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Effects of *Capsicum oleoresin* Inclusion on Rumen Fermentation and Lactation Performance in Buffaloes (*Bubalus bubalis*) during Summer: In Vitro and In Vivo Studies

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Abstract: This research aimed to evaluate the effects of *Capsicum oleoresin* (CAP) supplementation on rumen fermentation in vivo and In vitro, and lactation performance in buffaloes. In the experiment in vitro, 2 × 5 factorial design was carried out according to two temperatures (normal temperature: 39 °C; hyperthermal temperature: 42 °C) and five CAP concentrations (0 mg/L; 2 mg/L; 20 mg/L; 200 mg/L; 2000 mg/L). In the experiment in vivo, four multiparous mid-lactating Mediterranean buffaloes (body weight: 640.08 ± 17.90 kg) were randomly allocated to four treatments according to 4 × 4 Latin square design for CAP supplementation in four dosages (0 mg/kg, 10 mg/kg, 20 mg/kg, or 40 mg/kg of dry matter). The experiment's results In vitro showed that hyperthermal temperature affected all fermentation characteristics measured in this research. CAP decreased the pH, short-chain fatty acids concentration, and percentages of propionate, butyrate, isobutyrate, valerate, and caproate, while increasing the percentage of acetate and the ratio of acetate to propionate at normal temperature ($p \leq 0.05$). In the experiment in vivo, CAP decreased the percentage of propionate and quadratically affected acetate percentage in rumen fluid ($p \leq 0.05$). CAP reduced rectal temperature and respiratory rates ($p \leq 0.05$) and tended to increase dry matter intake quadratically ($p \leq 0.10$). For lactation performance, CAP increased milk yield and milk lactose yield ($p \leq 0.05$), and tended to increase milk protein yield ($p \leq 0.10$). In conclusion, CAP modified rumen fermentation characteristics in vivo and In vitro and had beneficial effects on lactation performance in buffaloes during summer.

Keywords: Buffaloes; *Capsicum oleoresin*; milk performance; rumen fermentation; Summer

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1. Introduction

As an essential domestic livestock, buffaloes are the second-largest dairy species in the world, consequent to its outstanding milk quality compared with dairy cows [1]. However, the warm ambient environment restricts productivity due to the geographical distribution of buffaloes (tropical and subtropical regions) and their inferior heat dissipation [2]. With the increased ambient temperature and the improvement of milk performance, the negative effect on buffaloes' production performance will be more severe [3]. As ruminants, the rumen is a vital digestive organ of buffaloes responsible for primary nutrient digestion and absorption. Regulating rumen fermentation could be considered an effective nutritional strategy to relieve heat stress [4].

Capsicum oleoresin (CAP) is the extract of pepper fruits, and its active ingredient is capsaicin. As a dietary polyphenol, capsaicin regulates metabolism [5] and is used as rumen

fermentation amendment in domestic animals [6]. It has been shown that CAP altered rumen fermentation under acidosis conditions [7]. In terms of performance, CAP improved the growth performance of beef [8,9], ewes [9], and the milk production performance of dairy cows [10]. Significantly, CAP might modify core body temperature, which cannot be neglected. Szolcsányi [11] verified that capsaicin could induce complex heat-loss response characteristics in warm ambient temperature. Thus, it suggested that CAP has a potentially beneficial effect on buffaloes against warm ambient environments. Research has studied the beneficial effects of CAP on dairy cows and buffalo calves [12,13]; however, research on the warm ambient environment of lactation buffaloes is still limited.

Therefore, this study aimed to investigate rumen fermentation characteristics under hyperthermal temperature In vitro and in vivo caused by CAP, as well as to explore the changes in lactation performance in lactation buffaloes. We hypothesized that CAP could alter rumen fermentation In vitro and in vivo, which might have salutary effects on milk performance.

2. Materials and Methods

This study consists of two parts, including In vitro and in vivo experiments. Both experiments were completed at Buffalo Farm of Jinniu Animal Husbandry Co. Ltd., Wuhan, China.

2.1. In Vitro Experiment

2.1.1. Experimental Design

To explore the dose–response and concentration threshold of CAP on the fermentation of buffalo rumen under normal and hyperthermal temperature In vitro, this experiment evaluated two temperatures (normal temperature: 39 °C; hyperthermal temperature: 42 °C) and five concentrations (0 mg/L; 2 mg/L; 20 mg/L; 200 mg/L; 2000 mg/L) for a 2 × 5 factorial design. The hyperthermal temperature at 42 °C is the rumen highest temperature under heat stress reported by Eihvalde et al. [14]. Before morning feeding, rumen fluid was collected from two Mediterranean lactating buffaloes through oral collectors and filtered through four layers of medical gauze. Two samples of rumen fluid were infused in prewarmed bottles to remove headspace and transported immediately to the lab on the farm. Two equal volumes of rumen fluid collected from each buffalo were pooled for inoculum preparation. The buffer was configured according to the report of Menke [15] and filled with CO_2 to maintain anaerobic conditions at 39 °C. The buffer was mixed 2:1 with rumen fluid, then, 50 mL of incubation solution was added to a 120 mL fermentation bottle, flushed with CO_2, and sealed with a stopper and aluminum cap. Each fermentable bottle contained 0.5 g of the same diet fed to the donor buffaloes. The experimental substrate consisted of corn silage, straw, and concentration mixture. The nutrient composition (percentage of dry matter, DM, 88.26% organic matter, 10.32% crude protein, 12.90% ether extract, 56.72% neutral detergent fiber, and 35.36% acid detergent fiber) met the nutritional requirement of lactating buffaloes based on the previous report [16]. According to the experimental design, CAP was configured into solutions with different concentrations before being added to the fermentation bottle. Bottle incubation solutions were divided into one control group (CON) and four CAP treatment groups in incubators at 39 °C or 42 °C for 24 h, respectively. The fermentation bottle was oscillated with 2 h interval. CAP product was purchased from Tianxu Food Additives Co., LTD. (MY1098, 10.0% capsaicin, Guangzhou, China). The treatment was made in sextuplicate.

2.1.2. Sampling and Analyses

The incubation solution was measured at the end of incubation period using pH meter (FE-20-FiveEasy PlusTM, Mettler Toledo, Shanghai, China), and collected for short-chain fatty acids (SCFA) and ammonia determination. For measuring SCFA, 200 µL of 25% metaphosphoric acid were added to 1 mL incubation solution, then centrifuged at 12,000× *g* for 15 min to separate the supernatant. Gas chromatograph (GC, 7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with capillary column (30 m × 0.25 mm

× 0.25 µm, DB–FFAP) and pyrophoric detector was used to analyze the supernatant. Ammonia was determined by the colorimetric method [17].

2.2. In Vivo Experiment

2.2.1. Animals and Treatments

The experiment was conducted from July to September 2021. Four multiparous mid-lactating Mediterranean buffaloes (body weight: 640.08 ± 17.90 kg) were used in 4 × 4 Latin squares and randomly assigned to one of four treatments: CAP of 0 mg/kg (CON), 10 mg/kg (10CAP), 20 mg/kg (20CAP), or 40 mg/kg (40CAP) of dry matter. The CAP was added in TMR by dissolving it in water. Each experimental period was 21d, including a 14d diet adaptation and 7d sampling period. Buffaloes were fed individually with 10% refusals and milked twice daily (5 a.m. and 5 p.m.). The diet consisted of corn silage, straw, and concentration mixture. The chemical composition is shown in Table 1. Buffaloes were housed in barns where fans were turned on when temperatures rose above 28 °C, and water was provided *ad libitum*.

Table 1. Chemical composition of the experimental diets in vivo.

Chemical Composition	Content (%)
% of dry matter	
Organic matter	88.74
Crude protein	13.26
Neutral detergent fibre	57.81
Acid detergent fibre	35.15

2.2.2. Sampling, Measurements, and Analyses

Every day, temperature and humidity in the barn were automatically monitored throughout (COS-04-X, Shandong Renke Measurement and Control Technology Co. LTD., Jinan, China). The temperature and humidity index (THI) was calculated based on the previous report [18]:

$$\text{THI} = (1.8 \times \text{temperature } ^\circ\text{C} + 32) - [(0.55 - 0.0055 \times \text{relative humidity } \%) \times (1.8 \times \text{temperature } ^\circ\text{C} - 26)].$$

In the sampling period, dry matter intake (DMI) was recorded on the first four days to avoid interference with subsequent sampling. Milk yield was recorded, and samples of each buffalo were collected from both milking sessions and aggregated according to the morning and night ratio volume on the fifth day. Milk samples were detected by the milk composition detector (CombiFoss FT+, Shanghai Jinmai Instrument Equipment Co., LTD, Shanghai, China) in Dairy Herd Improvement of Hubei province. Rectal temperature (RT) was measured at 2 p.m. on the sixth day using an electronic thermometer (SAT-1, Shangnong Electronic Technology Co., LTD., Linyi, China). The respiratory rates (RR) were calculated by observing the time of 10 flank movements and converted to breaths per minute [19] before RT measurement. Using an oral rumen tube to collect rumen fluid samples (50 mL) at 4h of the seventh day after the morning feeding and discarding the first 200 mL rumen fluid to avoid saliva interference during collection. The pH of the ruminal fluid was measured after draining through four layers of medical gauze using pH meter and stored at −20 °C until analyses. SCFA and ammonia concentration were measured in the same method as described for the In vitro experiment. Blood samples were collected via the jugular vein before rumen collection, and serum was obtained after centrifuged at 3000× *g* and stored at −20 °C for subsequent analyses. Serum non-esterified fatty acids (NEFA, catalog number A042-1) and glucose (catalog number A154-1) concentration were measured using commercial assay kits (Nanjing Jian Cheng Bioengineering Institute, Nanjing, China). Insulin (RX1600758B, detection range 1–32 mIU/L) and cortisol (RXJ1600793B, detection range 10–160 pg/mL) were measured using commercial ELISA kits (Quanzhou Ruixin

Biological Technology Co.,LTD., Quanzhou, China) under the instruction manual. The intra-assay and inter-assay coefficient of variation were less than 15%, and R^2 of the standard curve were greater than 0.99 for ELISA kits.

2.3. Statistical Analyses

All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). For In vitro data, the PROC MIXED procedure was used for analyses according to randomized compartmentalized design. The main effects include CAP levels, temperature, and their interactions. Using the PROC MIXED procedure to analyze in vivo data with a model that included treatment, period, fixed effects of treatment and period, as well as random effects of buffaloes. Linear and quadratic comparisons were used to examine the effect of including CAP on the response variables. The results of the two experiments are reported as least squares means and standard errors of means (SEM). The treatment effect was significant at $p \leq 0.05$, whereas trends were considered when $0.05 < p \leq 0.10$ by the Duncan test.

3. Results

3.1. In Vitro Experiment

The results of fermentation parameters In vitro are shown in Table 2. Hyperthermal temperature affected all fermentation parameters, specifically, decreased pH, short-chain fatty acids (SCFA) concentration, and percentage of propionate, butyrate, isobutyrate, and caproate of incubation fluid ($p \leq 0.05$). However, ammonia concentration, percentage of acetate and valerate, and the ratio of acetate to propionate were increased ($p \leq 0.05$). In addition to valerate percentage, CAP affected all the fermentation indicators ($p \leq 0.05$). Under normal temperature, CAP decreased the pH, SCFA concentration, and percentages of propionate, butyrate, isobutyrate, valerate, and caproate while increasing the percentage of acetate and the ratio of acetate to propionate ($p \leq 0.05$). Under hyperthermal temperature, CAP decreased ammonia concentration, the percentage of acetate, isobutyrate, and valerate, and the ratio of acetate to propionate, while CAP increased butyrate, propionate, and caproate percentage compared with the control group ($p \leq 0.05$).

Table 2. Fermentation parameters affected by temperature and *Capsicum oleoresin* (CAP) supplementation in vitro.

Item	Temperature (TEM)	*Capsicum oleoresin* (CAP)					SEM	*p*-Value		
		CON	2 mg/L	20 mg/L	200 mg/L	2000 mg/L		TEM	CAP	TEM × CAP
pH	39 °C	5.86 ^{xa}	5.72 ^{b}	5.73 ^{b}	5.70 ^{b}	5.74 ^{b}	0.02	<0.01	<0.01	0.01
	42 °C	5.71 ^{y}	5.67	5.68	5.72	5.68				
Ammonia (mmol/L)	39 °C	3.83 ^{y}	3.66	2.60	3.07	3.99	0.39	<0.01	<0.01	0.56
	42 °C	6.15 ^{xa}	5.16 ^{ab}	3.94 ^{b}	4.52 ^{ab}	5.00^{ab}				
SCFA (mmol/L)	39 °C	104.70 ^{xa}	100.68 ^{xa}	97.11 ^{ab}	86.42 ^{ab}	72.63 ^{b}	5.72	<0.01	<0.01	0.58
	42 °C	75.06 ^{y}	69.98 ^{y}	74.31	69.83	56.37				
SCFA proportion (%)										
Acetate	39 °C	59.84 ^{yc}	62.36 ^{yb}	63.40 ^{yb}	66.23 ^{ya}	68.03 ^{a}	0.52	<0.01	<0.01	<0.01
	42 °C	68.84 ^{xa}	67.74 ^{xa}	69.63 ^{xa}	69.09 ^{xa}	66.03 ^{b}				
Propionate	39 °C	26.53 ^{xa}	25.38 ^{xab}	24.71 ^{xb}	23.26 ^{xc}	22.01 ^{c}	0.27	<0.01	0.02	<0.01
	42 °C	20.02 ^{yb}	19.99 ^{yb}	20.63 ^{yb}	21.82 ^{yab}	22.61 ^{a}				
Butyrate	39 °C	11.61 ^{xa}	10.37 ^{ab}	10.15 ^{xb}	9.15 ^{bc}	8.72 ^{c}	0.26	<0.01	<0.01	<0.01
	42 °C	9.39 ^{yb}	10.38 ^{a}	8.50 ^{yb}	8.00 ^{bc}	9.72 ^{ab}				
Isobutyrate	39 °C	0.52 ^{xa}	0.46 ^{ab}	0.41 ^{xb}	0.30 ^{bc}	0.19 ^{c}	0.02	<0.01	<0.01	<0.01
	42 °C	0.33 ^{ya}	0.35 ^{a}	0.24 ^{ya}	0.21 ^{b}	0.26 ^{ab}				
Valerate	39 °C	0.75 ^{ab}	0.79 ^{a}	0.70 ^{ab}	0.54 ^{ab}	0.49 ^{b}	0.06	<0.01	0.86	0.07
	42 °C	0.82 ^{a}	0.90 ^{a}	0.53 ^{b}	0.43 ^{b}	0.62 ^{ab}				
Caproate	39 °C	0.75 ^{xa}	0.64 ^{ab}	0.63 ^{xab}	0.52 ^{b}	0.55 ^{yb}	0.03	0.04	<0.01	<0.01
	42 °C	0.58 ^{yb}	0.64 ^{ab}	0.47 ^{yb}	0.45 ^{b}	0.75 ^{xa}				
Ratio of acetate to propionate	39 °C	2.26 ^{yc}	2.46 ^{ybc}	2.57 ^{yb}	2.85 ^{ya}	3.09 ^{a}	0.05	<0.01	0.02	<0.01
	42 °C	3.44 ^{xa}	3.39 ^{xab}	3.38 ^{xab}	3.16 ^{xb}	2.93 ^{b}				

Note: ^{a,b,c} means within a row without a common superscript letter differ significantly at $p \leq 0.05$. ^{x,y} means within a column of each item without a common superscript letter differ significantly at $p \leq 0.05$.

3.2. In Vivo Experiment

3.2.1. THI

During the in vivo experiment, the THI of morning (7 a.m.), noon (2 p.m.) and afternoon (8 p.m.) were 69.39–82.03, 74.00–89.86, and 71.86–87.66, respectively. The daily average maximum and minimum THI were 87.08 and 72.64, respectively.

3.2.2. Rumen Fermentation Parameters In Vivo

For fermentation parameters in vivo (Table 3), CAP decreased the percentage of propionate ($p \leq 0.05$) and quadratically affected acetate percentage ($p = 0.04$). There was a linear trend of CAP affecting the butyrate percentage ($p = 0.10$). CAP did not affect the ratio of acetate to propionate compared with the CON, while the ratio of acetate to propionate in 40CAP was higher than that of 10CAP ($p \leq 0.05$). There was no difference in rumen fluid pH, ammonia concentration, SCFA concentration, and the percentage of isobutyrate, valerate and caproate.

Table 3. Fermentation parameters as affected by *Capsicum oleoresin* (CAP) supplementation in vivo.

Item	*Capsicum oleoresin*				SEM	*p*-Value		
	CON	10CAP	20CAP	40CAP		T	L	Q
pH	6.09	6.24	6.31	6.19	0.10	0.46	0.41	0.20
Ammonia (mmol/L)	7.23	6.25	6.18	6.33	0.77	0.58	0.33	0.37
SCFA (mmol/L)	125.35	127.98	131.28	120.50	2.98	0.84	0.78	0.46
SCFA proportion (mol/100 mol)								
Acetate	72.65	72.79	72.82	75.06	0.63	0.10	0.04	0.15
Propionate	15.48 ^{ab}	15.79 ^{ab}	16.11 ^{a}	14.26 ^{b}	0.37	0.05	0.09	0.03
Butyrate	9.38	9.04	8.90	8.56	0.31	0.36	0.10	0.99
Isobutyrate	0.51	0.55	0.52	0.41	0.05	0.21	0.12	0.14
Valerate	1.23	1.17	0.96	1.09	0.16	0.65	0.37	0.54
Caproate	0.72	0.66	0.70	0.61	0.04	0.27	0.12	0.71
Ratio of acetate to propionate	4.70 ^{ab}	4.62 ^{ab}	4.55 ^{b}	5.29 ^{a}	0.15	0.04	0.04	0.03

Note: T = treatment; L = linear effect of CAP; Q = quadratic effect of CAP. ^{a,b} means within a row without a common superscript letter differ significantly at $p \leq 0.05$.

3.2.3. Physiological Indicators, DMI, and Lactation Performance

For physiological indicators (Table 4), CAP reduced RT and RR of buffaloes ($p \leq 0.05$), and tended to quadratically increase DMI ($p = 0.07$). In milk performance, CAP increased milk yield and milk lactose yield ($p = 0.03$), and tended to increase milk protein yield ($p = 0.09$) with a quadratic effect ($p = 0.05$). CAP did not affect milk fat, milk lactose, total solids, non-fat solids, milk urea, and milk fat yield.

Table 4. Physiological indicators, dry matter intake, and milk performance as affected by *Capsicum oleoresin* (CAP) supplementation.

Item	*Capsicum oleoresin*				SEM	*p*-Value		
	CON	10CAP	20CAP	40CAP		T	L	Q
Physiological indicators								
Rectal temperature, °C	38.97 ^{a}	38.52 ^{ab}	38.44 ^{b}	38.95 ^{ab}	0.18	0.03	0.75	0.01
Respiratory rates, rpm	49.32 ^{a}	35.36 ^{b}	35.80 ^{b}	46.71 ^{ab}	3.28	0.05	0.21	0.66
Dry matter intake, kg	8.55	8.95	9.19	8.64	0.49	0.24	0.61	0.07
Milk yield, kg/d	7.10 ^{b}	7.83 ^{a}	8.00 ^{a}	7.38 ^{ab}	0.49	0.03	0.24	0.01
Fat, %	6.53	6.79	6.59	6.81	0.31	0.75	0.55	0.93
Protein, %	4.52	4.50	4.66	4.72	0.15	0.43	0.15	0.71
Lactose, %	5.00	5.07	5.08	5.13	0.08	0.48	0.15	0.85

Table 4. *Cont.*

Item	*Capsicum oleoresin*				SEM	*p*-Value		
	CON	10CAP	20CAP	40CAP		T	L	Q
Total solids, %	17.10	17.50	17.34	17.77	1.49	0.55	0.25	0.96
solid-not-fat, %	10.41	10.39	10.51	10.75	0.25	0.49	0.19	0.48
Urea, %	25.20	24.15	23.72	24.18	1.82	0.86	0.56	0.57
Milk solids yields								
Fat, kg/d	0.46	0.52	0.51	0.49	0.07	0.36	0.41	0.14
Protein, kg/d	0.32	0.35	0.37	0.34	0.03	0.09	0.10	0.05
Lactose, kg/d	0.36 ^{b}	0.40 ^{a}	0.41 ^{a}	0.38 ^{ab}	0.04	0.03	0.11	0.01

Note: T = treatment; L = linear effect of CAP; Q = quadratic effect of CAP. ^{a,b} means within a row without a common superscript letter differ significantly at $p \leq 0.05$.

3.2.4. Serum Indicators

As shown in Table 5, CAP increased serum glucose concentration ($p = 0.04$) with a quadratic effect ($p = 0.01$). CAP supplementation affected quadratically in serum cortisol concentration ($p = 0.04$). There was no difference in insulin and NEFA by CAP supplementation.

Table 5. Serum indicators as affected by *Capsicum oleoresin* (CAP) supplementation.

Item	*Capsicum oleoresin*				SEM	*p*-Value		
	CON	10CAP	20CAP	40CAP		T	L	Q
Insulin, mIU/L	7.01	6.31	7.93	6.40	1.33	0.81	0.97	0.76
Glucose, mmol/mL	4.19 ^{b}	5.58 ^{ab}	6.31 ^{a}	5.03 ^{ab}	0.44	0.04	0.11	0.01
NEFA, µmol/L	170.89	246.84	227.85	273.73	57.68	0.52	0.54	0.25
Cortisol, pg/mL	19.41	23.37	25.16	21.20	1.48	0.12	0.32	0.04

Note: T = treatment; L = linear effect of CAP; Q = quadratic effect of CAP. ^{a,b} means within a row without a common superscript letter differ significantly at $p \leq 0.05$.

4. Discussion

A warm ambient environment has a negative effect on buffalo performance. In the previous study, ambient temperature significantly affected the rumen temperature [20]. Therefore, it is imperative to study rumen fermentation under hyperthermal temperature and the resulting changes in milk performance to reduce the adverse impact of heat stress.

For the In vitro experiment, hyperthermal temperature affected all fermentation characteristics measured in this study. For instance, hyperthermal temperature decreased SCFA concentration while increasing ammonia concentration in the incubation solution, indicating that it restrained fermentation In vitro [21]. It is shown that the severe changes in rumen fermentation characteristics caused by hyperthermal temperature may be one of the crucial reasons for the productivity decrease in ruminants within a warm ambient environment. Hyperthermal temperature altered most bacteria and protozoa activity, resulting an increase in ammonia concentration and decrease in SCFA concentration [22]. In terms of SCFA composition, hyperthermal temperature increased the ratio of acetate to propionate by altering acetate and butyrate percentage, which will lead to increase milk fat under high THI [23]. The results showed the drastic effect of hyperthermal temperature on rumen fermentation in this study; however, the mechanism of temperature factors on rumen function needs further study, since there is little research about the effect of temperature effect on buffaloes' rumen fermentation In vitro.

In this study, CAP reduced ammonia concentration relative to CON under hyperthermal temperature In vitro at 20 mg/L; however, the ammonia concentration increased when the dose was higher than 20 mg/L, indicating the fermentation inhibition of high CAP concentration. On the other hand, there was no difference under hyperthermal temperature, although CAP decreased SCFA at physiological temperature. Therefore, CAP could

improve rumen fermentation characteristics with low concentration under hyperthermal temperature from the standpoint of ammonia and SCFA concentrations. In terms of SCFA composition, CAP had an opposite effect between physiological and hyperthermal temperature. CAP decreased the propionate percentage while increasing the acetate percentage and the ratio of acetate to propionate at physiological temperature, similar to the previous report [24]. However, the opposite effect was observed under hyperthermal temperature. From these results, the changes caused by CAP tended to be similar to the physiological fermentation results under hyperthermal temperature.

In contrast to the In vitro experiment, there was no difference in rumen pH by CAP in vivo, which is consistent with the previous report [25]. As one of the indicators of fermentation, modest rumen pH indicates rumen health. Although CAP did not affect rumen fermentation variables, it could regulate under certain circumstances. Castillo-Lopez et al. [26] reported that the improvement of CAP in simulating rumen acidosis was based on increasing the feeding time. It has been reported that the increased pH in rumen fluid of heifers under high concentrate diet with CAP was based on salivary secretion [27]. CAP did not affect SCFA and ammonia concentration; it was shown that CAP had no effect on the overall fermentation, which indicates the safety of CAP dosage. In this research, CAP increased propionate percentage in 20CAP, similar to the In vitro results. Meanwhile, the tendency to increase acetate percentage in 40CAP resulted in an increase of acetate to propionate ratio in 40CAP. Due to the effects of CAP on intake, feeding behavior, and salivary secretion of ruminates [26,28], fermentation variables fluctuated more at high doses in vivo than In vitro. Compared with the In vitro experiments, the fermentation variables in the experiments in vivo showed more fluctuation at high CAP doses.

For physiological variables, CAP decreased RT and RR, which are similar to those reported by Silva [29]. Capsaicin could cause hypothermia via transient receptor potential V1 (TRPV1) [30], so CAP decreased RR may be through anterior digestive tract stimulation rather than fermentation variables in the rumen. However, the capsaicin concentration used in this research is lower than the activate concentration in rumen epithelium (100 μM) [31]. Meanwhile, the previously reported temperature (37 °C) was lower compared to the physiological rumen temperature (39 °C) and the maximum rumen temperature under heat stress (42 °C), while the threshold of capsaicin activation in TRPV1 is temperature dependent [32]. On the other hand, it has been reported that CAP could increase DMI without considering changes in RT [7,33]. A negative correlation between RT and DMI was verified [34], so the decrease in RT may be responsible for the increase in the quadratic tendency of DMI by CAP addition. Therefore, due to the decrease of RT and the positive influence of DMI, CAP increased milk yield in this study, similar to previous study [35]. Additionally, rising milk production increases cortisol secretion [36], so 20CAP showed higher levels of cortisol. Because high CAP could slow down the feeding speed [26], the capsaicin concentration in the 40CAP group may be lower than 20CAP in the rumen, resulting in differences of fermentation variables and physiological variables. For the effect of fermentation variables on milk performance, CAP increased lactose yield due to an increase in the percentage of propionate in the fermentation variable; however, it did not affect lactose percentage [37,38]. Although the ratio of acetate to propionate in fermentation variables was different between the 20CAP and 40CAP, it was not shown in milk composition. In terms of serum indicators, CAP had no effect on insulin and NEFA, while it increased glucose concentration, contrary to previous studies [10,39]. This may be due to the increase of DMI, but the metabolism alteration in ruminants under warm ambient environment could be another noticeable reason [40].

5. Conclusions

In this experiment, CAP altered rumen fermentation variables In vitro and tended to be similar to the physiological fermentation results under hyperthermal temperature. Additionally, CAP supplementation altered rumen fermentation of buffaloes by increasing the percentage of propionate acid and the ratio of acetate to propionate in summer. The 20CAP

group had lower RR and RT compared with CON, leading to increase milk yield, milk protein yield, and lactose yield. This indicates that 20 mg/kg is the optimal supplemental dose in buffaloes.

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References

1. Pantoja, L.S.G.; Amante, E.R.; da Cruz Rodrigues, A.M.; da Silva, L.H.M. World scenario for the valorization of byproducts of buffalo milk production chain. *J. Clean. Prod.* **2022**, *364*, 132605. [CrossRef]
2. Kim Thanh, V.T.; Shi Chang, W. Differences in adaptation to tropical weather between buffaloes and cattle. *Ital. J. Anim. Sci.* **2007**, *6*, 1340–1343. [CrossRef]
3. Bernabucci, U.; Lacetera, N.; Baumgard, L.H.; Rhoads, R.P.; Ronchi, B.; Nardone, A. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. *Animal* **2010**, *4*, 1167–1183. [CrossRef]
4. Arowolo, M.A.; He, J. Use of probiotics and botanical extracts to improve ruminant production in the tropics: A review. *Anim. Nutr.* **2018**, *4*, 241–249. [CrossRef]
5. Silvester, A.J.; Aseer, K.R.; Yun, J.W. Dietary polyphenols and their roles in fat browning. *J. Nutr. Biochem.* **2019**, *64*, 1–12. [CrossRef]
6. Adaszek, Ł.; Gadomska, D.; Mazurek, Ł.; Łyp, P.; Madany, J.; Winiarczyk, S. Properties of capsaicin and its utility in veterinary and human medicine. *Res. Vet. Sci.* **2019**, *123*, 14–19. [CrossRef]
7. Fandiño, I.; Calsamiglia, S.; Ferret, A.; Blanch, M. Anise and capsicum as alternatives to monensin to modify rumen fermentation in beef heifers fed a high concentrate diet. *Anim. Feed. Sci. Technol.* **2008**, *145*, 409–417. [CrossRef]
8. Eidsvik, J.C.; McKinnon, J.J.; Blanchard, A.; Khelil, H.; Moya, D.; Penner, G.B. Effects of rumen-protected Capsicum oleoresin on dry matter intake, average daily gain, and carcass characteristics of finishing beef steers. *Appl. Anim. Sci.* **2022**, *38*, 335–342. [CrossRef]
9. Westphalen, M.F.; Carvalho, P.H.; Oh, J.; Hristov, A.N.; Staniar, W.B.; Felix, T.L. Effects of feeding rumen-protected Capsicum oleoresin on growth performance, health status, and total tract digestibility of growing beef cattle. *Anim. Feed. Sci. Technol.* **2021**, *271*, 114778. [CrossRef]
10. Oh, J.; Giallongo, F.; Frederick, T.; Pate, J.; Walusimbi, S.; Elias, R.; Wall, E.; Bravo, D.; Hristov, A. Effects of dietary Capsicum oleoresin on productivity and immune responses in lactating dairy cows. *J. Dairy Sci.* **2015**, *98*, 6327–6339. [CrossRef]
11. Szolcsányi, J. Effect of capsaicin on thermoregulation: An update with new aspects. *Temperature* **2015**, *2*, 277–296. [CrossRef]
12. An, Z.; Abdelrahman, M.; Zhou, J.; Riaz, U.; Gao, S.; Gao, S.; Luo, G.; Yang, L. Prepartum Maternal Supplementation of Capsicum Oleoresin Improves Colostrum Quality and Buffalo Calves' Performance. *Front. Vet. Sci.* **2022**, *9*, 935634. [CrossRef] [PubMed]
13. An, Z.; Luo, G.; Abdelrahman, M.; Riaz, U.; Gao, S.; Yao, Z.; Ye, T.; Lv, H.; Zhao, J.; Chen, C. Effects of capsicum oleoresin supplementation on rumen fermentation and microbial abundance under different temperature and dietary conditions In vitro. *Front. Microbiol.* **2022**, *13*, 1005818. [CrossRef] [PubMed]
14. Eihvalde, I.; Kairisa, D.; Sematovica, I. Long-term continuous monitoring of ruminal PH and temperature for dairy cows with indwelling and wireless data transmitting unit. *Parameters* **2016**, *3555*, 4829.
15. Menke, K.H. Estimation of the energetic feed value obtained from chemical analysis and In vitro gas production using rumen fluid. *Anim. Res. Dev.* **1988**, *28*, 7–55.

16. Bartocci, S.; Tripaldi, C.; Terramoccia, S. Characteristics of foodstuffs and diets, and the quanti-qualitative milk parameters of Mediterranean buffaloes bred in Italy using the intensive system: An estimate of the nutritional requirements of buffalo herds lactating or dry. *Livest. Prod. Sci.* **2002**, *77*, 45–58. [CrossRef]
17. Weatherburn, M. Phenol-hypochlorite reaction for determination of ammonia. *Anal. Chem.* **1967**, *39*, 971–974. [CrossRef]
18. National Research Council (U.S.). *A Guide to Environmental Research on Animals: National Academies*; National Research Council (U.S.): Rockville, MD, USA, 1971.
19. Li, G.; Chen, S.; Chen, J.; Peng, D.; Gu, X. Predicting rectal temperature and respiration rate responses in lactating dairy cows exposed to heat stress. *J. Dairy Sci.* **2020**, *103*, 5466–5484. [CrossRef]
20. Lees, A.; Lees, J.; Lisle, A.; Sullivan, M.; Gaughan, J. Effect of heat stress on rumen temperature of three breeds of cattle. *Int. J. Biometeorol.* **2018**, *62*, 207–215. [CrossRef]
21. Mahmood, M.; Petri, R.M.; Gavrău, A.; Zebeli, Q.; Khiaosa-ard, R. Betaine addition as a potent ruminal fermentation modulator under hyperthermal and hyperosmotic conditions In vitro. *J. Sci. Food Agric.* **2020**, *100*, 2261–2271. [CrossRef]
22. Khiaosa-Ard, R.; Mahmood, M.; Lerch, F.; Traintinger, F.-P.; Petri, R.M.; Münnich, M.; Zebeli, Q. Physicochemical stressors and mixed alkaloid supplementation modulate ruminal microbiota and fermentation In vitro. *Anaerobe* **2020**, *65*, 102263. [CrossRef]
23. Nasr, M.A. The impact of crossbreeding Egyptian and Italian buffalo on milk yield and composition under subtropical environmental conditions. *J. Dairy Res.* **2016**, *83*, 196–201. [CrossRef]
24. Cardozo, P.; Calsamiglia, S.; Ferret, A.; Kamel, C. Screening for the effects of natural plant extracts at different pH on In vitro rumen microbial fermentation of a high-concentrate diet for beef cattle. *J. Anim. Sci.* **2005**, *83*, 2572–2579. [CrossRef]
25. Fandiño, I.; Ferret, A.; Calsamiglia, S. Dose and combinations of anise oil and capsicum oleoresin as rumen fermentation modifiers In vitro and in vivowith high concentrate diets fed to Holstein beef heifers. *Anim. Feed. Sci. Technol.* **2020**, *260*, 114363. [CrossRef]
26. Castillo-Lopez, E.; Rivera-Chacon, R.; Ricci, S.; Petri, R.M.; Reisinger, N.; Zebeli, Q. Short-term screening of multiple phytogenic compounds for their potential to modulate chewing behavior, ruminal fermentation profile, and pH in cattle fed grain-rich diets. *J. Dairy Sci.* **2021**, *104*, 4271–4289. [CrossRef]
27. Ricci, S.; Rivera-Chacon, R.; Petri, R.M.; Sener-Aydemir, A.; Sharma, S.; Reisinger, N.; Zebeli, Q.; Castillo-Lopez, E. Supplementation with phytogenic compounds modulates salivation and salivary physico-chemical composition in cattle fed a high-concentrate diet. *Front. Physiol.* **2021**, *12*, 645529. [CrossRef]
28. Rodríguez-Prado, M.; Ferret, A.; Zwieten, J.; Gonzalez, L.; Bravo, D.; Calsamiglia, S. Effects of dietary addition of capsicum extract on intake, water consumption, and rumen fermentation of fattening heifers fed a high-concentrate diet. *J. Anim. Sci.* **2012**, *90*, 1879–1884. [CrossRef]
29. Silva, R. Suplementação de Vacas Leiteiras com Óleos Essenciais. Ph.D. Thesis, Universidade Federal de Lavras, Lavras, Brazil, 2017.
30. Inagaki, H.; Kurganov, E.; Park, Y.; Furube, E.; Miyata, S. Oral gavage of capsaicin causes TRPV1-dependent acute hypothermia and TRPV1-independent long-lasting increase of locomotor activity in the mouse. *Physiol. Behav.* **2019**, *206*, 213–224. [CrossRef]
31. Vennekens, R.; Vriens, J.; Nilius, B. Herbal compounds and toxins modulating TRP channels. *Curr. Neuropharmacol.* **2008**, *6*, 79–96. [CrossRef]
32. Chung, M.-K.; Wang, S. Cold suppresses agonist-induced activation of TRPV1. *J. Dent. Res.* **2011**, *90*, 1098–1102. [CrossRef]
33. Foskolos, A.; Ferret, A.; Siurana, A.; Castillejos, L.; Calsamiglia, S. Effects of capsicum and propyl-propane thiosulfonate on rumen fermentation, digestion, and milk production and composition in dairy cows. *Animals* **2020**, *10*, 859. [CrossRef]
34. Rejeb, M.; Sadraoui, R.; Najar, T.; M'rad, M.B. A complex interrelationship between rectal temperature and dairy cows' performance under heat stress conditions. *Open J. Anim. Sci.* **2016**, *6*, 24. [CrossRef]
35. Wall, E.; Bravo, D. 1554 Supplementation with rumen-protected capsicum oleoresin increases milk production and component yield in lactating dairy cows. *J. Anim. Sci.* **2016**, *94*, 755. [CrossRef]
36. Kumar, J.; Madan, A.K.; Kumar, M.; Sirohi, R.; Yadav, B.; Reddy, A.V.; Swain, D.K. Impact of season on antioxidants, nutritional metabolic status, cortisol and heat shock proteins in Hariana and Sahiwal cattle. *Biol. Rhythm. Res.* **2018**, *49*, 29–38. [CrossRef]
37. Stelwagen, K.; Wall, E.; Bravo, D. 1395 Effect of rumen-protected capsicum on milk production in early lactating cows in a pasture-based system. *J. Anim. Sci.* **2016**, *94*, 675. [CrossRef]
38. Grazziotin, R.; Halfen, J.; Rosa, F.; Schmitt, E.; Anderson, J.; Ballard, V.; Osorio, J. Altered rumen fermentation patterns in lactating dairy cows supplemented with phytochemicals improve milk production and efficiency. *J. Dairy Sci.* **2020**, *103*, 301–312. [CrossRef]
39. Silvestre, T.; Räisänen, S.; Cueva, S.; Wasson, D.; Lage, C.; Martins, L.; Wall, E.; Hristov, A. Effects of a combination of Capsicum oleoresin and clove essential oil on metabolic status, lactational performance, and enteric methane emissions in dairy cows. *J. Dairy Sci.* **2022**, *105*, 9610–9622. [CrossRef]
40. Rhoads, M.; Rhoads, R.; VanBaale, M.; Collier, R.; Sanders, S.; Weber, W.; Crooker, B.; Baumgard, L. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. *J. Dairy Sci.* **2009**, *92*, 1986–1997. [CrossRef]