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Research Article

Investigation of the Efficiency of CIDR-PG for Dehong Dairy Buffaloes under Thermoneutral and Heat Stress Conditions

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Abstract | The study investigated the efficiency of CIDR-PGF_2a-based protocol under thermoneutral (spring) and heat stress (summer) conditions. Dehong dairy buffaloes (128) were utilized to analyze the effects of the intravaginal progesterone-releasing insert (i.e., controlled internal drug release [CIDR]) and prostaglandin F_2a (PGF_2a) injection on reproductive performance under high temperatures. Each buffalo received a CIDR with 1.38 g progesterone (day 0) and a 0.6 mg PGF_2a injection on day 10 after CIDR insertion. The CIDR was removed on day 13 and then two artificial inseminations (AI) at 48 hr and 72 hr after the CIDR removal occurred. Average temperature-humidity index exceeding 72 during summer was greater than that in spring. Estrous rates of buffaloes were not significantly different between spring and summer. No difference in day 90 pregnancy/AI (pregnancy status at 90 d after AI) was found in multiparous buffaloes under thermoneutral and heat stress conditions; however, heifers (51.7%) showed greater day 90 pregnancy/AI in spring than in summer (39.6%) (p < 0.05). In total, buffalo day 90 pregnancy/AI in summer (34.1%) was lower than in spring (40.6%) (p <0.05). In conclusion, the administration of progesterone by CIDR and PGF_2a injection improve estrous response and day 90 pregnancy/AI in summer.

Keywords | CIDR-PG, Day 90 pregnancy/AI, Dehong dairy buffalo, Heat stress, Thermoneutral condition

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INTRODUCTION

Dehong buffaloes (*Bubalus bubalis*) have traditionally been used as draught power and for meat production; however, they are now becoming an important source of milk and meat production ever since the European Union conducted hybrid improvement of Dehong buffaloes using frozen sperm of Murrah and Nili-Ravi buffaloes in 1997 (Wang et al., 2007). There are currently 28,000 crossbred buffaloes (swamp × river) that are utilised for milking in Yunnan Province, China. Buffalo milk has a very rich nutritional content and the percentage of milk fat, protein, lactose and solids is 7.73%, 4.49%, 5.57%, and 18.07%, respectively (Tanpur et al., 2012). However, the reproductive performance of Dehong buffaloes is reduced under heat stress conditions. The optimum temperatures and humidity for growth and reproduction are 13–18 °C and 55–65%, respectively (Payne, 1990). Buffaloes, characterized by dark skin, sparse hair and low sweat gland density, are more sensitive to heat stress than cattle (*Bos taurus*) are. The sign of estrus is directly affected by heat stress in buffaloes and the incidence of anestrus is more serious than for cattle during summer in Indian (Kumar et al., 2014).

The main production area in China, Dehong Dai-Jingpo Autonomous Prefecture of Yunnan, is in a subtropical climatic region, which is characterized by high temperatures and high humidity in summer. Our data of dairy herd improvement indicates a higher daily milk yield (4.47 kg) was observed in Dehong dairy buffaloes in spring than for the summer (4.18 kg). More seriously, buffalo anestrus is a severe reproductive problem in hot-summer areas of China. Poor estrus signs cause failure of estrus detection and result in difficulty in determining the suitable time to artificially inseminate, which results in huge economic losses for the dairy industry (Perera, 2011). One previous study reported that the conception rate for river buffaloes (2n = 50) was improved by the injection of progesterone during summer (Kumar and Mandape, 2004). Another study reported CIDR + PG protocol had better efficiency in estrus and pregnancy rate for buffaloes than that of two intramuscular injections of PG (Zhao et al., 2010). They speculated PG plays single role in luteolysis and often causes pseudoestrus (Zhao et al., 2010). Hence, local farmers use an intravaginal progesterone-releasing insert called a controlled internal drug release (CIDR) insertion and prostaglandin $F_2\alpha$ ($PGF_2\alpha$) injection to increase the estrus and pregnancy rates during nonbreeding season, but the efficiency of CIDR depends on many factors including animal nutrition and health condition (Mellieon Jr et al., 2012). Dehong dairy buffaloes (2n=49) are the crossbred offspring of local swamp and river buffalo, and the actual efficiency of CIDR-$PGF_2\alpha$ is not available by now (Zhao et al., 2010). The present study aims to investigate the efficiency of the CIDR-$PGF_2\alpha$-based protocol under thermoneutral (spring) and heat stress (summer) conditions, and the research will benefit to improve the reproductive performances of Dehong dairy buffaloes during the hot-summer area of China.

MATERIALS AND METHODS

All animal handling procedures were approved by the Animal Ethics Committee under the Yunnan Province Animal Welfare Act 2007, China.

Animals, Housing and Management

The experiment was conducted at Dehong State (24.43° N; 98.57° E; 870 m altitude) from March to August 2015. Five buffaloes were loose housed in an open-sided barn (8.0 m × 2.4 m) with an anti-slip concrete floor and a loafing area (8.0 m × 10.0 m) where the concrete surface was connected to the open-sided barns. The indoor area was used as the bedding (feeding) area and the surface of the bedding floor was also made from concrete. The inside feed trough was parallel to the central feeding passage, and the drinking trough was located in the corner of the loafing area. Corn silage was offered to buffaloes and they obtained fresh drinking water ad libitum.

Environmental Measurements

Thermometers were secured 2.0 m above the bedding floor to record the temperature and relative humidity at 30 min intervals. The temperature-humidity index (THI) was calculated from temperature and relative humidity (Tucker et al., 2008):

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

where T is the air temperature (°C) and RH is the relative humidity (%).

Synchronisation of Estrus, Estrous Detection and Artificial Insemination

One group of buffalo synchronization of estrus and artificial insemination in spring was considered as the control group, and another group in summer was set as the experimental group. One hundred and twenty-eight healthy buffaloes (69 heifers and 59 multiparous buffaloes) with normal reproductive organ and body condition score of 3 out of 5 were utilized for synchronization of estrus. Buffaloes received a T-shaped CIDR that contained 1.38 g progesterone (DEC International NZ Ltd) about 50 days after calving, and the date of insertion was called day 0. Buffaloes received a 0.6 mg prostaglandin $F_2\alpha$ injection in the neck on day 10. The CIDR was removed on day 13, followed by detection of estrus and then two artificial inseminations (AI) at 48 h and 72 h after the CIDR removal occurred (Figure 1). Due to 8 buffaloes returning to estrus, only 120 buffaloes were detected as being in the pregnancy state on day 90 after AI.

Figure 1: Schedule of Synchronization of estrus with CIDR and PGF treatments and pregnancy detection for Dehong dairy buffaloes.

Estrus and Pregnancy Detection

Buffalo estrus detection was conducted using visual observation (mounting activity, vaginal mucus and swollen vulva) by a qualified breeder. Frozen-thawed semen exceeding 45% motility were utilized for the AI. Pregnancy status of the dairy buffaloes was checked by rectal palpation with the same breeder on day 90 after AI.

Statistical Analysis

The data were analyzed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). The season variable was defined as spring (thermoneutral condition) and summer (heat stress condition). A non-parametric Mann-Whitney *U* test was applied to compare the effect of season and parity (heifer and multiparous) on buffalo estrous response and day 90 pregnancy/AI (pregnancy status at 90 d after AI, binomial data). The interactions between seasonal variation and parity were analysed with binary logistic regression models. A probability level of $P \leq 0.05$ was considered statistically significant.

RESULTS

Weather variables including air temperature, humidity and calculated THI are summarized in Table 1 and their profiles are shown in Figure 2. The daily average ambient temperature exceeded 25 °C on only 13 out of 92 days in spring, but 41 out of 92 days in summer. Similarly, the daily average THI exceeding 72 occurred 20 out of 92 days in spring and 87 out of 92 days in summer.

Table 1: Environmental conditions in spring and summer

	Spring	Summer
Temperature (°C)	21.7±5.9 [a]	24.9±3.2 [b]
Relative humidity (%)	69.3±29.3 [a]	86.9±16.9 [b]
THI	67.6±6.9 [a]	74.9±3.3 [b]

Superscripts of different letters in the same row indicate a significant difference ($p<0.05$)

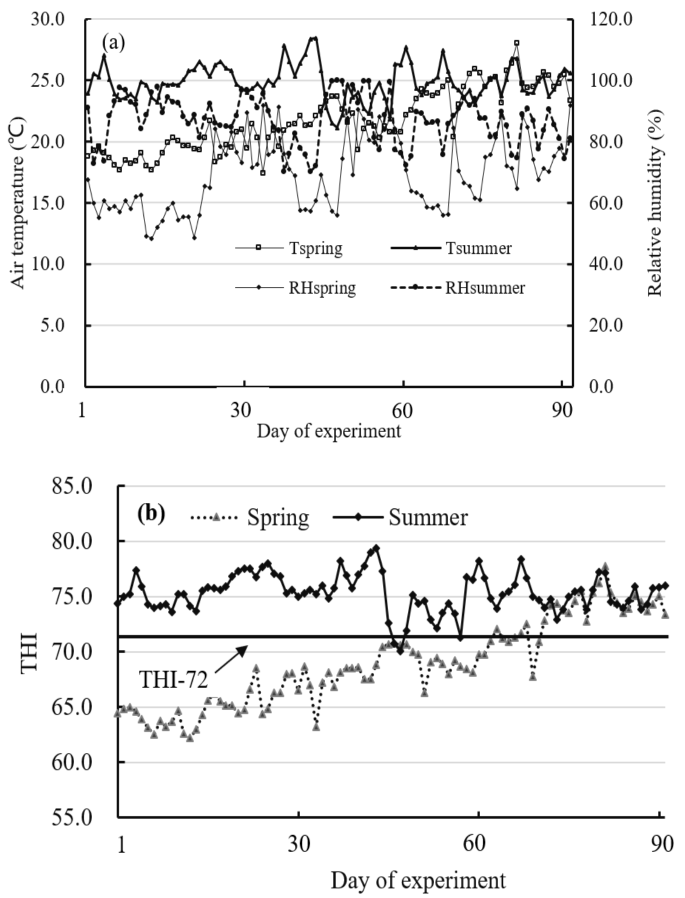

Figure 2: The profile of the indoor daily average temperature (°C), relative humidity (%) and THI. Tspring, Tsummer, RHspring and RHsummer indicated average temperature and relative humidity (%) in spring and summer, respectively.

Reproductive traits including estrous response and day 90 pregnancy/AI are presented in Table 2. The rates of estrus synchronization for buffaloes were 94.1% and 93.6% for the spring and summer group, respectively. No significant difference was found in estrous response (p = 0.35), indicating that the anestrous problem may be alleviated by decreasing the thermal sensibility of dairy buffaloes with hormone protocols. Day 90 pregnancy/AI of buffaloes in spring was greater than in summer. No difference was found in day 90 pregnancy/AI for multiparous buffaloes over the two seasons; however, the day 90 pregnancy/AI for heifers in spring was greater than that of their counterparts in summer ($p<0.05$) (Table 2). Binary logistic regression revealed no significant interaction between parity (heifer and multiparous) and seasonal (spring and summer) factors. The parity had greater effects (Wald = 3.55, 95% CI 0.97 – 4.55, p = 0.06) on day 90 pregnancy/AI compared to season classes (Wald = 0.73, 95% CI 0.62 -3.40, p = 0.39).

Table 2: Estrous response and day-90 pregnancy/AI (%) of dairy buffalo with CIDR + PG protocols in the spring and summer (Mean ± standard error)

		Spring	Summer
Mean age (month)		54.9±27.3	55.8±33.2
Mean parity		1.0±1.3 (0-5)	1.0±1.5 (0-6)
Estrous response %		94.1 (32/34) [a]	93.6 (88/94) [a]
Day-90 pregnancy/ AI, %	Heifer	57.1±12.8 (14) [a]	39.6±6.90 (48) [b]
	Multiparous	27.8±11.3 (18) [a]	27.5±7.6 (40) [a]
	Total	40.6% (13/32) [a]	34.1% (30/88) [b]

Superscripts of different letters in the same row indicate a significant difference ($p<0.05$)

DISCUSSION

The average ambient temperature and THI was lower during spring than summer, restricting estrous of buffaloes (Terzano et al., 2012). We applied CIDR-PGF2a-based protocols to assist estrus synchronization to alleviate the adverse effects of heat stress and seasonal variation on the estrous rate. High temperatures coupled with high relative humidity retards the early embryonic development and decreases the survival rate (Dutt and Jabaro, 1976), and the possible reason is the reactive oxygen species produced by heat stress causes oxidative stress and then inducing apoptosis and restraining embryonic development. We found greater day 90 pregnancy/AI of heifer buffaloes in spring (40.6%) than in summer (34.1%). Possible reasons for this are that heifer buffaloes lack the ability of thermal regulation and their ovarian activity and reproductive hormone profiles were also negatively affected by heat stress (EI-Sawaf et al., 1979; Singh et al., 2012). No interaction was found between parity (heifer and multiparous) and season (spring and summer) factors. Parity had a greater impact on day 90 pregnancy/AI than seasonal variation did, and the possible reason for this might be that CIDR-PGF_2a alleviated the negative effects of heat stress on the pregnancy rate for buffaloes.

There were more number hot days with daily THI values exceeding 72 in summer than that of the spring (Figure 2). THI exceeding 72 decreases the growth, milk yield and reproductive performance of buffaloes. Dash et al. (2015) reported that the threshold THI for pregnancy rate was identified as 75 in Murrah buffaloes (Dash et al., 2015). Although we cannot determine the threshold THI for pregnancy rate in Dehong dairy buffaloes, the average high THI in summer appeared to restrict their day 90 pregnancy/AI, especially for heifer buffaloes. Buffaloes show greater thermal sensitivity compared to cattle, and their oocytes are also more susceptible to heat stress (Neglia et al., 2003). Day 90 pregnancy/AI for heifer buffaloes in spring is greater than in summer (P <0.05), which agrees with previous research (Nakamura et al., 2012). Pinheiro et al. (2009) reported multiparous cows had higher pregnancy rates than primiparous cows, and the possible reason is that their experimental animal were Nellore cows (*Bos indicus*) that have greater thermal tolerance ability. Buffaloes received AI at 48 and 72 hr after hormone treatment, and the day 90 pregnancy/AI was 40.6% and 34.1% during spring and summer, respectively. The pregnancy rates in the two seasons for the present study were lower than the previous report (Haider et al., 2015), and the report indicated the optimal time is 48–60 h in CIDR-GnRH treatment and 60–72 hr in CIDR insertion for buffalo. The difference in pregnancy rate might be because the present study used the combination of CIDR-PGF_2a treatment and conducted AI at 48 hr and 72 hr. The estrous rate was improved with CIDR-PGF_2a protocols; however, the day 90 pregnancy/AI is still low, thereby indicating that the low status cannot be changed only with CIDR-PGF_2a protocols. The provision of shade and / or other cooling facilities may increase the efficiency of CIDR-$PGF_2\alpha$ for Dehong dairy buffaloes (Zhao et al., 2010). The reproductive hormone profiles at different stages can demonstrate the effects of CIDR-PGF_2a therapy on reproductive traits in different parity class and seasons.

In conclusion, the usage of CIDR-PGF_2a had better efficiency in estrous response and day 90 pregnancy/AI of dairy buffaloes; however, the day 90 pregnancy/AI of heifer buffaloes is still low under heat stress conditions. Owing to reproductive performance being influenced by different factors, not only hormone administration is required, but cooling facilities are also needed to obtain higher pregnancy rates for buffaloes during hot summers. In brief, CIDR-PGF_2a protocol may increase the estrus and pregnancy rate for crossbred dairy buffaloes during the non-breeding season in hot-summer area of China.

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

AUTHORS CONTRIBUTION

Zhaobing Gu designed the experiment and wrote the paper. Lin Li did the field experiments, and Huaming Mao analyzed the data.

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