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Effect of Dietary Betaine on Reproductive Performance of Karan Fries Cows during Hot Humid Season

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A B S T R A C T

The objective of this study was to investigate the effect of betaine supplementation in improvement of reproduction parameters in crossbred Karan Fries cows during hot humid season. Eighteen pre-parturient Karan Fries cows were selected and classified into two groups (n=9) as control and treatment (betaine supplemented) on the basis of body weight and parity. Control group was fed basal diet alone and in treatment group along with basal diet, 50g betaine anhydrous (betafin® S1) per cow per day was supplemented. Average Temperature Humidity Index (THI) during whole experimental period was 79.25 ± 0.34 units indicating that all experimental cows were under heat stress. The reproduction parameters studied during the experiment were incidence of retention of fetal membranes (RFM) and metritis, interval from calving to first observed estrus, calving to day of first service, first service conception rate, number of services per conception and service period. The results showed less number of cases of RFM and metritis (11.11%), a significant (P<0.05) reduction in interval from calving to first observed estrus (47.50 ± 7.60 days), calving to day of first service (75.25 ± 2.40 days), significant (P<0.05) increase in first service conception rate (22.22%), significant reduction (P<0.05) in number of services per conception (2.00 ± 0.24) and service period (114.44 ± 6.88 days) in treatment group. It was concluded that betaine supplementation in the diet of Karan Fries cattle results in significant improvement in reproductive performance during hot humid season.

Introduction

India is the largest milk producing country in the world with production of 163.7 million tonnes milk during 2016-17 (BAHS, 2017). Milk production from dairy animals has increased tremendously during the past few decades (FAO, 2011) due to great progress in management related aspects but reproductive performance of high producing dairy herds become dramatically poor (Patton *et al.*, 2006). With the emergence of high producing milch animals, reproductive performance of dairy animals is deteriorating which is further aggravated by the changing climatic conditions reflected in terms of rising ambient temperature all over the world particularly in tropical countries like India. These factors impose more focus over the research and development strategies to mitigate the detrimental effects of heat stress on production and reproduction performance. Heat stress results in lower fertility among dairy cows and affects 60% of the world cattle population (Wolfenson *et al.,* 2000). Hyperthermia due to heat stress directly affects cellular functions of various tissues of reproductive system (Wolfenson *et al.,* 2000). The effect of heat stress on the fertility of cattle is multifactorial (DeRensis and Scaramuzzi, 2003), as high ambient temperature causes reduction in appetite which directly leads to protracted period of negative energy balance (NEBAL). During NEBAL, the metabolism of animal gets disturbed and affects hypothalamic-pituitary-ovarian axis. All these factors lead to poor estrus expression, lengthens period from calving to first observed estrus, increase number of services per conception, poor oocyte quality, compromised uterine health and loss of embryo. Moreover, during summer months service period in Holstein cows has been found to be prolonged (Silva *et al.,* 2017). Incidence of RFM in dairy cattle has been found to be more in summer season which further affects fertility (Sharma *et al.,* 2017). Pregnancy rate in Holstein cattle declines, if there is slight increase in the THI values from threshold (El-Wishy, 2013). Conception rate in lactating dairy cows decreases during summer stress due to high THI during this period (Nabenishi *et al.,* 2011). Lactating Cows inseminated in the period when THI is above 72 causes decline in conception rate (Morton *et al.,* 2007). Heat stress directly affects reproductive organs by disturbing endocrine functions, follicular dynamics, oocyte maturation, hormonal secretions, uterine environment and embryonic development (Edwards and Hansen, 1997). The negative impact of heat stress not only affects the reproductive performance of dairy cows during hot humid season but it also increases incidence of sub-fertility in cows even after the period of hot humid season (Thatcher *et al.,* 2010). The delayed effect or carry over effect of heat stress further affects fertility of dairy animals.

Betaine, a tri-methyl glycine is a novel compound and has been found to ameliorate heat stress in rabbit (Hassan *et al.,* 2011), cattle (Zhang *et al.,* 2014), poultry (Nofal *et al.,* 2015), goat (Dangi *et al.,* 2016) and swine (Cabezon *et al.,* 2017). In ruminants, supplemental betaine has not been found to be completely metabolized by rum en microbes but some of it evades the rum en microbial metabolism to be absorbed and av ailable in intact form for performing various physiological functions (Mitchell *et al.,* 1979). Nakai *et al.,* (2013) proved in a study that 80% of the orally administered betaine to dairy cows reached to duodenum within 12 hours of its administration and is available for intact absorption in the intestine. In biological system, betaine serves as organic osmolyte. Betaine has both positive and negative charge on the molecule thus existing in Zwitterion form at neutral pH (Lever and Slow, 2010). It accumulates inside the cells and permits water retention and thus protects cells from dehydration during heat stress. Betaine also serves as an antioxidant and is found to be involved in impediment of oxidative stress generated during various adverse stress conditions (Ganesan *et al.,* 2010; Alirezaei *et al.,* 2014). Betaine acts as chaperone, as it repairs denatured proteins (Roth *et al.,* 2012) and interacts with molecular chaperones, the heat shock proteins. Betaine gradually reduces heat shock protein expression during heat stress acclimation in goat (*Capra hircus*) (Dangi *et al.,* 2016). The provision of nutritional supplements like betaine to ameliorate the adverse impact of heat stress will be an attractive, economic and novel approach particularly for dairy animals under influence of heat stress. Very few studies have been undertaken till date to assess the effect of supplemental betaine in improving production performance in dairy goats (Fernández *et al.*, 2004) and cattle (Peterson *et al.*, 2012; Zhang *et al.*, 2014) during high ambient temperature. In swine, literature is available regarding the heat stress ameliorating effect of betaine on production (Eklund *et al.*, 2005) and reproduction performance (Stewart *et al.*, 2015). Therefore, the present study was conducted to investigate the effect of dietary betaine on various reproduction parameters of Karan Fries cows during hot humid season.

Materials and Methods

Ethical approval

Experiment was approved and conducted under the established standard of the Institutional Animal Ethics Committee (IAEC) protocol number- 153/16.

Location of experiment

The study was conducted at Livestock Research Center of ICAR-National Dairy Research Institute, Karnal, Haryana, India. Karnal is situated at an altitude of 250 meter above mean sea level, latitude and longitude position being 29°42**"** N and 79°54**"** E, respectively. The maximum ambient temperature in summer goes up to 45°C and minimum temperature in winter comes down to 0°C with a diurnal variation in the order of 15-20 °C.

Selection of experimental animals

Eighteen pregnant Karan Fries cows were selected 21 days pre-partum from Livestock Research Center of the Institute in Karnal. It was ensured that the selected cows were free from any anatomical, physiological and infectious disorders.

Management of experimental animals

The experimental cows of both groups were maintained in individual pens. Cows of both groups were provided with similar conditions and maintained as per the standard conditions of feeding and management followed at NDRI. All the cows were fed a ration consisting of concentrate mixture and roughages. The Voluntary waiting period (VWP) for Karan Fries cows followed at Livestock Research Center is 60 days from calving. After VWP was over, the cows detected in estrus were subjected to first service only when the cervico-vaginal discharge was normal.

Experimental protocol

The selected cows were divided into two equal groups having nine cows in each group on the basis of parity and body weight, viz., C (Control) group and betaine supplemented or Treatment group. Control group was fed basal diet alone and treatment group was fed 50 g betaine (betafin® S1) every day per cow during the whole experimental period i.e. 21 days before and 91 days after calving. The experiment was conducted during hot humid period of summer months, viz., July, August, September and October.

Environment variables

Daily Temperature humidity index (THI) was calculated by recording the dry and wet bulb temperatures throughout the experimental period both in morning and evening by using the formulae: THI = 0.72 (Tdb+ Twb) + 40.6 (NRC, 1971)

Where, Tdb is dry bulb temperature (°C) and Twb is wet bulb temperature (°C). Maximum temperature ($T_{max.}$) (°C), minimum temperature ($T_{min.}$) (°C), maximum relative humidity ($RH_{max.}$) (%) and minimum relative humidity ($RH_{min.}$) (%) were recorded daily during the entire study period and enlisted in Table 1. The average $T_{max.}$ and $T_{min.}$ recorded during the experiment was 32.98 ± 0.19 °C and 23.43 ± 0.36 °C respectively. The average $RH_{max.}$ and $RH_{min.}$ recorded during the study was 88.87 ± 0.51% and 63.01 ± 1.51% respectively, indicating the typical hot and humid environment condition.

The values of $THI_{max.}$ and $THI_{min.}$ at fortnightly interval are presented in Table 2. The average THI recorded during the whole experimental period was 79.25 ± 0.34 units. Our observations of THI indicate an existence of heat stress in both groups of experimental cows during the entire study period because it exceeded the upper critical limit (72 THI units) for cross-bred cattle.

Estimation of Reproduction parameters

The reproduction parameters studied during the experiment were incidence of RFM and metritis, interval from calving to first observed estrus, calving to day of first service, first service conception rate, number of services per conception and service period.

Statistical analysis

Student's t-test was performed for comparison of various reproductive parameters between the control and treatment group using the SPSS statistical software program (version 21.0).

Results and Discussion

Results (Mean ± SE) pertaining to the effect of betaine supplementation on reproduction parameters in Karan Fries cows are presented in Table 3. In the present study, more number of cows (33.3%) of control group was suffered with RFM and metritis. Sharma *et al.,* (2017) in a retrospective study on dairy farm reported high incidence of retention of RFM in summer (29.2%) and spring (27.72%) as compared to during autumn (20.94%) and the incidence observed has been found to be more in pleuriparous cows. In a logistic regression analysis on the data collected from a herd of calved Holstein dairy cows, 39% more incidence of RFM was reported during warm season (Binabaj *et al.,* 2014).

Dubois and Williams (1980) reported an increase in the incidence of retention of fetal membranes and postpartum metritis in dairy cows calved during summer months (24.05 %) suggesting higher susceptibility of cows to post-partum uterine disorders during heat stress. Our results showed that the cows of betaine supplemented group had very low incidence (11.1%) of RFM and metritis. This may be attributed to anti-oxidant and immune-modulatory action of betaine along with its heat stress alleviating effect.

The interval from calving to day of first observed estrus was significantly (P<0.05) shorter in treatment group. Hansen and Arechiga (1999) found that the degree of anovulation and silent ovulations are increased during heat stress. Khodaei-Motlagh *et al.,* (2011) also found that due to overheating of body during high ambient temperature even the cows which are in estrus did not express it. Ramis *et al.,* (2011) found reduction in weaning to estrus interval in sows of betaine supplemented group as compared to control group. Sakatani *et al.,* (2012) stated that duration of estrus and its intensity is reduced during prolonged period of heat stress in dairy cows. These evidences support our results and possibly indicate the role of betaine in shortening the interval of first observed estrus after calving by reducing the adverse effect of heat stress on dairy cows.

The average intervals from calving to day of first insemination were significantly lower ($P<0.05$) in treatment group. Cavestany *et al.*, (1985) found that the average interval from calving to first service become increased during summer indicating the adverse impact of heat stress in lengthening the period from calving to first service. The calving to first service interval basically depends on various factors such as proper estrus expression, good uterine health etc. In our study, betaine supplemented cows showed shorter interval between calving and first insemination which indicates beneficial role of betaine in improving uterine health and strengthening of estrus expression intensity. Consequently, first insemination after calving was performed earlier in cows of treatment group. Moreover, cows suffering from RFM and postpartum uterine disease generally show lengthy calving to first service interval (Dubois and Williams, 1980) as was observed in control group of the present study.

The conception rate on first service was lower in cows of control group as compared to treatment group. It is well established that there are multiple factors which affect first service conception rate. In a retrospective study, it is reported that the conception rate to first service after calving is merely 27% in warm periods as compared to 44% in cold periods (López-Gatius, 2003). Cavestany *et al.*,(1985) observed decline in first service conception rate from 25% to as low as 7% when ambient temprature has increased from 29.7°C to 33.9°C during hot humid season in Holstein cows. Alnimer *et al.*, (2002) reported adverse effect of heat stress on the first service conception rate (18.8%) in dairy cows.

Betaine supplemented cows showed higher first service conception rate which may be due to heat stress alleviating properties of betaine and thereby, improves first service conception rate during adverse impact of heat stress. This may also be associated with the low incidence of RFM and metritis in treatment group suggesting the role of betaine in improving uterine health and subsequently first service conception rate in cows exposed to high ambient temperature.

Table.1 Monthly Mean ± S.E environmental variables of experimental period

Months	T_{max} (°C)	T_{min} (°C)	Tdb max (°C)	Tdb min (°C)	Twb max (°C)	Twb min (°C)	RH_{max} (%)	RH_{min} (%)
July'16	33.28 ± 0.56	26.40 ± 0.25	31.65 ± 0.56	27.85 ± 0.26	27.93 ± 0.33	26.50 ± 0.20	88.35 ± 1.00	72.16 ± 2.08
August'16	32.35 ± 0.33	25.48 ± 0.16	30.88 ± 0.44	27.11 ± 0.22	27.26 ± 0.22	25.89 ± 0.16	90.26 ± 0.90	75.16 ± 1.56
September' 16	33.21 ± 0.26	24.10 ± 0.16	32.49 ± 0.23	25.90 ± 0.16	26.97 ± 0.25	24.59 ± 0.17	89.37 ± 0.82	62.83 ± 1.33
October'16	33.07 ± 0.27	17.77 ± 0.72	32.00 ± 0.29	20.59 ± 0.64	22.58 ± 0.52	19.27 ± 0.66	87.52 ± 1.28	41.87 ± 2.39
Mean ± S.E	**32.98 ± 0.19**	**23.43 ± 0.36**	**31.75 ± 0.21**	**25.36 ± 0.32**	**26.18 ± 0.26**	**24.07 ± 0.32**	**88.87 ± 0.51**	**63.01 ± 1.51**

T_{max}- Maximum temperature; T_{min}- Minimum temperature; Tdb max- Maximum dry bulb temperature; Tdb min- Minimum dry bulb temperature; Twb max- Maximum wet bulb temperature; Twb min- Minimum wet bulb temperature; RH max- Maximum relative humidity; RH min- Minimum relative humidity

Table.2 Fortnightly Mean ± S.E THI of experimental sheds

Fortnight	THI min	THI max	Average THI
1	80.57 ± 0.47	84.23 ± 0.89	82.40 ± 0.60
2	79.04 ± 0.32	82.81 ± 0.70	80.92 ± 0.51
3	78.85 ± 0.40	81.97 ± 0.73	80.41 ± 0.50
4	78.68 ± 0.36	82.92 ± 0.57	80.80 ± 0.51
5	76.60 ± 0.25	83.27 ± 0.18	79.94 ± 0.64
6	77.30 ± 0.35	83.55 ± 0.50	80.43 ± 0.65
7	73.37 ± 1.18	82.17 ± 0.57	77.77 ± 1.04
8	65.49 ± 0.40	77.77 ± 0.34	71.63 ± 1.13
Mean ± S.E	**76.19 ± 0.45**	**82.31 ± 0.27**	**79.25 ± 0.34**

THI-Temperature Humidity Index; THI min- Minimum THI; THI max- Maximum THI

Table.3 Effect of betaine on reproductive performance of Karan Fries cows under heat stress

Reproduction Parameters	Control (n=9)	Treatment (n=9)
Incidence of RFM & metritis	3	1
Interval from calving to first observed estrus (days)	$71.50^{b} \pm 5.21$	$47.50^{a} \pm 7.60$
Calving to first service interval (days)	$102.25^{b} \pm 9.71$	$75.25^{a} \pm 2.40$
First service conception rate (%)	11.11	22.22
Number of services per conception	$2.89^{b} \pm 0.39$	$2.00^{a} \pm 0.24$
Service period (days)	$141.33^{b} \pm 12.97$	$114.44^{a} \pm 6.88$

RFM- Retention of fetal membranes
^{a,b}Means having different superscripts within a row differ significantly (P<0.05)

Number of services per conception was found to be significantly (P< 0.05) lower in treatment group. Cavestany *et al.*, (1985) observed that heat stress results in increased average number of inseminations per conception during summer months (4.5-5.3) as compared to winter months (2.3-3.5). García-Ispierto *et al.*, (2006) also observed that large number of pregnancy losses has occurred in cows conceived during summer than during winter. Ealy *et al.*, (1995) and Edwards and Hansen (1997) found adverse effects of elevated body temperature during heat stress on the embryonic development reflected as demise of more number of embryos and thus, it increases the number of services required for successful conception. Fedota *et al.*, (2017) reported reduction in number of inseminations required for successful conception in betaine supplemented dairy cows (1.2 ± 0.1) as compared to control group (1.8 ± 0.1) during heat stress condition. v an Wettere *et al.*, (2012) observed that there was significant (P<0.05) reduction in the returning rate of sows supplemented with betaine between day 15 and 30 post-insemination and subsequent increase in litter size. Our results regarding the effect of betaine supplementation in reducing the number of services required per conception may be associated with the low incidence of RFM and good uterine health in treatment group and are also in agreement with the findings of Fedota *et al.,* (2017) suggesting the favorable action of dietary betaine in improving conception rate with minimum services.

Service period was significantly (P <0.05) shorter in cows of treatment group. Silva *et al.,* (2017) reported prolonged average service period of 154.2 ± 12.5 days in Holstein cows in tropical savannah during hot humid season. Days open in Holstein Friesen cows has been observed to be more during summer months (167 to 173 days) as compared to other months (99 to 149 days) indicating the adverse effect of heat stress in protracting the service period (Cavestany *et al.,* 1985).

Turk *et al.,* (2015) observed that calving to conception interval in primiparous dairy cows during summer (159 ± 76.4 days) is longer than during winter (91 ± 46.7 days). There are multiple causes which determine the length of service period. In general, any disturbance during the process of uterine involution which may possibly be due to RFM, postpartum metritis, calving disturbances such as still birth, dystocia etc. has been found to have direct impact on increasing the service period. In control group of our study, incidence of RFM and metritis was high as compared to treatment group indicating positive effect of betaine in reducing service period by alleviating heat stress and possibly other causative factors during hot-humid season.

To our knowledge, it is perhaps the first study investigating the effect of betaine supplementation on reproductive performance in dairy cows. Improvement in reproduction parameters as evidenced in our study are in agreement with van Wettere *et al.,* (2012) and Ramis *et al.,* (2011) who also observed improved reproductive performance in betaine supplemented sows and gilts during heat stress condition.

It is concluded that betaine supplementation at the rate of 50 g per cow per day during hot humid season results in improved reproductive performance in Karan Fries cows possibly through ameliorating adverse impact of heat stress. However, further research will be needed with a large sample size to precisely validate the role of dietary betaine in improvement of reproductive performance of dairy cattle.

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