. While the mineral supplement did not affect any physiological variable in the afternoon, this treatment decreased breaths per min ($P = 0.01$) and most body surface temperatures ($P \leq 0.06$; head, shoulder, leg, right-flank, and udder) in the morning. There was no effect ($P = 0.37$) of the mineral supplementation on milk yield but increased ($P \leq 0.03$) the percentages of solids non-fat, protein, lactose, and density in the milk. In conclusion, Holstein cows' physiological thermoregulation and milk composition experiencing summer heat stress were improved by applying an injectable mineral supplement.

Keywords dairy cattle, heat stress, milk yield, phosphorus, selenium

1. Introduction

Heat stress can severely compromise dairy Holstein cows' healthiness and milk production efficiency by forcing them to make various physiological and metabolic adjustments to dissipate excess heat load and decrease endogenous heat production, respectively (Nardone et al 2010). Four basic routes of heat exchange have been reported in mammals: conduction, convection, radiation, and evaporation (Collier et al 2006). In heat-stressed dairy cattle, a redistribution of blood flow from internal organs to peripheral tissues along with subcutaneous vasodilation occurs to enhance heat exchange between skin and the surrounding environment, mainly through the first three routes (Rashamol et al 2018; Renquist 2019). However, when environmental temperatures are higher than the body's temperature, cows are required to significantly increase their respiratory rate (RR) to facilitate heat loss through pulmonary evaporation (Polsky and von Keyserlingk 2017). Concomitantly, heat-stressed dairy cows reduce their milk yield to decrease metabolic heat generation and prevent further increments in core body temperature (West 2003).

Along with reductions in milk yield, several reported that heat stress evokes changes in milk quality by reducing its content of protein, fat, solids non-fat, and lactose (Kadzere et al 2002; Gaafar et al 2011; Pragna et al 2017). Furthermore, heat stress can also disrupt the balance between pro-oxidant and antioxidant agents, altering concentrations of free radicals in the organism, and causing both cellular and mitochondrial oxidative damages (Belhadj et al 2016). Therefore, there is a need to develop strategies mitigating the effects of heat stress in dairy cattle.

Nutritional management strategies excel due to the flexibility that exists with the manipulation of nutrients offered to heat-stressed animals (Conte et al 2018). Among the nutrients that can be adjusted in diets formulated for hot weather feeding, special attention has been placed on minerals due to their important role as structural component of tissues, as well as their involvement in the synthesis and

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action of enzymes related to oxidative metabolism (Sejian et al 2014; Kumar 2015; Khorsandi et al 2016).

Mineral supplementation has proven to be helpful as a feeding strategy to alleviate the negative effects of heat stress by providing the missing minerals due to the reduced food intake (Sammad et al 2020). Administration of minerals in Holstein cows exposed to outdoor heat stress appears to increase their capacity to dissipate body thermal load (Conte et al 2018; Khare et al 2018). Supplementation of trace minerals such as Cu, Zn, Mn, and Co in heat-stressed Holstein cows increased solids non-fat and percentage of protein in milk (Khorsandi et al 2016). Similarly, Ayrshire cows supplemented with vitamin E and Se also improved both solids non-fat and milk protein (Guerra-Liera et al 2012).

In this context, we hypothesized that the parenteral supply of minerals could help to ensure the same availability to all treated animals and reveal the potential benefits of supplementing minerals under heat stress conditions. Therefore, the objective of this study was to determine the effects of applying an injectable mineral supplement containing phosphorus (P), selenium (Se), potassium (K), magnesium (Mg), and copper (Cu) on physiological responses, milk production and milk composition of Holstein cows exposed to outdoor heat stress.

2. Materials and Methods

The ethics committee of the Department of Agronomic and Veterinary Sciences of the Instituto Tecnológico de Sonora (ITSON) approved all procedures and animal care conditions performed in this study, based on national (NAM, 2010) and international (FASS, 2010) guidelines.

2.1. Experimental location

This study was conducted from June 11 to July 24 of 2018 in the Academic Unit of Research in Milk Production of ITSON. The farm is located at the Yaqui Valley, Sonora, in northwestern México (27° 29' N, 109° 56' W). The area's elevation is 46 m under sea level, with an average annual temperature of 23 °C and annual precipitation of 371.6 mm. During summer, the zone temperature and relative humidity can reach 48 ˚C and 70%, respectively, leading to a temperature-humidity index (THI) of approximately 85 units.

2.2. Animals and treatments

The study included sixteen Holstein cows with averages of 167 ± 45 days in milk (DIM) and body condition score (BCS) of 2.75 points according to the 1-5 scale, where 1 corresponded to a severe underconditioning and 5 to a severe overconditioning (Edmonson et al 1989). The experimental design was as a completely randomized block. Animals were blocked by parity (primiparous or multiparous) and then randomly assigned within block to the treatments (n= 8): 1) control group (CON) and 2) mineral-treated group (MTR). Cows in the MTR group received 3 im injections (10 mL/each) of a mineral supplement based on P, Se, K, Mg, and Cu (Fosfosan®, Laboratorios Virbac, Guadalajara, México). The first injection was given at the beginning of the experiment (day 0), the second application was performed 8 days after (day 8), and the third application was given 10 days later (day 18). All injections were given in the morning (between 08:00 and 10:00 h). No placebo was applied to CON group; however, all cows within this treatment were managed and restrained in a similar way as MTR cows during the application days.

2.3. Housing, cooling system and feeding practices

Cows were housed in shaded open pens (34 x 22 m) with approximately 8.5 m^2 of shade per cow. Shade was provided by a corrugated and galvanized iron sheet-roof at 4 m height, set from North to South. Dimensions of shades were 15 x 5.4 m. Both experimental groups were subjected twice-daily to a cooling system consisting of three ½ HP electric fans located at 2.73 m from the floor and 16 sprayers. Each cooling cycle included 3 min water aspersion (~15 L per cow/cycle) followed by 3 min of forced ventilation (Leyva-Corona et al 2016). All cows had free access to water and were fed a ration made up of 85% forage (60% corn silage, 25% alfalfa hay) and 15% of a commercial energy supplement plus vitamin and mineral premix. Diet was formulated to meet nutrient requirements of lactating Holstein cows weighing 600 kg and producing 28 kg of 3.5% fat corrected milk (NRC 2001). The chemical composition of the mixed diet was 15.3% of crude protein, 30.3% of neutral detergent fiber, and 1.2 Mcal of NE_L /kg dry matter.

2.4. Climatic data

Ambient temperature (AT, °C) and relative humidity (RH, %) data were collected from a nearby (~500 m) automatic weather station (Telemetry Gateway A840®, Adcon Telemetry Inc., Santa Rosa, CA, USA), equipped with the software AddVANTAGE 3.45® and sensors located 2 m from the ground. This information was used to estimate THI according to the formula proposed by Hahn (1999): THI = (0.81 x AT) + [(RH/100) x (AT − 14.4)] + 46.4.

2.5. Study variables

Physiological variables evaluated in this experiment were RR (bpm = breaths per min) and hair coat temperatures (HCT, °C) from the head, shoulder, rump, leg, right-flank, and udder. These variables were collected twice a day (05:00 and 17:00 h) and two-time weekly for 6 weeks. The RR was measured by visual counting of intercostal movements during 60 s using a stopwatch. Hair coat temperatures were measured with an infrared thermometer (model 568 Fluke®, Fluke Corp., WA, USA; accuracy ± 1°C) positioned approximately 3 m of distance from the animal.

Milk production variables included daily milk yield, which was calculated by summing the volume of milk produced by each cow from the two milking periods (06:00 and 18:00 h) of each sampling day. Information was retrieved from the electronic milking system of the farm (Metraton

21™, GEA, Westfalia-Surge Farm Technologies, Siemensstraße, Bönen Germany) using its associated software Dairy Plan®. At each milking, milk samples were collected directly from each cow's udder to measure milk temperature (MT, °C) using a digital thermometer (Delta Trak®, Pleasanton, CA, USA). Then, milk samples were transported to the molecular biology lab of ITSON to perform milk quality analyses, which involved the determination of fat (%), solids non-fat (%), density (kg/m^3), protein (%), lactose (%), salts (%) and freeze point (°C), using an ultrasonic milk analyzer (Milko-tester, model Master Pro, Bulgary). Results obtained from both morning and afternoon milking periods were averaged to obtain the milk quality indicators (MQI).

2.6. Statistical analysis

All statistical procedures were performed using SAS Version 9.4 (2014). Climatic variables were averaged using PROC MEANS. Assumptions of normality and homogeneity of variances were tested using PROC UNIVARIATE and PROC GLM, respectively. Physiological variables and milk production were subjected to an analysis of variance under a completely randomized block design with repeated measures over time using PROC MIXED. The model considered block, treatment, time (sampling day) and the treatment x time interaction as fixed effects for all variables.

Additionally, BCS and DIM were included as linear covariates. Cow nested within treatment was included as the random effect. Several variance-covariance structures were verified to fit the model, and the structure of variance components (VC) showed the best fit according to AIC and BIC criteria. Means were separated using the option LSMEANS/ PDIFF, considering significant differences when $P \leq 0.05$.

3. Results

3.1. Climatic conditions

Average values for AT, RH, and THI were 29.8 °C, 62.6 %, and 79.4 units, respectively, during the experimental period. In general, AT maintained values near to 25 °C during the nighttime (0:00 to 05:00 h), and then, between 06:00 and 14:00 h, it increased, reaching a maximum of 35 °C (Figure 1). In the case of RH, its circadian rhythm was inverse to that observed for AT, declining between 06:00 to 11:00 h, and remaining at approximately 45% from 11:00 to 17:00 h. THI maintained values above 80 units between 08:00 and 18:00 h.

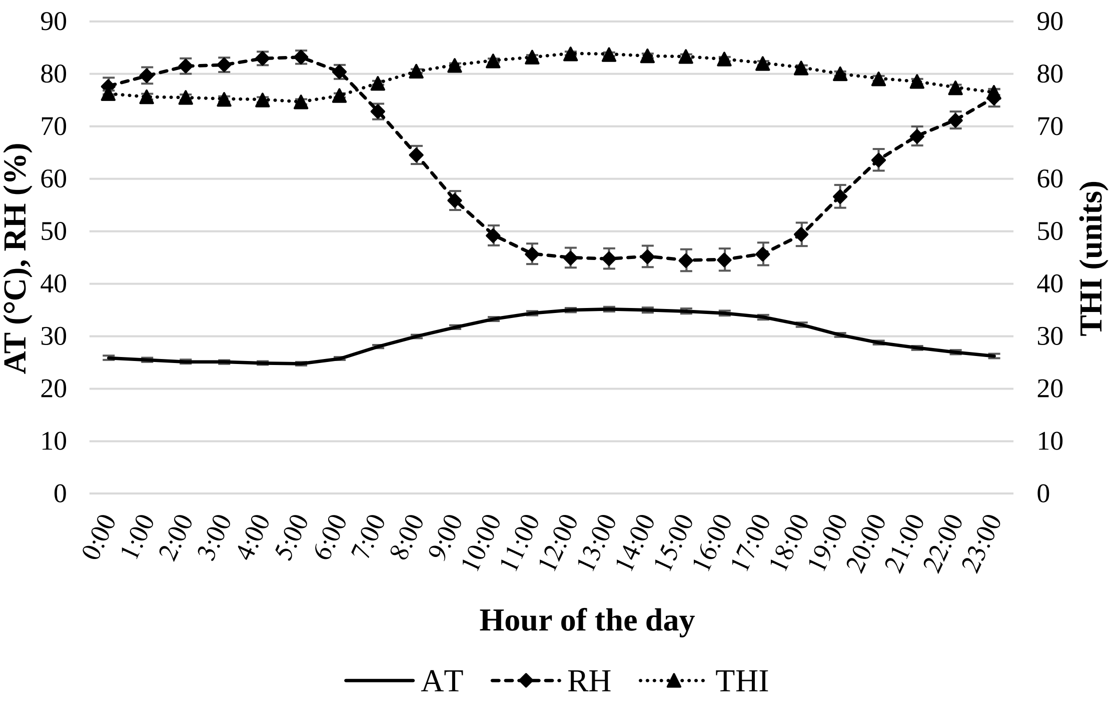

Figure 1 Hourly averages of ambient temperature (AT), relative humidity (RH) and temperature-humidity index (THI) during the experimental period.

3.2. Physiological variables

There were no significant interactions ($P \geq 0.21$) between treatments and time for any variable (Table 1). In the morning, the mineral treatment reduced RR ($P = 0.02$), as well as leg, shoulder, and udder temperatures ($P \leq 0.05$), while only tended to decrease ($P = 0.06$) head and right-flank temperatures. Supplementation of minerals had no influence ($P > 0.05$) on any physiological variable in the afternoons. Sampling day affected ($P < 0.01$) all physiological variables for both morning and afternoon.

3.3. Milk production variables

No effect ($P \geq 0.21$) of the treatment x time interaction was observed for any variable of milk production (Table 2).

Overall, mineral supplementation increased ($P \leq 0.03$) percentages of solids non-fat, protein and lactose, and density but decreased the freezing point ($P = 0.02$). In addition, mineral supplementation tended to decrease MT ($P = 0.08$) without affecting ($P \geq 0.18$) milk yield and fat and salt percentage. The individual effect of time affected ($P < 0.01$) only to milk yield and MT.

Table 1 Effects of the injectable mineral supplement on physiological variables of heat-stressed Holstein cows.

	Treatments (Tx)			*P*-value		
	MTR	CON	SEM*	Tx	Time	Tx ×Time
Morning (05:00 h)						
Respiratory rate (breaths per min)	59.10	63.23	1.12	0.01	<0.01	0.94
Head temperature (°C)	28.98	29.34	0.18	0.06	<0.01	0.69
Shoulder temperature (°C)	32.64	32.95	0.15	0.05	<0.01	0.81
Rump temperature (°C)	30.90	31.27	0.22	0.11	<0.01	0.97
Leg temperature (°C)	31.26	31.76	0.16	0.01	<0.01	0.69
Right-flank temperature (°C)	32.19	32.46	0.27	0.05	<0.01	0.20
Udder temperature (°C)	32.73	33.54	0.18	<0.01	<0.01	0.86
Afternoon (17:00 h)						
Respiratory rate (breaths per min)	68.30	67.48	1.18	0.49	<0.01	0.92
Head temperature (°C)	35.80	35.65	0.34	0.66	<0.01	0.77
Shoulder temperature (°C)	36.16	36.32	0.22	0.45	<0.01	0.92
Rump temperature (°C)	36.16	36.18	0.27	0.94	<0.01	0.81
Leg temperature (°C)	35.66	35.73	0.24	0.75	<0.01	0.68
Right-flank temperature (°C)	35.91	35.74	0.26	0.53	<0.01	0.97
Udder temperature (°C)	36.56	36.57	0.22	0.95	<0.01	0.28

*SEM= total standard error of means and time= sampling day

4. Discussion

As average TA (29.8 °C) was above the upper limit of the thermoneutral zone for dairy Holstein cattle and also THI exceeded 72 units, it was considered that Holstein cows in the current study were exposed to a heat stress environment, particularly of moderate type (79 to 88 units) according to Armstrong (1994). Similar results have been previously reported for summer months in the geographical region of study (Hernández-Cordero et al 2017; Leyva-Corona et al 2018). At night, TA dropped by around 6 °C; however, RH increased almost twice with respect to that observed in daylight hours; so, the cattle were experiencing heat stress 24 hours of the day.

In response to mineral supplementation, the heat-stressed cows showed decreased physiological activity in the morning (lower RR and HCT in most body regions). However, no physiological changes were observed in the afternoon. This physiological response differenced by daytime in mineral-treated cows could be due to TA and RH circadian fluctuations. Lactating dairy cows are susceptible to outdoor heat stress conditions, and their efficiency in dissipating body heat load decreases as the TA increases across the daylight hours. For the aforementioned, cows show a small gradient between TA and body temperature in the afternoon, which causes a reduction in cutaneous heat losses, and they are kept lying and panting under shade (Hernández-Rivera et al 2011).

Conversely, at night, cows take advantage of the reduction in TA and the null solar radiation to remain standing with increased RR outside the shade, which allows them to dissipate large amounts of body heat toward the environment through both the skin and respiratory tract (Gaughan et al 2000). Therefore, everything indicates that the administration of minerals, through their antioxidant effect (Conte et al 2018; Khare et al 2018), improved the nocturnal capacity of lactating Holstein cows to dissipate body thermal load acquired in sunshine hours. This caused that mineral-treated cows had a lower body heat load at dawn, reflected in less RR and HCT than control cows.

Oltramari et al (2014) reported that Se supplementation in heat-stressed dairy cows reduced RR and HCT (head, back, lower leg, and udder), particularly when the mineral source was inorganic. Similarly, heat-stressed Malpura ewes had lower RR when supplemented with mineral mixture (Zn, Cr, Co, and Se) and vitamin E (Sejian et al 2014). Collectively, these reports presented evidence of the possible protective effects of minerals and antioxidants on the respiratory tract of animals exposed to heat stress conditions.

Table 2 Effects of the injectable mineral supplement on milk yield, composition and temperature of heat-stressed Holstein cows.

Variable	Treatments (Tx)			*P*-value		
	MTR	CON	SEM	Tx	Time	Tx x Time
Daily milk yield (L)	15.94	16.54	0.64	0.36	<0.01	0.88
Milk temperature (°C)	36.27	36.40	0.07	0.08	<0.01	0.75
Fat (%)	2.55	2.44	0.09	0.28	0.30	0.97
Solids non-fat (%)	9.31	9.12	0.07	0.02	0.31	0.99
Density (kg/m^3)	26.60	26.06	0.20	0.02	0.29	0.99
Protein (%)	3.35	3.28	0.03	0.03	0.43	0.95
Lactose (%)	5.04	4.94	0.04	0.01	0.25	0.97
Salts (%)	0.72	0.71	0.01	0.18	0.38	0.94
Freeze point (°C)	-0.59	-0.57	0.00	0.02	0.38	0.98

*SEM= total standard error of means and time= sampling day.

In addition, the reductions in leg and udder temperatures following treatment with the mineral supplement appeared to be associated with and improved heat stress dissipation. It has been reported that legs contribute to heat dissipation in ruminants since, under heat stress conditions, blood gets redirected to extremities to promote heat loss via non-evaporative mechanisms (Nienaber and Hahn 2007). Similarly, the udder region has been considered in assessing the thermoregulatory ability of cows since it shows a moderate correlation with body core and rectal temperatures of animals challenged by warm environmental conditions (Garner et al 2016; Kaufman et al 2018).

Although milk yield and its fat and salts contents were not affected, mineral supplementation improved other milk components of the MTR cows compared to the CON cows. Particularly, milk from the mineral-treated cows had a greater percentage of solids non-fat, a variable whose values depend on the content of protein, lactose, vitamins, and minerals (Magariños 2000). The observed increase in protein and lactose percentages and the expected increase in mineral concentrations due to their parenteral administration could then explain changes in solids non-fat. Similar increases in solids non-fat were observed in heat-stressed Holstein cows supplemented with vitamins and complexes trace minerals such as Cu, Zn, Mn, and Co (Kincaid 2004; Khorsandi et al 2016). Guerra-Liera et al (2012) also reported increases in solids non-fat of Ayshire cows supplemented with vitamin E and Se.

Regarding density, the literature indicates a positive association between density and non-fat solids (Agudelo-Gómez and Bedoya-Mejía 2005), which explains the effect of minerals on milk density. It is worth noting that both milk yield and fat percentage depend on the feed intake (Conte et al 2018); however, it was not evaluated in the current study. Despite this, we speculate that there were no changes in feed intake due to mineral supplementation as a possible explanation for milk yield results and fat percentage.

Concerning the milk protein percentage, cows in the MTR group showed higher values than those in the CON group. This result is in agreement with a report in which the protein percentage of milk increased in a linear fashion when supplementing dairy cows with minerals like P, Cu, Zn, and Mn in a 100, 150, and 200 % of the requirements established by NRC (De Boer et al 1981). This result also agreed with Khorsandi et al (2016), since in their study, the heat-stressed cows supplemented with an intraruminal bolus that contained vitamins and minerals deposited a higher percentage of protein in milk. Guerra-Liera et al (2012) also reported an increase in protein percentage of Ayrshire cows supplemented with vitamin E and Se. In the same study, the authors reported that treated cows also deposited a higher percentage of lactose in their milk. A result that parallels the findings of our study where the MTR group showed a higher percentage of lactose compared to the CON group. Furthermore, Mutoni et al (2012) also reported an increment in lactose percentage of Sahiwal cows supplemented with vitamin E, Cu, and Zn. Similarly, Brzóska and Sala (2001) reported increments in milk lactose of Holstein cows supplemented with Cu.

Milk's freeze point was also influenced by the minerals supplemented in this study since the MTR group showed a lower freeze point than CON group. This result agreed with reports that indicated that milk's freeze point depends almost exclusively on milk's lactose, salts, and minerals content (Agudelo-Gómez and Bedoya-Mejía 2005; Zagorska and Ciprovica 2013). The increases in lactose percentage and the expected increase in milk's mineral concentrations due to their parenteral administration could help explain the slight drop in the milk's freeze point. Finally, the tendency observed in reducing MT could be associated with the decrease in udder temperature since both of them present a positive and strong correlation (Perano and Gebremedhin 2015). The significant effect of time (day of measurement) in DMY and MT was considered normal and expected because both variables depend on time-associated factors like DIM and the particular climatic conditions of each measurement.

5. Conclusions

In conclusion, the parental supplementation of minerals (i.e., P, Se, K, Mg, and Cu) improved the physiological thermoregulation capacity of lactating Holstein cows under a summer heat stress ambient, particularly at night and lasting until dawn. Additionally, this heat stress mitigation strategy was beneficial in improving milk composition without affecting its yield. These results suggested that parenteral mineral supplementation could be considered as a useful nutritional management strategy intended to mitigate the adverse effects observed in heat-stressed Holstein dairy cattle. However, further research is recommended to replicate these results in larger dairy cattle herds exposed to heat stress conditions.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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