

Effect of capsaicin supplementation on lactational and reproductive performance of Holstein cows during summer

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Abstract

Context. Summer ambient temperature and humidity are major factors negatively influencing the physiology and the productive and reproductive efficiency of dairy cows. Various management and breeding approaches may be useful for maintaining productivity in dairy cows during summer to minimise these effects.

Aims. The experiment was designed to test a natural phytochemical supplementation of capsaicin (CPS), followed by a specific oestrus synchronisation protocol as a means to maintain productivity and reproductive performance of Chinese Holstein cows during summer in Hubei province, China.

Methods. Lactating Chinese Holstein cows ($n = 109$) were randomly divided into four groups and supplemented with 0 (control, $n = 27$), 20 (CPS-20, $n = 26$), 40 (CPS-40, $n = 28$) and 60 (CPS-60, $n = 28$) mg of capsaicin (CPS) per kg of total mixed ration respectively for continuous 30 days initiated on 10 July 2018. Milk production and composition were measured over 45 days from the start of CPS supplementation. After CPS supplementation, all cows were synchronised through a modified Ovsynch oestrus synchronisation protocol, and reproductive variables were recorded. Metabolic parameters were measured on the days before CPS supplementation, artificial insemination (AI) and 50 days post-AI.

Key results. Highest ($P < 0.05$) milk production were found in the CPS-40 group, and highest total solids and milk fat in the CPS-20 and CPS-40 groups. Milk urea nitrogen and milk fat were generally increased in CPS supplemented cows compared with the control group. Significant increases ($P < 0.05$) in oestrus response, ovulatory follicle size, ovulation rate and pregnancy rates were observed in the CPS-40 group compared with other groups. Glucose, lipoprotein esterase and aspartate aminotransferase were increased ($P < 0.05$) in CPS-40 and CPS-60 supplemented groups at the time of AI and 50 days post-AI.

Conclusions. CPS supplementation has the capacity to maintain milk yield, milk composition and serum metabolites in Chinese Holstein dairy cows during summer. The synergistic effect of CPS and the modified oestrus synchronisation protocol also improved reproductive variables of the cows.

Implications. Adoption of CPS as a supplement along with oestrus synchronisation could be a useful and economical strategy for dairy herd keepers to improve herd performance during summer.

Keywords: capsaicin, oestrus synchronisation, summer season, Holstein cows.

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Introduction

Low fertility and production consequences are usually observed in dairy cows over summer in south of China owing to high

ambient temperature and humidity. The effects of hot summer weather on dairy cows are not limited to tropical areas but also extend to cattle herds reared in mild climates. Generally, the low

productivity in cows during summer is directly linked to metabolic functions, which in turn reduces lactational and reproductive performance (Bernabucci *et al.* 2014; Schüller *et al.* 2017). Additionally, increased blood circulation to the body surface for heat dissipation under hot conditions leads to insufficient blood supply to the digestive, reproductive and lactation systems of exposed animals. This phenomenon affects productive and reproductive efficiency in dairy animals (Das *et al.* 2016).

Various regimens are employed under commercialised and conventional dairy systems to combat conditions of high ambient temperature and humidity in dairy cows. Numerous reports illustrate the use of different dietary supplements to augment the productivity in dairy cows during summer heat conditions (Cheng *et al.* 2016; Hall *et al.* 2016; Cheng 2018; Nasiri *et al.* 2018). Oriental folk medicines have been widely used in the Asian region for the treatment of various diseases, including reproductive disorders, over the centuries (Cheng 2018). These traditional medicines are usually based on essences and secondary metabolites such as polysaccharides, saponins and essential oil (Cheng 2018). Capsaicin (CPS) is an active compound in *Capsicum* spp. and has a phenolic structure. It has been tested as an antimicrobial agent for enhancing the rumen fermentation (Jeeatid *et al.* 2018), as a stimulant for digestive enzyme secretion (Oh *et al.* 2017) and as a modulator for glucose homeostasis via an insulin secretion mechanism (Oh *et al.* 2017). Increased metabolic rate in response to CPS ingestion can be a good indicator for regulation of reproductive hormone production and secretory pattern.

The use of oestrus synchronisation protocols has been considered beneficial for improving oestrus response and pregnancy rates in dairy cows in summer (De Rensis *et al.* 2015; El-Tarabany and El-Tarabany 2015; Garciaispierto *et al.* 2018). Synchronisation regimens have been modified for successful ovulation induction in order to promote the efficacy of fixed-time artificial insemination (AI) (Dirandeh 2014; Dirandeh *et al.* 2015; Voelz *et al.* 2016; Garciaispierto *et al.* 2018). However, ovulation synchronisation protocols have not yet been tested in Chinese Holstein cows under conditions of high ambient temperature and humidity. For the present study, we hypothesised that fertility, metabolic functions and milk production could be maintained by the application of a modified Ovsynch protocol (Abulaiti *et al.* 2019) in combination with CPS as a dietary supplement in dairy cows under conditions of high ambient temperature and humidity. Hence, the study was designed to determine the beneficial effects of CPS on production and fertility in Chinese Holstein dairy cows under summer conditions of high ambient temperature and humidity.

Materials and methods

Animal care

The study was approved by the Ethical Committee of the Hubei Research Center of Experimental Animals (Approval ID: SCXK (Hubei) 20080005). All experimental protocols were performed in accordance with the guidelines of the Committee of Animal Research Institute, Huazhong Agricultural University, China.

Experimental site and climatic conditions

The experiment was conducted during summer 2018, from 10 July to 24 August, at Hubei Tong Shun Co., Hubei province, China (30°32'N, 111°51'E). Hubei province has mountainous topography and is in the humid subtropical zone of central China. Climatic conditions are cool to cold in winter (average temperature 1–5°C) and hot in summer (average temperature 27–35°C, reaching 40°C during July and August). Average rainfall in Hubei province is 800–1600 mm (300–700 mm in summer vs 30–190 mm in winter) and maximum rainfall is recorded during the monsoon season.

During the experiment, the daily ambient temperature (T°C) and relative humidity (RH) were noted, and temperature–humidity index (THI) was calculated (Bohmanova *et al.* 2007) using the following formula:

$$\text{THI} = (0.8 \times T^{\circ}\text{C}) + (\text{RH}/100) \times (T^{\circ}\text{C} - 14.4) + 46.4$$

Daily variations in T°C, RH and THI are presented in Fig. 1, with respective averages 35.6°C ($\pm 0.2^{\circ}\text{C}$), 78.1% ($\pm 1.3\%$) and 91.4 (± 0.3) throughout the trial. The observed THI indicated severe heat stress for cows during the period of observations.

Husbandry practices

In total, 109 multiparous, lactating (34 ± 12.5 days in milking) Chinese Holstein cows with optimum body condition score (2.5–3.0) and average bodyweight (557.1 ± 89.5 kg) were selected. The selected cows were clinically and physically healthy with a normal history of calving and reproductive soundness. All of the cows were fed with total mixed ration (TMR) according to NRC recommendation (Table 1). *Ad libitum* fresh and clean water was provided routinely. The cows were housed in an open shed with a cemented roof top and two sides fenced by galvanised wire mesh. Exhaust fans for ventilation, sprinklers for shower and grooming brush facilities were also available at the farm. A tail-to-tail stall feeding system was in practice. Each cow was allocated an area of ~ 3.7 m² along with a manger 0.6 by 0.9 m.

Experimental design

The experimental cows ($n = 109$) were randomly divided into four groups and supplied with 0 (control, $n = 27$), 20 (CPS-20, $n = 26$), 40 (CPS-40, $n = 28$) and 60 (CPS-60, $n = 28$) mg of CPS (Guangzhu Pumai Biotechnology, China) per kg of TMR daily for continuous 30 days initiated on 10 July and terminate on 9 August 2018.

Milk production and milk composition

Cows were milked three times a day at 03:00, 11:00 and 19:00 daily. Milk yield was estimated in litters by using calibrated jars, and milk yield averages were calculated over intervals of 5 days from the start of supplementation to Day 45 of the trial. Milk composition parameters including total solids, lactose, fat, protein, somatic cell count, and urea nitrogen were determined by Milk Composition Analyzer (type 78110; Foss, Denmark).

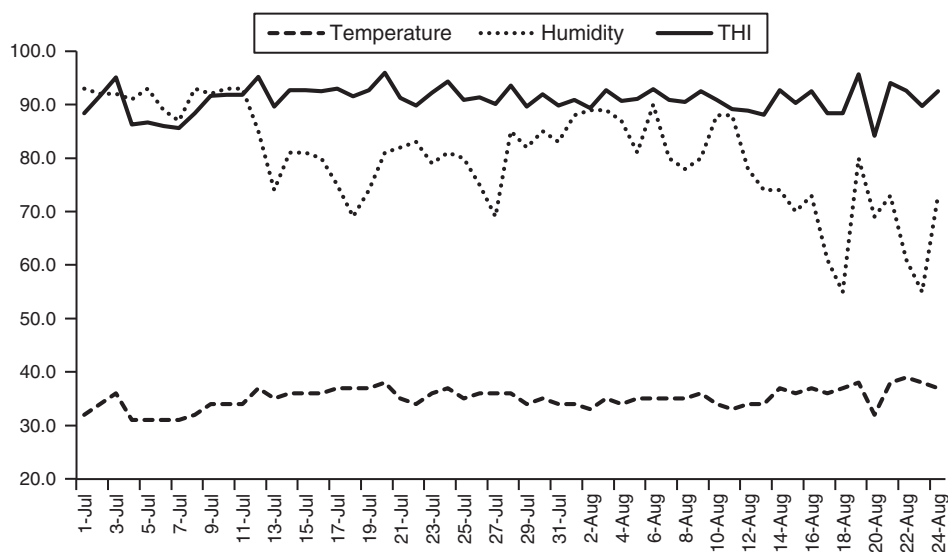


Fig. 1. Daily variations of temperature ($^{\circ}\text{C}$), humidity (%) and temperature–humidity index (THI) during the study period.

Table 1. Ingredients and composition of total mixed ration and concentrate for experimental cows in summer
Formulated according to NRC recommendations

Ingredient	Crude protein (%)	Dry matter (%)	Quantity
<i>Total mixed ration</i>			
Straw	2	90	3.5 kg
Lucerne clover	16	90	2.5 kg
Silage	8	28	21 kg
Soy pulp	17	15	8 kg
Concentrate part 1	17.1	88	8.5 kg
Total			43.5 kg
Total dry matter			19.96 kg
Concentrate			43%
<i>Concentrate</i>			
Maize	7.8	88	53%
Soybean meal	43	88	19%
Palm meal	34	88	10.5%
Wheat bran	11	88	11%
Premix	0	95	5%
Sodium bicarbonate	0	90	1%
Dicalcium phosphate	0	90	0.5%
Total			100%
Crude protein			17.1%

Modified Ovsynch oestrus synchronisation protocol and pregnancy diagnosis

Following the CPS supplementation from 10 July to 9 August, the cows were initially intramuscularly injected with gonadotropin-releasing hormone (GnRH, 200 μg) on 10 August (Day 0), followed by an intramuscular injection of prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$, 0.5 mg; Ningbo Sansheng Pharmaceutical Ltd, China) on Day 7 of treatment. The

second injection of GnRH (200 μg) and the first intramuscular injection of mifepristone (0.4 mg/kg, Hubei Yun Cheng Sai Technology, China) were given simultaneously on Day 9 of treatment. The cows were subjected to insemination 16 h post the second injection of GnRH. An intramuscular injection of human chorionic gonadotropin (hCG, 2000 IU, Ningbo Sansheng Pharmaceutical Ltd, China) was administered for each animal 5 days post-AI.

Ultrasonography (Desktop B-type veterinary ultrasound scanner, WED-9618-v, equipped with LV2-3/6.5 MHz rectal probe; Shenzhen Well D Medical Electronics, Guangdong, China) was used to monitor follicle development. The cows were scanned twice a day starting from Day 6 (the day before $\text{PGF}_{2\alpha}$ treatment) until Day 12 (at 72 h after the second injection of GnRH) of treatment. Ovulation was considered by the sudden disappearance of dominant follicles at an ultrasonographic examination compared with the previous scan (Liu *et al.* 2016). The cows were observed for signs of oestrus visually twice a day (06:00 and 18:00). Pregnancy diagnosis was performed by ultrasonography 30 days post-AI.

Estimation of metabolic activity

Blood samples were collected from each cow at the start of CPS supplementation, at the time of AI, and 50 days after AI. Metabolic activity of serum glucose, lipoprotein esterase (LPL) and aspartate aminotransferase (AST) were assessed with ELISA kits (Shanghai Enzyme-linked Biotechnology Co.). Blood glucose values were assessed by use of the MI076792 bovine blood glucose test kit. LPL estimation was performed by the MI607622 bovine lipoprotein esterase test kit (sensitivity 3 $\mu\text{g}/\text{mL}$, intra-assay variation <10%, inter-assay variation <15%); AST determination was done by the MI063776 aspartate aminotransferase kit (sensitivity 0.5ng/mL, intra-assay variation <8%, inter-assay variation <10%).

Statistical analyses

Data were analysed using statistical software (SPSS version 17.0.1, Chicago, IL, USA). One-way analysis of variance (ANOVA) was applied to compare the diameter of the ovulatory follicle and growth rate of dominant follicles among groups. Two-way ANOVA was applied for the analysis of milk production, milk composition and metabolic activity at different intervals among the groups. The chi-square test was applied for comparison of pregnancy, oestrus and ovulation across the groups using Prism-6 software package (GraphPad Software). Values of $P < 0.05$ were considered statistically significant.

Results

Effects of CPS on milk production and milk composition

There was no effect of CPS on milk production among the groups during the first 2 weeks of supplementation. However, the CPS-40 group had significantly higher ($P < 0.05$) milk production than the control and CPS-60 groups on Days 20, 25, 30, 35, 40 and 45, and higher ($P < 0.05$) milk production than the CPS-20 group at most of these dates (Table 2). With regard to milk composition (Table 3), CPS-20 and CPS-40 groups had higher ($P < 0.05$) total solids than the control group on Day 20. Cows in CPS-60 group had higher ($P < 0.05$) lactose levels in milk than cows in group CPS-20 at both sampling times and higher ($P < 0.05$) lactose levels than cows in group CPS-40 on Day 20. There was no difference in milk protein level among treatment groups. Cows in groups CPS-20 and CPS-40 had higher ($P < 0.05$) milk fat than those in control group at both

sampling times, and higher ($P < 0.05$) milk fat than those in group CPS-60 on Day 20. Milk urea nitrogen increased ($P < 0.05$) in groups CPS-20 and CPS-60 on Day 20 relative to the control. All CPS-supplemented groups had numerically higher somatic cell count than the control at both sampling times (Table 3).

Effects of CPS on reproductive performance of cows

The effect of CPS supplementation on reproductive variables in synchronised cows was presented in Table 4. The CPS-40 group showed greater ($P < 0.05$) oestrus response than other treatment groups. The CPS-40 group had significantly higher ($P < 0.05$) ovulation rate than other treatment groups, and higher ($P < 0.05$) ovulatory follicle diameter than the control group. However, duration of oestrus, follicle growth rate, and interval from second GnRH injection to oestrus and ovulation were similar ($P > 0.05$) across all groups. A higher ($P < 0.05$) pregnancy rate was observed in the CPS-40 group than control and CPS-20 groups.

Effects of CPS on metabolic activities in cows

Cows in groups CPS-40 and CPS-60 had higher ($P < 0.05$) serum glucose levels than those in the control at the time of AI and at 50 days post-AI (Table 5). Cows in all CPS groups had significantly higher ($P < 0.05$) LPL levels than the control cows at both sampling times. However, only the CPS-40 treatment group had higher ($P < 0.05$) serum AST than the control at either sampling time.

Table 2. Daily milk yield (kg) of cows supplemented with capsaicin (CPS) at different doses in summer
Within a row, means followed by the same letter are not significantly different ($P > 0.05$)

Day of CPS supplementation	Control	CPS-20	CPS-40	CPS-60	P-value
5	11.5 ± 0.88	10.6 ± 1.09	11.5 ± 1.31	11.32 ± 1.47	0.0808
10	10.5 ± 1.71	11.4 ± 0.91	11.2 ± 1.1	11.25 ± 1.44	0.1041
15	12.64 ± 1.73a	10.9 ± 1.25b	11.6 ± 1.28ab	11.4 ± 0.84b	0.0005
20	11.25 ± 1.54b	12.00 ± 1.33b	14.25 ± 1.44a	12.35 ± 2.57b	<0.0001
25	11.33 ± 1.11c	12.5 ± 2.03bc	13.75 ± 1.01a	12.8 ± 1.49b	<0.0001
30	11.45 ± 0.87b	12.96 ± 1.71b	16.24 ± 1.45a	13.6 ± 1.20b	<0.0001
35	12.5 ± 2.21b	13.17 ± 1.3ab	14.32 ± 1.03a	12.85 ± 2.62b	0.0238
40	12.75 ± 1.17b	13.3 ± 1.41b	15.63 ± 0.54a	13.5 ± 0.87b	<0.0001
45	12.71 ± 1.34b	13.65 ± 1.68b	16.3 ± 0.66a	13.25 ± 0.63b	<0.0001

Table 3. Milk composition on Days 20 and 30 in cows supplemented with capsaicin (CPS) at different doses in summer
Within a row, means followed by the same letter are not significantly different ($P > 0.05$)

Milk parameter	Day 20				Day 30			
	Control	CPS-20	CPS-40	CPS-60	Control	CPS-20	CPS-40	CPS-60
Total solids (%)	10.2 ± 0.5b	11.2 ± 0.8a	11.2 ± 0.9a	10.5 ± 0.8ab	9.7 ± 1.2	10.5 ± 0.9	10.1 ± 0.9	9.9 ± 1.1
Lactose (%)	4.6 ± 0.4a	4.3 ± 0.4b	4.3 ± 0.5b	4.5 ± 0.3a	4.7 ± 0.2ab	4.4 ± 0.5b	4.6 ± 0.5ab	4.8 ± 0.2a
Protein (%)	2.7 ± 0.4	3.1 ± 0.4	3.2 ± 0.5	3.0 ± 0.4	3.0 ± 0.4	3.4 ± 0.5	3.4 ± 0.6	3.3 ± 0.5
Fat (%)	2.3 ± 0.4b	3.0 ± 0.7a	3.2 ± 0.9a	2.5 ± 0.6b	2.2 ± 1.1b	3.1 ± 0.7a	3.3 ± 0.4a	2.7 ± 0.7ab
Urea nitrogen (mg/dL)	20.4 ± 1.3b	21.7 ± 1.3a	21.3 ± 0.6ab	22.3 ± 0.8a	18.8 ± 2.5	19.9 ± 1.1	20.2 ± 1.3	18.9 ± 1.4
Somatic cell count (10^4 /mL)	13.9 ± 5.7	17.2 ± 11.9	19.50 ± 11.5	20.3 ± 9.4	14.1 ± 16.6	18.7 ± 16.8	19.0 ± 11.7	19.5 ± 17.4

Table 4. Reproductive variables in cows treated with modified Ovsynch regimen after supplementation with capsaicin (CPS) in summer
Within a row, means followed by the same letter are not significantly different ($P > 0.05$)

Variables	Control ($n = 27$)	CPS-20 ($n = 26$)	CPS-40 ($n = 28$)	CPS-60 ($n = 28$)
Oestrus response (%)	63.0 (17/27) ^b	57.7 (15/26) ^b	78.6 (22/28) ^a	60.7 (17/28) ^b
Interval to oestrus after second GnRH (h)	8.8 ± 1.0	9.9 ± 1.0	8.3 ± 0.9	8.4 ± 0.8
Duration of oestrus (h)	15.5 ± 0.7	15.7 ± 0.9	15.3 ± 0.6	15.4 ± 0.5
Ovulation rate (%)	55.6 (15/27) ^b	53.8 (14/26) ^b	75.0 (21/28) ^a	57.1 (16/28) ^b
Interval to ovulation after second GnRH (h)	24.2 ± 1.3	24.3 ± 0.2	23.4 ± 0.7	23.7 ± 0.5
Pregnancy rate (%)	33.3 (9/27) ^b	34.6 (9/26) ^b	53.6 (15/28) ^a	39.3 (11/28) ^{ab}
Diameter of first detecting follicle (mm)	7.0 ± 1.9	8.9 ± 1.7	6.4 ± 1.1	8.3 ± 2.4
Ovulatory follicle diameter (mm)	11.5 ± 1.5 ^b	12.1 ± 1.7 ^{ab}	13.8 ± 0.4 ^a	12.3 ± 1.9 ^{ab}
Follicle growth rate (mm/day)	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1

Table 5. Variation in serum glucose, lipoprotein esterase (LPL) and aspartate aminotransferase (AST) in cows on Day 0 of supplementation with capsaicin (CPS), the day before artificial insemination (AI), and 50 days post-AI

Within a column, means followed by the same letter are not significantly different ($P > 0.05$)

Parameter	Treatment group	Day 0 of supplementation	At time of AI	50 days post-AI
Glucose (mmol/L)	Control	5.61 ± 0.80	4.75 ± 0.43 ^c	5.55 ± 0.96 ^c
	CPS-20	5.27 ± 1.10	5.74 ± 1.20 ^{bc}	6.67 ± 0.45 ^b
	CPS-40	5.05 ± 0.71	6.99 ± 0.57 ^a	7.86 ± 0.61 ^a
	CPS-60	5.64 ± 0.39	7.24 ± 0.51 ^a	7.64 ± 0.69 ^a
LPL (U/L)	Control	667.57 ± 88.78	581.08 ± 84.08 ^b	634.12 ± 78.85 ^b
	CPS-20	571.86 ± 77.85	695.08 ± 57.17 ^a	734.12 ± 56.91 ^a
	CPS-40	635.47 ± 116.88	746.09 ± 76.39 ^a	789.07 ± 72.84 ^a
	CPS-60	598.37 ± 90.65	719.79 ± 70.99 ^a	768.64 ± 65.67 ^a
AST (mU/mL)	Control	11.25 ± 1.32	10.63 ± 0.76 ^b	11.55 ± 2.39 ^b
	CPS-20	11.16 ± 1.39	10.99 ± 0.75 ^b	11.87 ± 0.74 ^b
	CPS-40	11.46 ± 0.98	12.58 ± 1.69 ^a	13.47 ± 0.73 ^a
	CPS-60	11.35 ± 1.25	12.13 ± 0.72 ^{ab}	13.22 ± 0.36 ^{ab}

Discussion

Generally, the negative effects of heat stress are displayed by dairy cows in summer when THI values exceed 80 (Kadzere *et al.* 2002; Bohmanova *et al.* 2007). We recorded a higher THI value (91.0 ± 0.3), which indicates severe heat stress, and observed values that are higher than documented in earlier reports for dairy cows (Liu *et al.* 2020; Dehghan-Banadaky *et al.* 2013; Cheng *et al.* 2016). The THI values remained relatively constant during the experimental trial under local conditions. These extreme values of THI might be linked to location of study site, in the humid subtropical zone where the summer season coincides with higher humidity level.

Variation in milk production or milk composition is common in cows under heat stress condition (Ravagnolo and Misztal 2000; Joksimovic-Todorovic *et al.* 2011). There is an increasing trend with heat stress affecting not only the physiology, feed intake and milk production, but also reproduction efficiency of dairy cattle (Chen *et al.* 2018; Shenhe *et al.* 2018). Under heat stress, a sharp decrease in milk yield is observed in high producing dairy cows (Zimbelman *et al.* 2010; Dunn *et al.* 2014), and this

reduction in milk yield is directly linked to low feed intake and reduced digestibility or absorption of nutrients (Boonkum and Duangjinda 2015). It is also reported that reduced flow of nutrients to the udder affects milk production and composition (Puggaard *et al.* 2014). Lower secretion of T_3 (triiodothyronine) or T_4 (thyroxine) affects protein metabolism by suppressing the appetite, and the release of prolactin is also influenced in heat-stressed cows (Cheng *et al.* 2016; Hall *et al.* 2016). Earlier use of GABA (gamma-aminobutyric acid) or betaine improved milk production and milk composition in cows during summer heat conditions (Cheng *et al.* 2016; Hall *et al.* 2016) because these compounds are involved in the metabolism of protein and enhancement of digestibility. CPS has also been used as a supplement for cows to test lipopolysaccharide (LPS) and glucose tolerance; however, no prominent effect on milk yield or milk composition was noted (Blanck *et al.* 2014; Oh *et al.* 2017). Unchanged milk production after CPS supplementation might increase the energy demand by the immune system following LPS challenge or glucose resistance test (Oh *et al.* 2017). In the present study, high milk production with improved milk fat, protein, lactose and urea nitrogen was

achieved in CPS-supplemented cows. Ingestion of CPS in heat-stressed cows might improve the utilisation protein by enhancing rumen efficiency, thereby increasing milk protein and milk urea nitrogen level. The above findings suggest that CPS as a dietary supplement can effectively augment the gastrointestinal environment with an improvement in microbial protein synthesis in cows.

The application of exogenous hormones for the development of follicles with successful ovulation following fixed-time AI is a useful technique for reducing infertility rates in dairy cows during summer (Bilgen and Özenç 2009; Khodaeimotlagh *et al.* 2011; Dirandeh *et al.* 2015). In the present study, effects of the modified Ovsynch protocol in combination with mifepristone and hCG were evaluated on follicular dynamics, oestrus response and pregnancy rate in heat-stressed dairy cows. A better oestrus response with successful ovulation (60%) and pregnancy (40%) rates was achieved during the study. The present data on fertility reveal higher rates than in previous reports where ovulation synchronisation protocols were applied during the heat stress period (Dirandeh 2014; De Rensis *et al.* 2015; Dirandeh *et al.* 2015; El-Tarabany and El-Tarabany 2015). The higher pregnancy rates obtained in present study might be linked with the use of hCG injection post-AI. Accessory corpus luteum (CL) formation and increased CL life in response to hCG injection could be the pregnancy enhancing factor in the present study. The high progesterone secretion following hCG could reduce the incidence of early embryonic mortality during summer. Similar reports are documented where hCG was used for induction of ovulation in heat-stressed anoestrous cows (De Rensis *et al.* 2008; Honparkhe *et al.* 2009; Fischertenhagen *et al.* 2010; Garciaispierto *et al.* 2018). In addition, post-AI hCG in an Ovsynch protocol is reported as an appropriate strategy to improve fertility in cows during summer (de Rensis *et al.* 2008; Honparkhe *et al.* 2009).

On the other hand, achievement of optimal fertility responses following the Ovsynch protocol could be related to CPS supplementation of cows before initiation of the protocol in the present study. Earlier reports indicate that supplementation with dietary yeast significantly improved the pregnancy rates in heat-stressed cows (Dehghanbanadaky *et al.* 2013; Nasiri *et al.* 2018). Those authors speculated that improved metabolic status after yeast supplementation led to development of large ovulatory follicles with high circulation of steroid hormones in heat-stressed cows. CPS is also involved in the regulatory mechanism of the body, boosting the immune response with increased vascularity to internal tissues (Yoshioka *et al.* 2000; Sung Hyen *et al.* 2013). In this context, it has been speculated that the ingestion of CPS might increase the blood flow towards the internal organs (i.e. reproductive system), improving follicle development, meiotic maturation and embryonic development in heat-stressed cows. Incorporation of CPS in the diet of high producing cows could be an ameliorative strategy to improve the application of ovulation synchronisation protocols in terms of oestrus response, ovulation occurrence and pregnancy rate during stressful conditions of the summer season.

The present data on serum glucose, LPL and AST show that CPS has the capacity to improve their levels by enhancing the metabolic functions. The higher serum glucose level at breeding and post-breeding times in heat-stressed cows could be due to CPS supplementation improving the rumen fermentation efficiency. This higher level of glucose at or after AI is a good indicator for early embryo survival and maintenance of pregnancy. In addition, optimum serum glucose is the precondition for lactose synthesis, which maintains the osmotic pressure of milk. This indicates that better serum glucose levels with CPS supplementation enhanced lactose production and subsequent milk yield in lactating cows. The liver efficiency indicators AST and LPL also benefited by CPS in the diet, although, the variation in LPL or AST at the time of AI or post AI were observed which indicates that CPS supplementation might improve the metabolic activity of cows rather than the pathological level (Oh *et al.* 2017; Şekeroğlu *et al.* 2018). Hence, the provision of CPS efficiently regulated the different body functions without affecting liver functions.

Conclusions

In conclusion, CPS supplementation has the capacity to enhance milk production and maintain the metabolic function of cows during the heat exposure period. Moreover, supplementation with CPS is a useful strategy for improving follicular dynamics, oestrus, ovulation and pregnancy rate in synchronised cows during summer.

Conflicts of interests

The authors declare no conflicts of interest.

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