



Feeding encapsulated pepper to dairy cows during the hot season improves performance without affecting core and skin temperature

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ABSTRACT

Peppers (*Capsicum* spp.) contain capsaicin, an organic compound with a group of alkaloids that has shown thermoregulation properties in humans and mice, and may influence glucose and lipid metabolism in ruminants. An experiment was conducted to evaluate different doses of a feed additive containing encapsulated pepper on milk yield and composition, dry matter intake, feed sorting index, total-tract apparent digestibility of nutrients, purine derivatives excretion, and serum concentrations of urea-N and glucose, N excretion, respiration rate, rectal temperature, and skin temperature in different regions (forehead, face, and rumen). Thirty-six Holstein cows (150 ± 102.1 d in milk and 29.3 ± 5.81 kg/d milk yield) were used in a 9-wk randomized complete block ($n = 12$) design experiment. Following a 2-wk covariate period, cows were blocked according to parity, days in milk, and milk yield and were randomly assigned to the following treatments: 0 (CAP0), 0.75 (CAP75), or 1.5 (CAP150) g/d of a feed additive containing encapsulated pepper (1 g/kg, Capcin; Nutri-Quest) added to the concentrate along with minerals. Treatment differences were evaluated through orthogonal contrasts (CAP0 vs. CAP75 + CAP150 or CAP75 vs. CAP150). The average temperature-humidity index during the experiment was 72.0 ± 2.07 . Dry matter intake was greater in cows fed a feed additive containing encapsulated pepper (CAP) treatments (CAP75 and CAP150) compared with CAP0. Cows fed CAP150 tended to have greater dry matter intake than those in CAP75 group. Feeding CAP decreased sorting for feed particles with size between 8 and 4 mm. An interaction effect between treatment and week was observed for crude protein digestibility whereas cows fed CAP150 had the greatest digestibility on the third week of ex-

periment. Orthogonal contrasts did not detect differences in serum concentrations of glucose and urea-N, or purine derivatives excretion. Nitrogen excretion (as % of N intake) in milk, urine, and feces was not altered by treatments. Feeding CAP increased yields of 3.5% fat-corrected milk, fat, protein, and lactose. A tendency toward greater milk protein content was observed for cows fed CAP150 than CAP75. No differences were detected on respiration rate, rectal temperature, and skin temperature of cows. A feed additive containing encapsulated pepper fed at 0.75 or 1.5 g/d can improve yield of fat-corrected milk and milk solids by increasing feed intake without affecting nutrient digestibility and body temperature of lactating cows during the hot season.

Key words: capsaicin, essential oil, feed additive, phytonutrient

INTRODUCTION

Peppers (*Capsicum* spp.) are mainly commercialized based on their pungency, which is due to the presence of 6 chemically related compounds known as capsaicinoids (Deepa et al., 2007). Among capsaicinoids present in peppers, the most abundant is capsaicin (trans-8-methyl-N-vanillyl-6-nonamide), accounting for 71% of the total capsaicinoids in most of the pungent varieties (Barbero et al., 2014). Capsaicin has exhibited antimicrobial (Adaszek et al., 2019), anti-inflammatory (Kim et al., 2003), and anti-oxidant properties (Kempiah et al., 2005), which are of particular interest in dairy nutrition, especially when cows are exposed to high temperature-humidity index (THI). Oxidative stress, inflammation response, and endocrine imbalance of lactating dairy cows often occur during the hot summer in comparison with thermoneutral seasons (Li et al., 2021).

There is evidence in literature that capsaicin may influence ruminal microbiota in cows and nutrient digestion in mammals. Authors fed increasing doses (0, 0.25, 0.5, or 1.0 g/d) of an additive with 1.2% capsaicinoids to dairy cows, and reported positive quadratic effect on abundance of *Butyrivibrio*, *Faecalibacterium*,

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Synergistaceae, *Dorea*, and negative quadratic effect on *Prevotella* and *Roseburia* genera (Oh et al., 2015). The phenolic group (vanillyl) present in capsaicin may inhibit bacteria growth by reducing membrane stability because of its hydrophobicity (Burt, 2004). Some phenolic compounds are likely resistant to microbial degradation in the rumen, hence can reach the small intestine in a biologically active form. In dairy cows, capsaicin ruminal escape was reportedly estimated between 15 and 33% depending on the dose provided (Oh et al., 2016). In humans and rats, capsaicin stimulates gastric emptying and decreases blood leptin levels, resulting in a greater food intake (McCann et al., 1988; Debreceni et al., 1999; Hsu and Yen, 2007). Feeding capsaicin to rats has also increased pancreatic secretion of digestive enzymes including lipase, amylase, trypsin, and chymotrypsin (Platel and Srinivasan, 1996, 2000), which may improve degradation and absorption of nutrients in the small intestine.

Capsaicin has also shown thermoregulation properties (Adaszek et al., 2019). Earlier studies have shown that capsaicin may activate heat sensitive transient receptor potential vanilloid (TRPV1) channels, leading to a decline in rectal temperature of rodents under a thermoneutral environment (Jancsó-Gábor et al., 1970; Donnerer and Lembeck, 1983). Aligning with these outcomes, studies conducted with humans revealed that TRPV1 channels contribute to thermal hyperaemia (Wong and Fieger, 2010) and cutaneous vasodilation (Wong and Fieger, 2012). The TRPV1 channels are expressed in a wide range of central and peripheral tissues; specifically, TRPV1 is highly expressed in the brain stem, mid-brain, hypothalamus, and limbic systems (Edwards, 2014). Peripherally, TRPV1 is expressed in the vagal and spinal sensory nerves, stomach, and adipose tissue (Christie et al., 2018).

Most studies that evaluated feed additives containing capsaicinoids had few replications per treatment and tested the short-term effects (i.e., Latin square or change-over designs) on milk yield and nutrient digestibility (Tager and Krause, 2011; Oh et al., 2015, 2017; Foskolos et al., 2020). Thus, it is somewhat expected that those studies did not have enough power to detect differences in milk yield. This study was conducted to evaluate different doses of a commercial feed additive containing encapsulated pepper (rich in capsaicin) on DMI and apparent digestibility of nutrients, purine derivative excretion, N use, milk composition, and physiological parameters (respiration rate, rectal temperature, and skin temperature in different regions) in dairy cows. We hypothesized that encapsulated pepper would improve nutrient digestibility, increasing feed intake and milk yield, and altering physiological parameters of dairy cows in a dose-dependent manner.

MATERIALS AND METHODS

This study was carried out between January and April 2020 in the Laboratório de Pesquisa em Bovinos de Leite (Laboratory on Dairy Cattle Research; Pirassununga, Brazil) under the approval of the Ethics Committee on Animal Use from the School of Veterinary Medicine and Animal Sciences, University of São Paulo (São Paulo, Brazil; protocol #1036240320).

Treatments and Design

Thirty-six Holstein cows (150 ± 93.0 DIM, 29.3 ± 5.81 kg/d milk yield, 660 ± 85.9 kg BW), of which 9 were primiparous and 27 multiparous (13 pregnant cows), were used in a randomized complete block ($n = 12$) design. Cows were blocked according to parity, DIM, and milk yield, and randomly assigned to the following treatments: 0 (CAP0), 0.75 (CAP75), or 1.5 (CAP150) g/d of a feed additive containing encapsulated pepper (Capcin; NutriQuest) added to the concentrate along with minerals. Doses were determined based on outcomes reported by Oh studies (Oh et al., 2013, 2015, 2017). Note that greater doses than 1.5 g/d of capsaicinoids did not show positive effects or even decreased the performance of dairy cows (Oh et al., 2013). Diets were formulated for cows consuming 24.0 kg DM/d; thus, the diet content of CAP was 0.003 and 0.006% for CAP75 and CAP150 treatments, respectively. The feed additive is a brownish powder with a strong odor and contains 5 g/kg of capsaicinoids. According to manufacturer's information, Capcin contains 10 g/kg (minimum) of encapsulated pepper in addition to palm oil and dextrose. Palm oil and dextrose are intentionally mixed with pepper to minimize its pungent taste and possible negative effects in DMI. Dextrose and pepper are homogenized and coated with heated palm oil through fluidized bed granulation method to form the encapsulated pepper.

Cows were allowed a 2-wk period for adaptation to the basal diet and data of DMI, milk yield and composition, blood metabolites, and BW were recorded for covariate purposes. After this adaptation period, cows received treatments during the following 9 wk. Cows were fed twice daily (0700 and 1300 h) a TMR formulated according to NRC (2001) requirements estimates (Table 1). The feed provided was adjusted daily, targeting refusals between 5 and 10%. Corn silage DM content was analyzed 3 times a week for dietary adjustments. Cows were housed in a barn containing individual pens with sanded beds, feed bunks, fans, and free access to water. Two data loggers (TagTemp Stick; Novus) were placed at 2-m height in each side of the barn to measure every hour the air temperature and

air relative humidity throughout the experiment. The THI was calculated according to Mader et al. (2006) as follows: $\text{THI} = (0.8 \times \text{air temperature, } ^\circ\text{C}) + [(\text{relative humidity, } \%) \times (\text{air temperature, } ^\circ\text{C}, - 14.6) + 46.4]$.

Samples of feed ingredients were collected 3 times a week and pooled by week for traditional wet chemistry analyses. Briefly, feed samples were dried in a forced-air oven (55°C for 72 h) and ground in Willey knives mill with 2-mm or 1-mm screen. Samples were analyzed for contents of DM (method 930.15; AOAC International, 2000), ash (method 942.05; AOAC International, 2000), OM (DM – ash), and CP (N \times 6.25; Kjeldahl method 984.13; AOAC International, 2000). Neutral detergent fiber (Van Soest et al., 1991) was analyzed using α -amylase (TE-149 fiber analyzer; Tecnal Equipamentos para Laboratório Inc.), and ADF and lignin (method 973.18) were analyzed according to AOAC International (2000). Neutral detergent insoluble N and ADIN were determined according to Licitra et al. (1996). Starch content in feed samples was analyzed using an enzymatic degradation method (Amyloglicosidase; Novozymes Latin America Ltda.) and glucose absorbances measure on spectrophotometer (SBA-200, Celm) according to Hendrix (1993). Samples of TMR were collected twice a week for particle size distribution (Maulfair and Heinrichs, 2012).

Nutrient Intake, Sorting Index, and Apparent Digestibility

Individual orts were weighed, and samples were collected daily and pooled by week and cow for further chemical analyses and nutrient intake determination. Orts were analyzed for contents of DM, OM, NDF, and CP as previously described. Individual orts samples from 2 consecutive days were collected weekly for particle size distribution and evaluation of sorting index according to Silveira et al. (2007). The sorting index was calculated using the following equations:

$$\text{Expected intake (kg/d)} = \text{intake}[(\text{kg as-fed}) / \text{d}] \times P_{\text{TMR}} (\text{kg} / \text{kg}),$$

$$\text{Observed intake (kg/d)} = [\text{offered (kg/d)} \times P_{\text{TMR}} (\text{kg/kg})] - [\text{refusals (kg/d)} \times P_{\text{refusals}} (\text{kg/kg})], \text{ and}$$

$$\text{Sorting index} = \frac{\text{observed intake (kg/d)}}{\text{expected intake (kg/d)}}.$$

The intake corresponding to each sieve was expressed as the percentage of the total estimated intake, where

Table 1. Ingredients and chemical composition of the experimental diet

Item	Diet
Ingredient, % DM	
Corn silage	48.0
Ground corn	17.4
Soybean meal, 46% CP solvent	11.6
Citrus pulp	8.19
Whole raw soybean	6.47
Dried distillers grains with soluble	5.34
Mineral mixture ¹	1.29
Sodium bicarbonate	0.82
Limestone	0.73
Salt	0.26
Chemical, % DM	
DM, % as-fed	44.2
OM	93.1
NDF	34.1
ADF	20.8
Starch	26.3
CP	16.7
Lignin	3.81
Neutral detergent insoluble protein	1.86
Acid detergent insoluble protein	1.16
Ether extract	3.42
NE _L , ² Mcal/kg DM	1.62
Particle size distribution, % as-fed	
>19 mm	7.98
19–8 mm	41.5
8–4 mm	19.7
<4 mm	30.8

¹Contained, per kilogram, 215 g of Ca, 15 g of Co, 700 mg of Cu, 10 mg of Cr, 20 g of S, 600 mg of F, 40 mg of I, 20 g of Mg, 1,600 mg of Mn, 20 mg of Se, 70 g of Na, 2,500 mg of Zn, 200,000 IU of vitamin A, and 50,000 IU of vitamin D₃.

²Estimated according to NRC (2001).

P_{TMR} is the TMR particle size and P_{refusals} is the particle size distribution of refusals. The sorting index equaling 1 means no sorting, <1 indicates sorting against the particular particle size, and values >1 shows that cows sorted for a specific particle size.

Daily fecal output (kg of DM) was estimated based on indigestible NDF (iNDF) intake and its concentration in feces. Fecal samples were collected directly from the rectum of cows during 3 consecutive days in 9-h intervals (8 samples) on wk 3, 6, and 9 of the experiment. Fecal samples were pooled by week and cow for further chemical analyses (DM, OM, NDF, and CP). Samples of feed, orts, and feces (ground at 2 mm) were placed in non-woven fabric bags (5 \times 5 cm; 20 mg of DM/cm²) and incubated in the rumen of 2 cannulated cows for 288 h (Huhtanen et al., 1994; Casali et al., 2008). After the incubation period, bags were retrieved from the rumen and washed in running tap water until bleached. Next, samples were submitted to neutral detergent treatment for iNDF determination. Digestibility of DM and nutrients were calculated using the following equations:

$$\text{DM digestibility (\%)} = 100 - \left[100 \times \left(\frac{\% \text{ iNDF intake}}{\% \text{ iNDF in feces}} \right) \right]$$

and

$$\text{Nutrient digestibility (\%)} = 100 - \left[100 \times \left(\frac{\% \text{ iNDF intake}}{\% \text{ iNDF in feces}} \right) \times \left(\frac{\% \text{ nutrient in feces}}{\% \text{ nutrient intake}} \right) \right]$$

Serum Metabolites, Excretion of Purine Derivatives, Milk Composition, and Nitrogen Use Efficiency

Blood samples were collected on the third day of wk 3, 6, and 9 of experiment by puncture of tail vessels, 4 h after the morning feeding. Blood samples were also collected on the last day of covariate period. Blood samples were collected in vacuum tubes without anticoagulant and centrifuged after clotting (15 min and $2,000 \times g$ at 22°C). Serum was harvested and analyzed for glucose and urea using commercial colorimetric kits (Bioclin), with absorbances measured on a spectrophotometer (SBA-200; Celm).

Urine samples were collected by stimulation of urinating at the same time points of fecal samples. Aliquots of urine samples (10 mL) were diluted in sulfuric acid solution (40 mL at 0.036 *N*) to avoid purine derivatives (PD) degradation and uric acid precipitation. Samples were analyzed for creatinine concentration using a commercial kinetic kit (K-067; Bioclin) and absorbance measured on a spectrophotometer (SBA-200; Celm). Urine output was estimated considering a daily creatinine excretion of 29 mg/kg of BW (Valadares et al., 1999). Body weight of cows were measured every week during 2 consecutive days before the morning feeding and after milking. Urine samples were also analyzed for allantoin concentration, according to Fujihara et al. (1987), and uric acid using a commercial kit (K-052; Bioclin). Milk samples were collected on the fourth day of every week and deproteinized with trichloroacetic solution (25%) according to Broderick and Clayton (1997). Afterward, samples were analyzed for allantoin according to Fujihara et al. (1987).

Nitrogen intake was calculated dividing the CP intake by 6.25, and N in milk was calculated dividing milk protein yield by 6.38. Nitrogen in feces and urine samples were determined (Kjeldahl method 984.13) according to AOAC International (2000).

Physiological Parameters

Measurements of physiological parameters (respiration rate and rectal temperature) and skin temperature

from different regions (forehead, face, and rumen) were performed for 2 consecutive days on wk 3, 6, and 9 of experiment in the pens twice daily (1000 h and 1700 h). Respiration rate was measured using a stethoscope and observing the flank movements during 1 min. Rectal temperature was measured using a digital thermometer. Surface temperature was measured using an infrared thermometer (0.95 emissivity and 0.1°C resolution) according to manufacturer's recommendation (Ray MXP4PU; Raytek Raynger).

Statistical Analysis

Data were submitted to ANOVA as repeated measures using SAS 9.4 (SAS Institute Inc.) according to the following model:

$$Y_{ijklm} = \mu + A_i + P_j + B(a)_{kl} + \omega_{ijkl} + T_m + T_m \times A_i + \varepsilon_{ijklm},$$

where $\omega_{ijkl} \approx N(0; \sigma_c^2)$ and $\varepsilon_{ijklm} \approx N(0; \sigma_e^2)$; Y_{ijklm} = observation on animal l , given treatment i , at period j , in block k ; μ = overall mean, A_i = fixed effect of the i th treatment ($i = 1$ to 3); P_j = fixed effect of the j th period ($j = 1$ to 9); $B(a)_{kl}$ = random effect of the l th animal ($l = 1$ to 36) within the k th block ($k = 1$ to 12); T_m = fixed effect of the m th week ($m = 1$ to 9); ε_{ijklm} = random error associated with each observation; N = Gaussian distribution, σ_c^2 = estimated variance associated with cows; and σ_e^2 = estimated residual variance. Covariate data were included in the model as a fixed effect. Variance-covariance matrixes were tested [CS, CSH, AR(1), ARH(1), TOEP, TOEPH, and UN)] and the best fit was chosen by Bayesian method. Orthogonal contrasts were used to evaluate treatment differences (CAP0 vs. CAP75 and CAP150; or CAP at 0.75 g/d vs. 1.5 g/d). Significance was considered when $P \leq 0.05$ and tendency when $0.05 < P < 0.10$.

RESULTS

During the experiment the average air temperature was $19.2 \pm 5.51^\circ\text{C}$, the average air relative humidity was $69.5 \pm 13.4\%$, and the THI was 64.5 ± 7.08 (Supplemental Figure S1; <https://doi.org/10.6084/m9.figshare.20323515.v1>; Takiya, 2022). Intake of DM (either as kg/d or % BW) and OM were greater ($P \leq 0.049$) for cows fed CAP treatments (CAP75 and CAP150) compared with CAP0 (Table 2). Cows fed CAP150 tended to have greater ($P = 0.058$) DMI than those under CAP75 treatment. Feeding CAP resulted in lower sorting ($P = 0.002$) for feed particles with size between 8 and 4 mm. Greater sorting ($P = 0.016$) for feed particles

Table 2. Nutrient intake, sorting index, and total-tract apparent digestibility in dairy cows fed different doses of a feed additive containing encapsulated pepper during the hot season (n = 12 per treatment)

Item	Treatment ¹				P-value ²			
	CAP0	CAP75	CAP150	SEM	C1	C2	Time	Treatment × time
Intake, kg/d								
DM	24.9	25.3	26.2	0.48	0.049	0.058	<0.001	0.970
OM	23.0	23.6	24.4	0.47	0.028	0.105	<0.001	0.978
NDF	8.48	8.66	8.95	0.171	0.042	0.113	<0.001	0.903
CP	4.26	4.34	4.52	0.095	0.113	0.163	<0.001	0.999
DM, % BW	3.72	3.81	3.94	0.074	0.030	0.116	<0.001	0.997
Sorting index ³								
>19 mm	0.910	0.957	0.944	0.022	0.159	0.673	0.002	0.115
19–8.0 mm	0.986	0.990	0.986	0.003	0.622	0.328	0.179	0.915
8.0–4.0 mm	1.001	0.982	0.993	0.003	0.002	0.016	<0.0001	<0.0001
<4.0 mm	1.036	1.032	1.035	0.005	0.691	0.636	0.097	0.524
Apparent digestibility, %								
DM	66.6	67.0	67.8	0.86	0.440	0.530	<0.001	0.078
OM	68.2	68.4	69.2	0.94	0.623	0.515	0.005	0.083
CP	68.8	69.4	71.5	0.91	0.172	0.109	<0.001	0.029
NDF	50.1	49.0	50.1	1.07	0.679	0.425	0.035	0.083

¹Treatments included 0 (CAP0), 0.75 (CAP75), or 1.5 (CAP150) g/d of a feed additive containing encapsulated pepper (Capcin; NutriQuest) added to the concentrate along with minerals.

²Orthogonal contrasts: CAP0 versus CAP75 + CAP150 (C1); or CAP at 0.75 g/d versus 1.5 g/d (C2).

³No sorting = 1, values <1 indicates sorting against, and values >1 indicates sorting for particles on the particular particle size range; sorting index was calculated according to Silveira et al. (2007).

with size between 8 and 4 mm was observed in cows fed CAP150 compared with those fed CAP75. Although orthogonal contrasts did not detect differences in total-tract apparent digestibility of nutrients, a tendency for an interaction effect between treatment and time ($P \leq 0.083$) was observed for digestibility of DM and NDF (Figure 1). An interaction effect between treatment and time ($P = 0.029$) was observed for CP digestibility, whereas cows fed CAP150 had greater digestibility on the third week of experiment when compared with the other treatments.

Orthogonal contrasts did not detect ($P \geq 0.331$) differences in serum concentrations of glucose and urea-N (Table 3). An interaction effect between treatment and time ($P = 0.024$) was observed for serum urea-N concentration (Figure 2). Treatments did not affect ($P \geq 0.330$) PD excretion. Neither milk N, fecal N, nor urine N were affected by CAP. Milk yield tended to be greater in cows fed treatments with CAP than those in control (CON or CAP0; Table 4). Feeding treatments with CAP resulted in greater ($P \leq 0.037$) yields of FCM, fat, protein, and lactose compared with CON. A trend toward greater ($P = 0.086$) lactose content was observed in cows fed CAP. No treatment differences were detected on feed efficiency. Cows fed CAP150 tended to have greater ($P = 0.094$) milk protein content than those fed CAP75. Feeding CAP treatments did not affect respiration rate, or rectal and skin temperature of

cows (Table 5). No treatment differences were detected on BW and BW change.

DISCUSSION

We hypothesized that different doses of CAP would improve nutrient degradation/absorption and performance of cows, in addition to influencing body and surface temperature in a dose-dependent manner. Indeed, cows fed CAP had greater DMI and FCM yield than cows in CON; however, no differences were detected on nutrient digestibility or body surface temperature. Because CAP has shown thermoregulation and antioxidant properties, we decided to supplement CAP during the hot season. The average THI observed in this study was 72, whereas the maximum THI observed was 76.1, and during 35 d of experiment the average daily THI was >72. The critical THI threshold for cows ranges from 68 to 72 (Herbut et al., 2018), and are based on rectal temperature and respiration rate (Pinto et al., 2020). During the warm summer months, milk yield can decrease between 10 to 35% across the United States (St-Pierre et al., 2003). It is well known that exposing cows to a high THI environment reduces DMI and milk yield of dairy cows; however, the drop in DMI accounts for only about 50% the decrease in milk synthesis in terms of nutrient supply (Wheelock et al., 2010). Thus, an environmental thermal load can affect

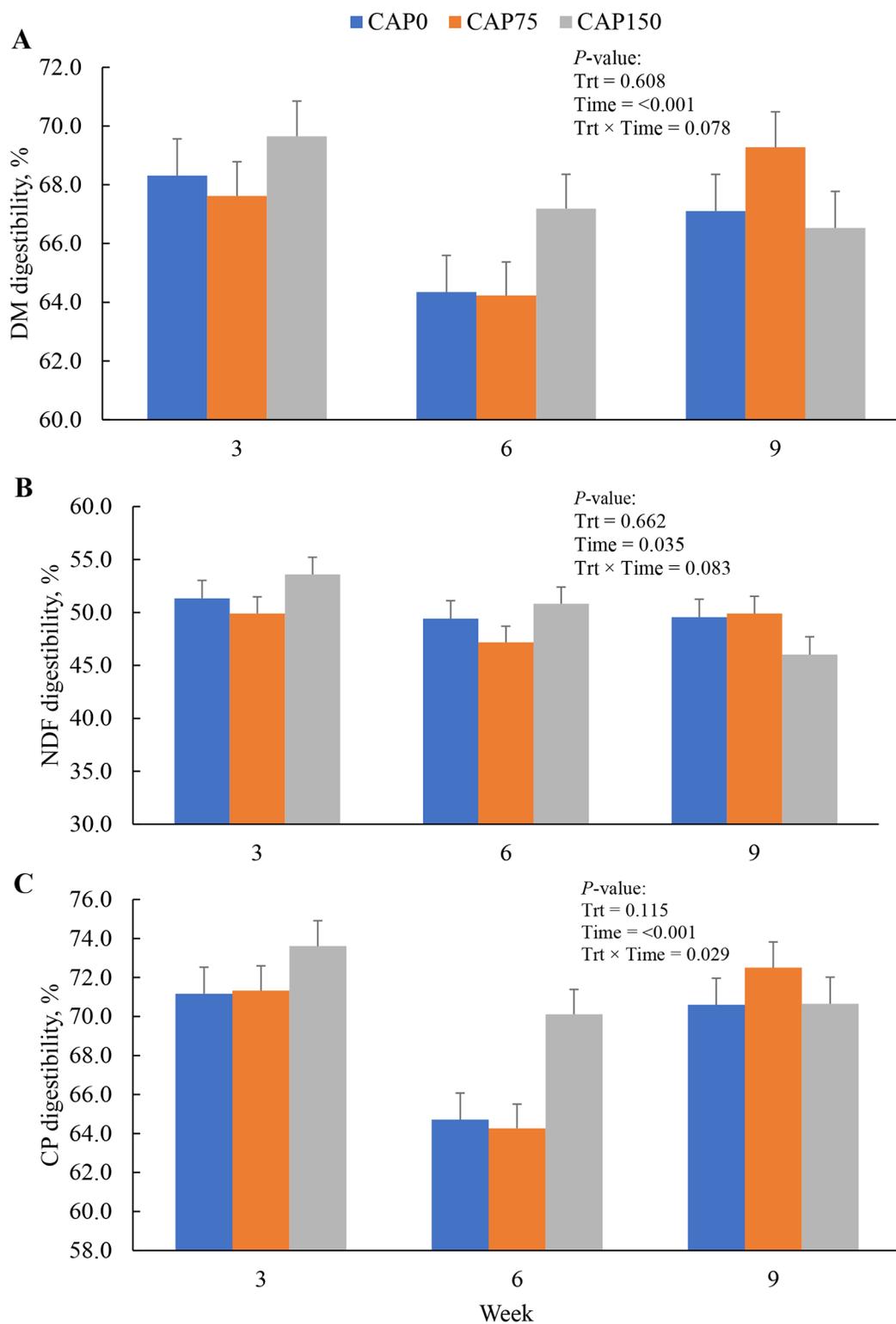


Figure 1. Total-tract apparent digestibility of DM (A), NDF (B), and CP (C) in dairy cows fed different doses of a feed additive containing encapsulated pepper during the hot season. Treatments included 0 (CAP0), 0.75 (CAP75), or 1.5 (CAP150) g/d of capsaicin (Capcin; NutriQuest), added to the concentrate along with minerals. Error bars are SE. Trt = treatment.

Table 3. Serum metabolites, purine derivatives excretion, and efficiency of N utilization in dairy cows fed different doses of a feed additive containing encapsulated pepper during the hot season (n = 12 per treatment)

Item	Treatment ¹				P-value ²			
	CAP0	CAP75	CAP150	SEM	C1	C2	Time	Treatment × time
Serum metabolite, mg/dL								
Glucose	53.0	55.9	56.5	1.85	0.331	0.824	0.090	0.108
Urea-N	14.9	15.5	15.1	0.64	0.469	0.973	0.052	0.024
Purine derivatives, mmol/d								
Urine allantoin	287	309	292	23.1	0.608	0.561	0.129	0.635
Urine uric acid	188	219	184	19.2	0.526	0.139	<0.001	0.191
Milk allantoin	16.3	17.1	13.7	2.44	0.764	0.330	<0.001	0.691
Total	481	548	492	39.1	0.398	0.271	0.005	0.307
Nitrogen use efficiency, g of N/g of N intake								
Milk N	0.230	0.240	0.240	0.01	0.453	0.978	<0.001	0.282
Fecal N	0.352	0.343	0.321	0.01	0.156	0.159	<0.001	0.020
Urine N	0.249	0.284	0.249	0.02	0.558	0.284	0.024	0.457

¹Treatments included 0 (CAP0), 0.75 (CAP75), or 1.5 (CAP150) g/d of a feed additive containing encapsulated pepper (Capcin; NutriQuest) added to the concentrate along with minerals.

²Orthogonal contrasts: CAP0 versus CAP75 + CAP150 (C1); or CAP at 0.75 g/d versus 1.5 g/d (C2).

milk yield by mechanisms not dependent on reduced intake of nutrients.

In the current study, increased intake of cows fed CAP might be related to differences in DM and CP digestibility throughout the experiment. Cows fed CAP150 had greater DM and CP digestibility, especially in wk 3 and 6 of experiment, in comparison with other treatments. The reasons for greater digestibility when feeding CAP are unclear but can be associated with CAP effects on ruminal microbial population (Oh

et al., 2015), pancreatic digestive enzymes secretion (Platel and Srinivasan, 1996, 2000), gastric emptying (Debrececi et al., 1999), or blood leptin levels (Hsu and Yen, 2007). Contrasting with the current study, feeding different doses of CAP (0, 0.25, 0.5, or 1.0 g/d; containing 1.2% capsaicinoids) had no effect on DMI and digestibility of nutrients in dairy cows (Oh et al., 2015). However, when cows were fed different doses of a rumen-protected CAP product (0, 0.1, or 0.2 g/d; containing 0.93% capsaicinoids), DM digestibility linearly

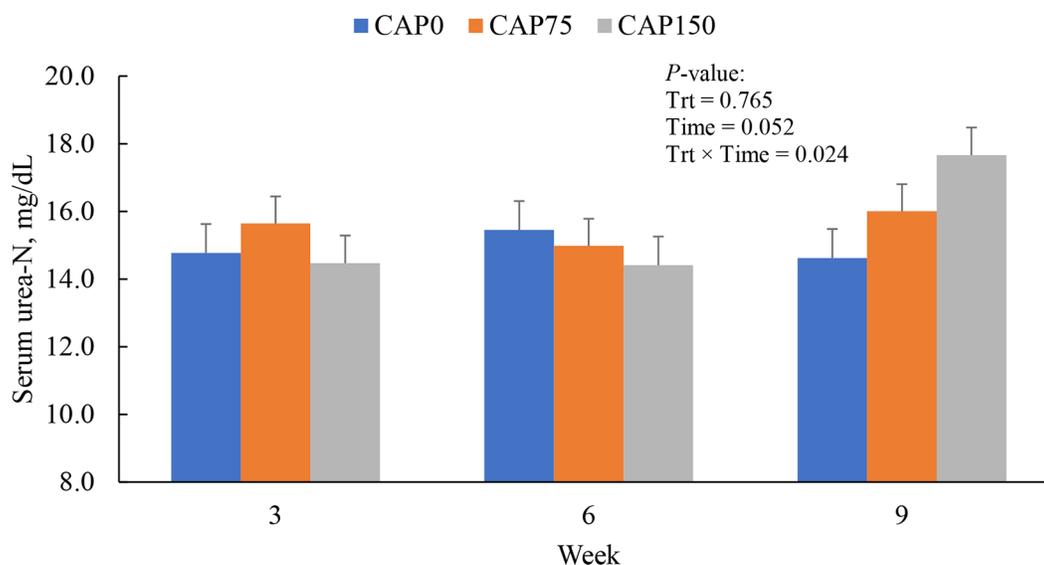


Figure 2. Serum urea-N concentration in dairy cows fed different doses of capsaicin during the hot season. Treatments included 0 (CAP0), 0.75 (CAP75), or 1.5 (CAP150) g/d of a feed additive containing encapsulated pepper (Capcin; NutriQuest), added to the concentrate along with minerals. Error bars are SE. Trt = treatment.

Table 4. Milk yield and composition, and feed efficiency in dairy cows fed different doses of a feed additive containing encapsulated pepper during the hot season (n = 12 per treatment)

Item	Treatment ¹				P-value ²			
	CAP0	CAP75	CAP150	SEM	C1	C2	Time	Treatment × time
Yield, kg/d								
Milk	30.2	31.2	31.6	0.699	0.081	0.553	<0.001	0.992
3.5% FCM	33.9	35.9	36.5	0.832	0.005	0.724	<0.001	0.697
Fat	1.28	1.37	1.40	0.036	0.002	0.502	<0.001	0.392
Protein	1.02	1.05	1.08	0.027	0.037	0.361	<0.001	0.937
Lactose	1.52	1.59	1.62	0.040	0.022	0.474	<0.001	0.728
Composition, %								
Fat	4.32	4.40	4.43	0.053	0.148	0.696	<0.001	0.126
Protein	3.39	3.38	3.42	0.013	0.435	0.094	<0.001	0.711
Lactose	5.06	5.09	5.12	0.019	0.086	0.257	<0.001	0.706
Feed efficiency								
Milk yield ÷ DMI	1.22	1.25	1.21	0.020	0.688	0.221	<0.001	0.944
FCM ÷ DMI	1.37	1.43	1.39	0.022	0.110	0.209	<0.001	0.791
BW, kg	675	670	676	3.01	0.522	0.148	<0.001	0.699
BW change, kg/wk	2.57	1.65	2.82	0.536	0.627	0.122	0.266	0.277

¹Treatments included 0 (CAP0), 0.75 (CAP75), or 1.5 (CAP150) g/d of a feed additive containing encapsulated pepper (Capcin; NutriQuest) added to the concentrate along with minerals.

²Orthogonal contrasts: CAP0 versus CAP75 + CAP150 (C1); or CAP at 0.75 g/d versus 1.5 g/d (C2).

increased (Oh et al., 2017). Other authors reported a tendency for increased DMI when feeding 250 mg/d of CAP to dairy cows (Foskolos et al., 2020).

One can ask whether CAP might have had an effect in ruminal fermentation, but this study did not detect differences in concentrations of glucose and urea-N in serum, PD excretion, or milk fat percentage, which are parameters that indicate changes in ruminal fermentation. Despite its antimicrobial property, CAP has not altered ruminal fermentation, PD excretion (marker of microbial protein synthesis), MUN, and milk fat percentage in dairy cows (Tager and Krause, 2011; Oh et al., 2015, 2017, 2018; Foskolos et al., 2020). In contrast with observed in earlier studies (Oh et al., 2013, 2015), CAP increased FCM yield and tended to improve milk yield, regardless of the dose tested. In a completely

randomized experiment conducted during the summer season, authors supplemented low producing cows with increasing doses of capsaicin (0, 20, 40, or 60 mg/d) during early lactation (30 DIM until 60 DIM) and started to observe positive effects of capsaicin supplementation (40 mg/d) in milk yield (+ 3 kg/d) after 20 d of treatment supply, whereas the higher milk yield was maintained even after the treatment supply was ended (Abulaiti et al., 2021). In the current study, the average increase in DMI (+0.85 kg/d) of cows fed CAP would theoretically supply extra 1.38 Mcal/d. Considering that the NE_L required to produce 1 kg of milk (4% fat) is 0.74 Mcal, the extra DMI could increase milk yield by 1.86 kg/d. In the current study, feeding CAP numerically increased milk yield by 1.2 kg/d. Oh et al. (2017) also reported a trend toward greater milk yield

Table 5. Physiological parameters and skin temperature in dairy cows fed different doses of a feed additive containing encapsulated pepper during the hot season (n = 12 per treatment)

Item	Treatment ¹				P-value ²			
	CAP0	CAP75	CAP150	SEM	C1	C2	Time	Treatment × time
Respiration rate, bpm	52.5	54.5	53.8	1.83	0.486	0.793	<0.001	0.709
Rectal temperature, °C	38.5	38.5	38.5	0.07	0.719	0.856	<0.001	0.466
Skin temperature, °C								
Forehead region	29.4	29.3	29.4	0.29	0.963	0.915	<0.001	0.417
Face region	31.9	32.0	32.1	0.27	0.728	0.656	<0.001	0.337
Rumen region	32.0	32.1	32.3	0.19	0.220	0.459	<0.001	0.639

¹Treatments included 0 (CAP0), 0.75 (CAP75), or 1.5 (CAP150) g/d of a feed additive containing encapsulated pepper (Capcin; NutriQuest) added to the concentrate along with minerals.

²Orthogonal contrasts: CAP0 versus CAP75 + CAP150 (C1); or CAP at 0.75 g/d versus 1.5 g/d (C2).

(+1.5 kg/d) when feeding CAP to dairy cows. Other properties of capsaicin that were not assessed in the current study may also be involved with improvements in milk yield. For instance, feeding CAP to lactating sheep increased serum superoxide dismutase activity, a biomarker of antioxidant status (Cunha et al., 2020). It is well established that oxidative stress is induced after cows are exposed to high THI environments, impairing milk production (Guo et al., 2021).

Under high THI environments, blood circulation is increased in body surface for heat dissipation leading to a decrease in blood supply to the gut and mammary gland, which may compromise nutrient absorption and milk synthesis. To the best of our knowledge, this study was the first to evaluate the effects of feeding CAP on rectal temperature and skin temperature of dairy cows. Capsaicinoids have demonstrated varied effects on thermoregulation in mammals. For instance, capsaicin can activate warm-sensitive receptors and visceral organs (Szolcsányi, 2015). Systemic application of capsaicin elicits activation of afferent pathways for heat-loss responses and nociception (Hori, 1984; Caterina, 2007). Heat-losses responses (activation of heat effectors and inhibition of heat gain mechanisms) to capsaicin treatment have been described in rats, rabbits, goats, guinea pig, and dogs (Szolcsányi, 2015). Because of the positive effects of capsaicin on improving heat-losses, we measured core body temperature, skin temperature, and respiration rate of cows exposed to a relatively high THI. However, this study did not detect CAP effects on physiological parameters evaluated. The lack of CAP responses might be related to the fact that cows were not experiencing heat stress. Authors have reported that rectal temperature of cows experiencing heat stress increased to values between 38.6 and 40.4°C (Wheelock et al., 2010). In the current study cows had an average rectal temperature of 38.5°C.

CONCLUSIONS

Feeding encapsulated pepper increased feed intake and FCM yield without altering feed efficiency, nutrient digestibility, PD excretion, N use efficiency, and physiological parameters of cows exposed to relatively high THI. Different doses of feed additive containing encapsulated pepper (0.75 or 1.50 g/d) had minor effects on parameters evaluated in this study, including a tendency for greater feed intake and milk protein content for cows fed 1.5 g/d than those fed 0.75 g/d. Thus, under the conditions of this experiment, feeding encapsulated pepper improved performance of dairy cows.

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