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Effects of a Commercial Blend of Phytogenic Compounds and Prebiotic on the Performance of Mid-Lactation Dairy Cows Exposed to Heat-Stress

Research Article

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ABSTRACT

The efficacy of a commercial additive containing carvacrol, anethole, limonene, and fructo-oligosaccharides on heat stress (HS) abatement in lactating dairy cows was evaluated. Control pen (n=92, DIM=91±53.1 d, average milk yield=37.0 kg) without feed additive (control) and phytogenic supplemented pen (n=112, DIM=122±46.8 d, average milk yield=36.1 kg) (PS) were fed a basal total mixed ration, while PS group received 15 g of additive into 2 equal parts top-dressed daily for 5 wks. Feed intake, milk production, rectal temperature, respiratory rate, fecal score, number of cows on feed bunk, and blood biochemistry were determined. The inclusion of PS increased feed intake ($P<0.05$) though it did not alter milk production. While the fecal score improved, the respiratory rate and concentration of thyroxine decreased decreased in PS-supplemented cows. Feeding PS increased the number of cows on bunk feed at 0330 and 0730. Overall, supplementation of PS helped alleviate some negative effects of HS though production performance was not affected.

KEY WORDS ambient temperature, lactating cows, phytogenic compounds, prebiotic.

INTRODUCTION

Heat stress (HS) is a serious problem troubling the global dairy industry because of associated decreases in milk production (Polsky and von Keyserlingk, 2017), compromised reproduction, increased culling rate (St-Pierre *et al.* 2003), suppressed immune (Shan *et al.* 2018) and endocrine systems and increased susceptibility to various diseases (Das *et al.* 2016). When the animals cannot maintain their body thermal balance, it may evoke physiological changes causing suppression of normal metabolic functions (Hao *et al.* 2016). Scientific forecasts predict that the intensification of the greenhouse emission and global warming will increase the environmental temperature by more than 5.8 ˚C by the end of the 21st century (IPCC, 2007), and heat stress (HS) may continue to become one of the most important factors affecting the profit of dairy industry (Hao *et al.* 2016). Thus, most scientists are interested in alleviating HS adverse effects on feed intake, milk production and reproductive performance. Management strategies such as cooling system and improving ventilation in barns (Polsky and von Keyserlingk, 2017), feeding more concentrate supplemented with fat (Moallem *et al.* 2010), supplementing diets with ionophores (Baumgard *et al.* 2011) and other feed additives (Cheng *et al.* 2014) are often used during warm summer months in dairy cattle farms to mitigate the detrimental effects of HS. Alternatively, herbal additives and prebiotics have nutritional and medicinal properties compared to classic feed additives with no residues, which could be a part of HS combating strategies (Hao *et al.* 2016). Essential oils (EO) have been shown to possess strong antimicrobial effects against a wide range of micro-

organisms, including bacteria, fungi, and protozoa (Bodas *et al.* 2012), but to date, a few research have studied the effects of plant-derived components on performance, blood parameters and behavior of dairy cow during HS.

Available evidence proposes this hypothesis that feeding dairy cattle with particular plant extracts and EO may have the potential to reduce the harmful effects of HS (Pan *et al.* 2014; Reza-Yazdi *et al.* 2014; Min *et al.* 2019). According to research, supplemented dairy cows during heat exposure with 0.25 g/kg *Radix bupleuri* extract increased milk production, milk protein, and fat yield by 8, 8.9 and 10%, respectively, compared to control. In addition, cows fed with plant extracts had a lower respiratory rate (Pan *et al.* 2014). Feeding 2 grams of a traditional blend of cinnamaldehyde, eugenol, peppermint, coriander, cumin, and lemongrass to cows in 60 days in milk exposed to HS increased increased dry matter intake (DMI), milk production and acetate to propionate ratio relative to that of the control (Reza-Yazdi *et al.* 2014). In contrast, feeding a mixture of capsicum, cinnamaldehyde, and eugenol extract to lactating dairy cows did not alter DMI and milk production while improving digestibility of fiber and reducing milk fat percentage (Boyd *et al.* 2012).

Meanwhile, considerable efforts have been devoted to evaluating the efficiency of prebiotic for modulating the gut microflora of non-ruminants. Still, research focusing on prebiotics in ruminant nutrition is rare (Bagheri *et al.* 2009). It was found that prebiotics, such as inulin, exhibit desirable modifications in non-ruminants gut microflora and are fermented by *Bacteroides* spp. (Umucalilar *et al.* 2010). Since the latter forms one of the most abundant phyla in the rumen core microbiome (Petri *et al.* 2013), modulation of ruminal fermentation is expected after inulin supplementation (Umucalilar *et al.* 2010). Reduced rumen ammonia nitrogen and methane and increased microbial protein synthesis in ruminants and live weight gains in calves reported by feeding inulin (Samanta *et al.* 2013).

Although phytogenic compounds are studied extensively to determine their effects on performance, ruminal fermentation, health and metabolic status of ruminants (Cardozo *et al.* 2006; Khiaosa-ard and Zebeli, 2013; Hashemzadeh-Cigari *et al.* 2014), sparse research is available examining the effect of prebiotics and herbal additives for abating negative effects of HS on dairy cattle.

Accordingly, the objective of the current study was to evaluate the effect of a commercial product containing fructooligosaccharides and EO on body temperature, production variables and blood parameters in heat-stressed lactating cows.

MATERIALS AND METHODS

Animals, diets and experimental design

Two hundred and four primiparous Holstein cows were housed in large free stall barns. Control pen (n=92, DIM=91±53.1 d, average milk yield= 37.0 kg) without feed additive (control) and phytogenic supplemented pen (n=112, DIM=122±46.8, average milk yield=36.1 kg) (PS) were fed a basal total mixed ration (TMR), while the PS group received 15 g per cow per day of a phytogenic-contained supplement Digestarom P.E.P® (containing carvacrol, anethole, limonene and fructooligosaccharides, biomine, GmbH, Austria) for 5 wks. The supplement was divided into two equal parts and top-dressed in the morning and the evening feedings. Cows were fed *ad libituim* three times per day (07:30, 14:30, 19:00 h) and had free access to freshwater. Basal ration ingredients and composition are presented in Table 1. Diet was formulated to meet nutrient recommendations (NRC, 2001).

Table 1 Ingredients of the basal diet

Composition	Content
Ingredient (g/kg dry matter)	
Alfalfa hay	110
Corn silage	250
Beet pulp	35
Molasses	10
Bakery by-products	28
Supplemental saturated fat	17
Whole cottonseed	50
Ground corn grain	130
Ground barley grain	166
Corn gluten meal	10
Soybean meal	100
Roasted soybean	30
Heat-treated soybean meal	25
Calcium carbonate	5
Salt	3
Dicalcium phosphate	2.5
Sodium bicarbonate	12.5
Magnesium oxide	1.5
Vitamin-mineral permix[1]	10
Bentonite	4.5
Chemical composition (g/kg dry matter)	
Crude protein	155
Neutral detergent fiber	310
Ether extract	50.8
NE_l (Mcal/kg)	1.53

[1] Formulated to provide (per kg): vitamin A: 1300000 IU; vitamin D: 360000 IU; vitamin E: 12000 IU; Zn: 16000 mg; Se: 80 mg; I: 150; Fe: 800 mg; Co: 120 mg; Mn: 10000 mg and Cu: 4000 mg.

Sample collection and analysis

Group TMR intake and orts were recorded daily over five weeks. Also, TMR samples were collected weekly, oven-

dried at 70 ˚C for 48 h and stored for later analyses. Ten cows were randomly selected from each pen to measure other variables.

All the parameters were determined on the same cows (except milk production). Ambient temperature and humidity were recorded daily by a thermo-humidity meter installed in the middle of the barn at 2 m height in each pen. Temperature-humidity Index was calculated by the following formula (Nascimento *et al.* 2019):

$$THI = (0.81 \times T + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)$$

Where:
T: dry bulb temperature (˚C).
RH: relative humidity (%).

The number of cows on the feed bunk was enumerated at hourly intervals on days 25 and 31 for 24 h. Rectal temperature was recorded in 10 cows of each group using a digital thermometer (MAKI-D-1002) twice at 1100-1200 and 1300-1400 on days 25 and 30. The respiratory rate was monitored three times (1200, 1600, 2300 h) in 10 animals of each treatment by counting the numbers of flank movements per min (Pan *et al.* 2014). Also, on days 25 and 30, a 1-5 fecal scoring system was used for the evaluation of fecal consistency with 1 for watery or extremely loose and 5 for extremely hard feces (Bagheri *et al.* 2009).

Cows were milked at 07:00, 16:00 and 0030 h. The milk yield was recorded for all 204 cows on d 0 and 35 of the experiment. Milk samples were preserved with potassium bichromate and stored at 4 ˚C until analyzed for fat, protein, and somatic cell count (SCC) using near infra-red reflectance spectroscopy analyzer (Foss 605B Milko-Scan, Foss Electric, Hillerød, Denmark).

Blood samples were obtained via coccygeal venipuncture on day 30 before the noon feeding and serum was harvested and stored at -20 ˚C until analyzed for serum parameters. Concentrations of thyroxine (T4) and triiodothyronine (T3) were analyzed by ELISA (Ideal Tashkhis® kit, Iran); glucose and cortisol (Monobind®, Iran) and β-Hydroxybutyric acid (BHBA) and Insulin (Randox, UK and Demeditec, Germany, respectively) were measured by commercial kits.

Rectal fecal grab samples were collected from 10 cows in each pen on day 30, dried by oven at 70 ˚C for 48 h and ground through a 2 mm screen. The fecal and TMR samples were analyzed for acid insoluble ash as an internal marker for calculating the apparent nutrient digestibility (942.05 AOAC, 2002). The crude protein (976.05) and ash (942.05) contents of TMR and feces, as well as ether extract (954.02) of TMR were analyzed using AOAC (2002) methods. Heat stable alpha-amylase enzyme (A3306, sigma – Aldrich, Steinheim, Germany) was used for neutral detergent fiber (NDF) measurement according to van Soest *et al.* (1991). Acid detergent fiber (ADF) was determined according to AOAC (2002).

Statistical analysis

A completely randomized design was used in this project. Data were analyzed using the MIXED procedure of SAS (SAS, 1999) with the TREATMENT considered as a fixed effect, while COW was the random variable. Data repeated over time were analyzed using the REPEATED statement with time of sampling as a repeated measure. Normality of distribution of response variables was tested using PROC UNIVARIATE of SAS. The treatment least squares means were compared when the treatment effect in the statistical model approached significance ($P \leq 0.05$). Tendencies were discussed where $0.05 < P < 0.10$. Milk production on d 0 (i.e. the start of the trial) was used as covariate variable for milk production on d 35.

RESULTS AND DISCUSSION

The average temperature and humidity index (THI) was 79 ± 5 throughout the trial period. It was observed that the DMI was increased by 3.6 percent in the PS ($P<0.05$). However, milk yield, compositions and SCC were not affected by phytogenic supplement (Table 2).

Until now, there is paucity of data about the effects of prebiotics on milk production of dairy cows, early studies showed that the feeding 15 g of mannan-oligosaccharides alone did not affect yield and compositions of milk (Bagheri *et al.* 2009). In agreement, Boyd *et al.* (2012) reported no change in milk production of dairy cows fed a mixture of capsicum, cinnamaldehyde, eugenol and fermentation metabolites from *Aspergillus niger*. In contrast, some studies reported a higher milk production by the addition of a plant extract to the diet of heat-stressed cows (Pan *et al.* 2014; Reza-Yazdi *et al.* 2014; Shon *et al.* 2018).

Dairy cow comfort zone has been reported to be between 5 to 25 ˚C (NRC, 1981). When the ambient temperature rises above 25 ˚C, the cow cannot cool herself adequately. In addition to the high environmental temperature, relative humidity contributes to uncomfortable conditions for lactating cows. A dairy cow experiences HS when THI exceeds 72 (Armstrong, 1994; Bohmanova *et al.* 2007). Heat stress can directly alter ruminal microbiome independent of its effect on DMI. It has been shown that the relative abundance of *Streptococci*, *Ruminobacter*, *Treponema* and unclassified *Enterobacteriaceae* and *Bacteroidaceae* increased and that of *Acetobacter* decreased significantly in heat-stressed cows (Zhao et al. 2019).

Table 2 Effects of phytogenic supplementation (PS) on feed intake, milk production and composition in heat-stressed dairy cows

Item	Treatments		SEM	P-value
	Control	PS		
Dry matter intake (kg/d)	19.3[b]	20.0[a]	0.167	0.00
Milk yield (kg/d)	34.4	33.9	0.420	0.45
Components (%)				
Fat	3.77	3.69	0.081	0.48
Crude protein	2.73	2.76	0.027	0.42
Somatic cell count (SCC) log ^{10}/mL	4.83	4.81	0.048	0.78

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Anaeroplasma spp. that degrade cell walls of gram-negative bacteria, produce extracellular protein-degrading enzymes and have a role in protein turnover are also shown to increase in the rumen of heat-stressed cows (Joblin and Naylor, 2002), whereas *Ruminococcus spp.* that have a major role in acetate production and cellulose degradation are suppressed (Kone *et al.* 1989). Bagheri *et al.* (2018), reported adding 80 mg/g dry matter of *Scrophularia striata* hydroalchoholic extract to rumen supported rumen microbiome diversity and increased *Fibrobacter bacteria* in comparison to monensin. Although microbial communities were not determined in the current study, changes of microbial communities and fermentation parameters by plant derived compounds in HS could be considered as a possible explanation. Therefore, maintaining a greater DM intake in cows fed with PS in this trial is presumed to result from modulating the ruminal microbiota of these cows under the heat stress conditions.

The most important result of the present trial was a 700 g increase in feed intake in PS cows. This might have occurred through increased number of cows on feed bunk (Figure 1), which might have been arisen from increased meal frequency, size or both and/or in response to result of stimulatory effects of EO on the hunger centers in the brain, which in turn arises from odorous entity of these compounds (Franz *et al.* 2010).

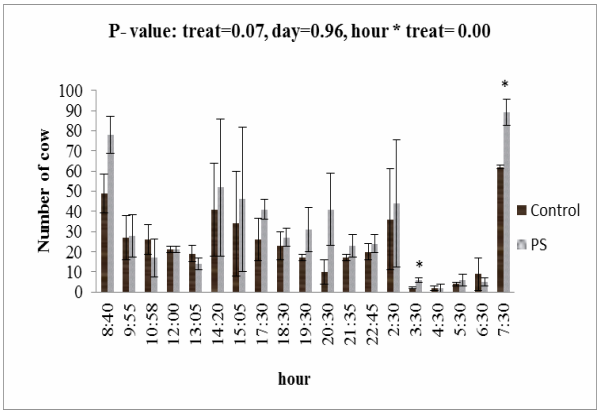

Figure 1 Effects of phytogenic supplementation (PS) on number of cow on feed bunk

Further studies are needed to better understand the mechanism of action of phytogenic compounds on feed intake. Increased DMI in heat-experienced cows with feeding EO has been reported previously (Reza-Yazdi *et al.* 2014). In contrast to the current study, some research reported DMI was not affected by citrus extract (Havlin and Robinson, 2015).

Dry matter (P=0.21) and crude protein digestibility (P=0.17) did not change significantly by feeding PS (Table 3) which showed that PS did not affect negatively the nutrient digestibility. This agrees with previously reported data showing no change in nutrient digestibility in heat-stressed dairy cows fed with *Radix bupleuri* extract (Pan *et al.* 2014) and citrus extract (Havlin and Robinson, 2015). Likewise, lack of effect of cinnamaldehyde, eugenol and capsicum on total tract digestibility of nutrients in dairy cows has been reported (Tager and Krause, 2011).

The respiratory rate tended to be lower in PS treatment compared to control (Table 3, P=0.09). In contrast, rectal temperature was 0.2 ˚C lower in control (P=0.01). Although PS-supplemented cows had a higher rectal temperature, it presumably was not high enough to reduce the DMI and milk yield. It has been reported that decrease in feed intake occurs when rectal temperature rises beyond 1 ˚C (Rejeb *et al.* 2016). On the other hand, slight increase in rectal temperature (0.2 ˚C) of PS-fed cows might have been due to higher feed intake, thus greater production of heat fermentation (Baumgard *et al.* 2011). Reduction of respiratory rate in PS- supplemented treatment might be translated to better welfare of cows improving health of the heat-stressed dairy cows (Pan *et al.* 2014). In contrast, feeding of citrus extract to cow ration in hot weather had no effect on respiratory rate and panting score (Havlin and Robinson, 2015).

Feeding PS improved fecal scores (P=0.09). More consistent fecal scores in PS treatment indicated a lower risk of over-passage of undigested nutrients to the lower gut. Although the number of cows on feed bunk was higher for PS-fed group during warm hours (1420, 1505, 1730 h), these differences were significant only at 0330 and 0730 (P<0.05, Figure 1).

Table 3 Effects of phytogenic supplementation (PS) on apparent digestibility of nutrients, respiratory rate (RR) and rectal temperature (RT) in heat-stressed dairy cows

Item	Treatments		SEM	P-value		
	Control	PS		treat	day	Treat × day
Dry matter digestibility (%)	77.6	80.1	1.22	0.21		
Crude protein digestibility (%)	75.9	78.5	1.10	0.17		
RR (breath/min.)	68.0	63.0	2.44	0.09	0.01	0.67
RT (ºC)	38.5[a]	38.7[b]	0.08	0.01	0.00	0.49
Fecal score	2.8	3.0	0.04	0.09	0.58	0.70

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of phytogenic supplementation (PS) on serum parameters and hormones in heat-stressed dairy cows

Item	Treatments		SEM	P-value
	Control	PS		
Glucose (mg/dL)	49.8	52.8	2.03	0.31
Beta-Hydroxybutyric acid (mmol/L)	0.40	0.42	0.041	0.78
T3 (ng/mL)	1.81	1.83	0.158	0.92
T4 (µg/dL)	5.08[a]	4.22[b]	0.260	0.03
Cortisol (µg/dL)	1.14	1.84	0.306	0.12
Insulin (µIU/mL)	11.42	10.54	2.105	0.77

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Higher number of cows on feed bunk in the PS-fed group at 0730 might be related to delivery of the morning meal at this time. Unfortunately, there is not enough information about the effects of phytogenic compounds on feeding behavior of dairy cows. However, a shortened length of the first meal has been reported by feeding 85 mg cinnamaldehyde and 140 mg eugenol in lactating cows 43 ± 29 DIM (Tager and Krause, 2011).

Blood glucose, BHBA, T3, cortisol and insulin were similar between treatments, though T4 concentration decreased by feeding PS (P=0.03, Table 4). Heat-stressed cows usually experience hypoglycemia and higher glucose disposal rate. This suggests that higher utilizetion of glucose in extra-mammary tissue may have a major role in lower milk production during HS (Baumgard *et al.* 2011).

Further, there is evidence showing that HS directly affects milk production such that it is independent of feed intake (Wheelock *et al.* 2010). In the present experiment, no change in blood glucose concentration could explain the absence an effect on milk production in PS-supplemented cows.

CONCLUSION

This trial revealed that feeding of PS increased feed intake, changed intake behavior and decreased T4 in heat-stressed Holstein cows. Further research on behavioral and physiological changes may help better understand the mode of action of these compounds in cows exposed to HS.

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