



Dietary betaine reduces incidence of follicular cyst in post-partum Karan Fries cows during hot-humid season

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ABSTRACT

The objective of this study was to investigate the heat stress ameliorating effect of dietary betaine in reducing the risk of development of follicular cyst in dairy cows during hot-humid season. Eighteen pre-parturient Karan Fries (KF) cows in their 2nd to 5th parity were selected and assigned to 2 groups of 9 cows each on basis of parity and body weight. One group of cows was kept as control and other as treatment (50 g betaine/day/cow). Environmental variables and temperature humidity index (THI) of sheds were recorded during the experimental period. Body weight, dry matter intake (DMI), plasma non esterified fatty acids (NEFA) and cortisol levels were measured in both groups at fortnightly interval. Trans-rectal B-mode real time ultrasonography was performed weekly for diagnosis of follicular cyst. Results showed nonsignificant increase in mean DMI (1.86 ± 0.12 kg vs 1.79 ± 0.12 kg per 100 kg body weight) and overall body weight (416.76 ± 10.24 kg vs 411.33 ± 11.07 kg) in treatment group compared to control group. Plasma NEFA concentrations were lower in treatment group as compared to control (329.42 ± 29.08 μ mol/L vs 407.05 ± 15.68 μ mol/L). Plasma cortisol concentrations were significantly lower in treatment group as compared to control group (4.91 ± 0.68 ng/ml vs 6.89 ± 0.49 ng/ml). In control group, 22.22% cows had follicular cyst on their ovaries whereas no follicular cyst was found in betaine supplemented cows. It can be concluded that supplementation of 50 g betaine/cow/day reduces the incidence of follicular cyst along with reduction in plasma NEFA and cortisol levels.

Key words: Betaine, Cortisol, DMI, Follicular cyst, Karan Fries, NEBAL

Cystic ovarian follicles are the significant cause of reduced fertility in dairy cows due to prolonged service period and thereby calving interval. This condition thus reduces reproduction efficiency in dairy cattle and is an indispensable cause of economic losses (Bartlett *et al.* 1986). The incidence of ovarian follicular cyst in dairy cattle may range from 6–30% (Kesler and Bartlett *et al.* 1986, Opsomer *et al.* 1998). However, in indigenous breeds of dairy cows, the incidence is lower ranging from 1–3.70% (Rao 1991). The occurrence of this condition is frequently found during first 30 to 60 days postpartum. This pathological condition is a consequence of disturbed hypothalamic-pituitary-gonadal (HPG) axis which regulates follicular development and its ovulation (De Silva and Reeves 1988). Heat stress directly and indirectly influences HPG axis (De Rensis and Scaramuzzi 2003). During hot months, heat stress decreases appetite and dry matter intake (West *et al.* 2003) thereby prolonging period of negative energy balance (NEBAL). Owing to effect of prolonged

period of NEBAL on HPG axis of high producing dairy cows under heat stress, the incidence of anovulation and cystic ovarian follicles get increased (Roche and Diskin 2001). Stressors like heat stress increase plasma cortisol concentrations and their elevated concentrations favoring development of cystic follicles by acting at two levels, one at pituitary level through alteration of luteinizing hormone (LH) release (Kamel *et al.* 1987) and other at local follicular level through alteration in steroid synthesis and declining concentration of LH receptors (Hsueh and Kubajak 1984).

Various investigations regarding the improvement of reproductive efficiency of dairy cows has been conducted by decreasing the deadly effect of heat stress on reproduction through use of many feed supplements and additives. Among these, betaine is one of the novel and potent candidates, which plays a significant role in alleviating the adverse effect of heat stress effect during warm season in cows (Zhang *et al.* 2014), poultry (Nofal *et al.* 2015), goat (Dangi *et al.* 2016), and swine (Cabezon *et al.* 2017). In ruminants, bioavailability of dietary betaine is almost 80% after its evasion from ruminal microbial digestion (Nakai *et al.* 2013). It performs various vital intracellular activities such as osmolytic action, osmoprotectant and methyl group donation (Eklund *et al.*

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2005). It exists as Zwitter ion at neutral pH and thus accumulates inside the cells where it holds water molecule and thus protects cells from dehydration under various stress conditions (Lever and Slow 2010). It also improves feed intake in dairy cows during heat stress thus salvage them from impaired metabolic effects of NEBAL (Zhang *et al.* 2014).

The pathological condition of cystic ovarian follicles adversely affects the reproduction efficiency of dairy cows. The summer season further increases the frequency of its occurrence especially in newly calved dairy cows by protracting the period of NEBAL and decreases in feed intake. Therefore, present study was proposed to investigate the effect of betaine supplementation on the incidence of follicular cyst in association with plasma cortisol and NEFA levels in post-partum KF cows during hot-humid season.

MATERIALS AND METHODS

Animal ethics approval: Experiment was approved and conducted under the established standard of the Institutional Animal Ethics Committee (IAEC), constituted as per the article number 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India (Reg. No. 1705/GO/ac/CPCSEA Dt. 3/7/2013). IAEC Reg. No. for the present experiment was 153/16.

Location of experiment: The study was conducted at Livestock Research Center of the institute. Karnal is situated at an altitude of 250 m 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 and management of animals: The study was conducted during hot-humid season from July to October. For this study, 18 pre-parturient KF cows were selected from Livestock Research Center of the Institute in Karnal. Cows selected for the experiment were in their second to fifth parity. Cows were weighed before the start of experiment. Thereafter, the cows were assigned to 2 groups of 9 cows each on the basis of parity and body weight. Body weight of cows of both groups was measured at fortnight interval up till 63 days after calving. One group of cows was kept as control (0 g betaine/day/cow) and other as treatment (50 g betaine (betafin® S1)/day/cow). The cows were housed in individual stalls. All the cows were given similar conditions during the whole experimental period. It was ensured that the selected animals for study were free from any anatomical, physiological and infectious disorders.

During the experiment, cows were maintained as per the standard conditions of feeding and management followed at ICAR-NDRI. The feeding was given 2 times daily, i.e. during morning (0700 h) and evening (1700 h). All the cows were fed a ration consisting of concentrate mixture and roughages. During feeding, 50 g betaine was top-dressed in the concentrate mixture in the feed of each

cow of treatment group whereas no betaine was supplemented in the feed of control group cows. Fresh tap water was available for drinking throughout the time to all the cows.

Environmental variables: Daily maximum temperature ($T_{\max}$; °C), minimum temperature ($T_{\min}$; °C), maximum relative humidity ($RH_{\max}$; %) and minimum relative humidity ($RH_{\min}$; %) were recorded during the entire study period.

Temperature humidity index (THI) was calculated by recording the dry and wet bulb temperatures throughout the experimental period daily in morning and evening by using the formulae

$$THI = 0.72 (T_{db} + T_{wb}) + 40.6 \text{ (NRC 1971)}$$

where T_{db} is dry bulb temperature (°C) and T_{wb} is wet bulb temperature (°C).

Estimation of dry matter intake (DMI): Fortnightly DMI of KF cows were estimated during pre-partum and post-partum by recording the feed offered and feed refusal. A total of 5 fortnight periods were investigated for recording DMI in both groups. The DM content of different feed composition was estimated fortnightly. The DMI of KF cows in both the group (control and treatment) at fortnightly interval were recorded for 3 consecutive days and average was considered as DMI of the fortnight. DMI intake of each cow of both groups was calculated as DMI/100 kg body weight.

Blood sampling and analysis: Blood samples were collected at fortnight interval at 0700 h before feeding in sterile EDTA vacutainer tubes (BD Vacutainer®). Blood sample (5 ml) was drawn from jugular vein puncture of each cow in both groups, with intention of minimum disturbance to cows. Blood plasma was harvested by immediately centrifuging the EDTA containing blood samples at 2,500 g for 15 min at 4°C. Plasma samples were stored at –20°C.

Blood plasma NEFA concentrations were estimated at fortnight interval (21 days pre-partum to 35 days post-partum) by using 'Bovine NEFA ELISA kit' (Shanghai Sun red Biologicals, China). The sensitivity of NEFA assay was 1.662 ng/ml and assay range was 2 ng/ml to 600 ng/ml.

Similarly, blood plasma cortisol concentrations were also assessed at fortnight interval (21 days pre-partum to 49 days post-partum) by using Bovine Cortisol ELISA kit (Shanghai Sun red Biologicals, China). The sensitivity of cortisol assay was 1.779 µmol/L and assay range was 2 µmol/L -600 µmol/L.

Determination of ovarian cyst by ultrasonography: Both right and left ovaries of each and every cow of both groups were monitored for diagnosis of any follicular cyst with the help of ultrasonography (USG). USG was performed weekly, starting from second week post-partum up to 9 weeks. Trans-rectal approach was followed using a B-mode, real-time portable ultrasound scanner equipped with two kinds of linear array transducer, a 7.5 MHz and a 5 MHz (Prosound- 2 ALOKA Ltd., Japan).

Statistical analysis: For statistical analysis, student's t-

test was performed for statistical comparison between the control and treatment group at different days after parturition when comparing fortnightly body weight, DMI/100 kg body weight, plasma cortisol and plasma NEFA. Two way ANOVA was performed for analyzing the changes in DMI within days post-partum. Post hoc difference within days post-partum was examined by the Duncan test. Bivariate Pearson Correlation analysis was done for estimating the association between NEFA and DMI as well as NEFA and body weight. The statistical analysis was performed by using SPSS statistical software program (version 21.0). The data were reported as mean $\pm$ SE and differences were regarded as significant at $P<0.05$.

RESULTS AND DISCUSSION

The overall mean of environmental temperature varied from minimum temperature of $23.43\pm0.36^{\circ}\text{C}$ to a maximum temperature of $32.98\pm0.19^{\circ}\text{C}$. The overall mean of RH of the environment varies from minimum RH of $63.01\pm0.51\%$ to a maximum RH of $88.87\pm0.51\%$ (Table 1). THI varies from maximum of 82.31 ± 0.27 units and minimum of 76.19 ± 0.45 units (Table 2). The values of environmental variable and THI of the sheds recorded during the present study reveals that all the cows of our

Table 1. Fortnightly mean $\pm$ SE environmental variables of experimental period

Fortnight	T _{min} ($^{\circ}\text{C}$)	T _{max} ($^{\circ}\text{C}$)	RH _{min} (%)	RH _{max} (%)
1	26.59 $\pm$ 0.42	33.88 $\pm$ 0.90	69.27 $\pm$ 2.43	87.00 $\pm$ 1.38
2	26.23 $\pm$ 0.28	32.72 $\pm$ 0.70	74.25 $\pm$ 3.08	89.63 $\pm$ 1.41
3	25.71 $\pm$ 0.21	31.77 $\pm$ 0.58	78.00 $\pm$ 2.71	90.80 $\pm$ 1.49
4	25.26 $\pm$ 0.22	32.90 $\pm$ 0.31	72.50 $\pm$ 1.52	89.75 $\pm$ 1.07
5	24.47 $\pm$ 0.11	32.57 $\pm$ 0.31	66.87 $\pm$ 1.46	89.07 $\pm$ 1.45
6	23.73 $\pm$ 0.26	33.84 $\pm$ 0.34	58.80 $\pm$ 1.71	89.67 $\pm$ 0.83
7	20.82 $\pm$ 0.98	34.06 $\pm$ 0.15	49.47 $\pm$ 3.59	88.13 $\pm$ 1.88
8	14.92 $\pm$ 0.25	32.14 $\pm$ 0.39	34.75 $\pm$ 1.99	86.94 $\pm$ 1.80
Mean $\pm$ SE	23.43 $\pm$ 0.36	32.98 $\pm$ 0.19	63.01 $\pm$ 0.51	88.87 $\pm$ 0.51

T_{max}, Maximum temperature; T_{min}, minimum temperature; RH_{max}, maximum relative humidity; RH_{min}, minimum relative humidity.

Table 2. Fortnightly mean $\pm$ SE THI of experimental sheds

Fortnight	Db min ($^{\circ}\text{C}$)	Wb min ($^{\circ}\text{C}$)	Db max ($^{\circ}\text{C}$)	Wb max ($^{\circ}\text{C}$)	THI min	THI max	THI (Mean $\pm$ SE)
1	28.50 $\pm$ 0.35	27.01 $\pm$ 0.32	32.47 $\pm$ 0.82	28.13 $\pm$ 0.50	80.57 $\pm$ 0.47	84.23 $\pm$ 0.89	82.40 $\pm$ 0.60
2	27.24 $\pm$ 0.32	26.15 $\pm$ 0.22	30.89 $\pm$ 0.75	27.74 $\pm$ 0.43	79.04 $\pm$ 0.32	82.81 $\pm$ 0.70	80.92 $\pm$ 0.51
3	27.13 $\pm$ 0.36	25.99 $\pm$ 0.21	30.34 $\pm$ 0.71	27.12 $\pm$ 0.32	78.85 $\pm$ 0.40	81.97 $\pm$ 0.73	80.41 $\pm$ 0.50
4	27.09 $\pm$ 0.28	25.80 $\pm$ 0.23	31.39 $\pm$ 0.51	27.39 $\pm$ 0.30	78.68 $\pm$ 0.36	82.92 $\pm$ 0.57	80.80 $\pm$ 0.51
5	25.67 $\pm$ 0.16	24.33 $\pm$ 0.22	32.12 $\pm$ 0.20	27.15 $\pm$ 0.14	76.60 $\pm$ 0.25	83.27 $\pm$ 0.18	79.94 $\pm$ 0.64
6	26.13 $\pm$ 0.26	24.84 $\pm$ 0.25	32.85 $\pm$ 0.40	26.80 $\pm$ 0.48	77.30 $\pm$ 0.35	83.55 $\pm$ 0.50	80.43 $\pm$ 0.65
7	23.41 $\pm$ 0.76	22.11 $\pm$ 0.88	32.99 $\pm$ 0.15	24.75 $\pm$ 0.72	73.37 $\pm$ 1.18	82.17 $\pm$ 0.57	77.77 $\pm$ 1.04
8	17.95 $\pm$ 0.35	16.61 $\pm$ 0.23	31.08 $\pm$ 0.43	20.55 $\pm$ 0.16	65.49 $\pm$ 0.40	77.77 $\pm$ 0.34	71.63 $\pm$ 1.13
Mean $\pm$ SE	25.36 $\pm$ 0.32	24.07 $\pm$ 0.32	31.75 $\pm$ 0.21	26.18 $\pm$ 0.26	76.19 $\pm$ 0.45	82.31 $\pm$ 0.27	79.25 $\pm$ 0.34

Db min, Minimum dry bulb temperature; Db max, maximum dry bulb temperature; Wb min, minimum wet bulb temperature; Wb max, maximum wet bulb temperature; THI, temperature humidity index; THI min, minimum THI; THI max, maximum THI.

experiment were under heat stress. McDowell *et al.* (1976) classified THI as comfortable (≤ 70), stressful (71–78) and extreme distress (> 78). The overall THI of our experimental sheds during the period of experiment was recorded as 79.25 ± 0.34 units which are far above the comfort zone which indicates that cows experienced severe heat stress during the period of experiment.

The overall mean of DMI during 5 fortnights in control group was 1.79 ± 0.12 kg/100 kg body weight and in treatment group was 1.86 ± 0.12 kg/100 kg body weight with no significant difference was found between control and treatment group (Table 3). However, mean DMI intake in cows of both groups increase from 1.56 ± 0.009 kg/100 kg body weight at 21 days before parturition to 2.17 ± 0.041 kg/100 kg body weight at 35 days after parturition which significantly ($P<0.05$) differed within days post-partum. The concentrations of NEFA in blood plasma of control cows at 21 days postpartum were 419.66 ± 64.03 $\mu\text{mol/L}$ vs. 249.60 ± 66.12 $\mu\text{mol/L}$ in treatment group which significantly ($P<0.05$) differed (Table 4). At 35 days postpartum in control cows, the plasma NEFA concentrations were 384.06 ± 63.54 $\mu\text{mol/L}$ vs. 235.94 ± 50.99 $\mu\text{mol/L}$ in treatment group which also significantly ($P<0.05$) differed. The overall mean NEFA concentrations during the 5 fortnight periods were lower in treatment group as compared to control group (329.42 ± 29.08 $\mu\text{mol/L}$ vs. 407.05 ± 15.68 $\mu\text{mol/L}$). However, the difference was non-significant. The overall mean body weight was more in treatment group (416.76 ± 10.24 kg vs. 411.33 ± 11.07 kg) as compared to control group. However, difference was non-significant. In the present study, incidence of follicular cyst was 22.22% in control group (Table 5). However, no case was reported in betaine supplemented group.

High ambient temperature causes subfertility in dairy cows which may be directly and indirectly associated with large number of factors (De Rensis *et al.* 2017). The reason for declined fertility during heat stress in cows may be associated with reduction in DMI and prolonged NEBAL. One of the most important causes of infertility in dairy cows is the development of cystic ovarian condition and this condition in general, most commonly found in cows which

are in NEBAL (Beam and Butler 1999). A large number of cows with cystic ovaries remain in prolonged anestrus which results in lengthened service period and thus calving interval (Fourichon *et al.* 2000). All these factors decrease reproductive efficiency of dairy cows. The susceptibility for development of ovarian cyst increases during summer months (Lopez-Diaz and Bosu 1992, Stradaioli *et al.* 1994, Ronchi *et al.* 2001). López-Gatius *et al.* (2002) reported 2.6 times more incidence of development of follicular cyst in cows calving during warm season. Beam and Butler (1999) reported that negative energy balance which is indicated by high concentrations of NEFA and BHBA affects ovarian function. Heuer *et al.* (1999) observed that cows which undergo NEBAL are more susceptible to development of cystic ovarian follicles. Diskin *et al.* (2003) and Lucy (2003) stated that NEBAL affects both at the level of hypothalamic-pituitary-ovarian axis and at ovarian follicles by altered metabolic and endocrine mechanisms. Ronchi *et al.* (2001) observed that heat stress causes 23% reduction in DMI, along with development of cystic follicles in heifers. Baumgard *et al.* (2006) also stated that heat stress

Table 3. Fortnightly mean±SE DMI/100 kg body weight of KF cows

Days	Control (kg)	Treatment (kg)	Mean±S.E
-21	1.56±0.09	1.57±0.13	1.56 ^a ±0.009
-7	1.54±0.05	1.68±0.13	1.60 ^{ab} ±0.068
7	1.72±0.05	1.73±0.13	1.72 ^{abc} ±0.002
21	2.04±0.13	2.11±0.11	2.07 ^{bc} ±0.036
35	2.13±0.17	2.21±0.10	2.17 ^c ±0.041
Mean±S.E	1.79±0.12	1.86±0.12	

^{a,b,c}Means having different superscripts within a column differ significantly (P<0.05).

Table 4. Fortnightly mean±SE plasma NEFA level (µmol/L) of KF cows

Days	Control	Treatment
-21	341.94±55.81	339.68±70.72
-7	440.48±58.76	357.77±75.06
0	443.03±59.83	384.62±67.14
7	413.14±71.25	408.88±69.31
21	419.66 ^b ±64.03	249.60 ^a ±66.12
35	384.06 ^b ±63.54	235.94 ^a ±50.99
Mean±S.E	407.05±15.68	329.42±29.08

^{a,b}Means having different superscripts within a row differ significantly (P<0.05).

Table 5. KF cows suffered from condition of follicular cyst

Experimental cows	Total cows (n)	Cows with follicular cyst	Percentage of cows suffered with follicular cyst
Control	9	2	22.22%
Treatment	9	0	0%

causes reduction in energy intake in lactating cow, which results in most of the cows entering in the state of NEBAL which is particularly more pronounced during early part of lactation and is generally associated with metabolic loads for high production performance. In our study, we found that DMI was lower in control group as compared to betaine supplemented group. However, the difference was non-significant. The numerical increase in DMI in betaine supplemented cows may be due to the heat stress alleviating properties of betaine which improve appetite and DMI in treatment group. Our results pertaining to increase in DMI in cows of treatment group are in agreement with Zhang *et al.* (2014) who found significant increase in feed intake in betaine supplemented dairy cows as compared to control. Richards *et al.* (1989) reported that in Hereford cows, reduced feed intake results in high levels of NEFA in blood plasma along with reduction in their body weights. Our results showed that cows of control group had reduced mean DMI, low mean body weight and high NEFA concentrations in blood plasma as compared to betaine supplemented cows. These conditions probably made dairy cows to be more susceptible for the development of follicular cyst. Our results are consistent with Wang *et al.* (2010) who found decreased plasma concentrations of NEFA in betaine supplemented lactating cows. Zhang *et al.* (2014) found nonsignificant increase in body weight of betaine supplemented cows as compared to control group during heat stress.

Bivariate Pearson correlation was analyzed between DMI and NEFA as well as body weight and NEFA. Bivariate Pearson correlation showed negative association between DMI and NEFA in both groups (Table 6). The value of correlation coefficient was weak negative for control group ($r=-0.070$; $P>0.05$) as compared to treatment group ($r=-0.312$, $P>0.05$). Stronger negative correlation between DMI and NEFA for treatment group as compared to control group indicated that value of NEFA declines at faster rate with increase in DMI in treatment group and thus betaine probably plays an important role in improving energy balance in cows of treatment group during heat stress. The value of correlation coefficient was more positive between NEFA and body weight control group ($r=0.265$, $P>0.05$) as compared to treatment group ($r=0.173$; $P>0.05$) which indicated that despite increase in body weight with increase in period from parturition, decline in NEFA was slow in control group. This revealed that betaine has favorable effect in improving the energy balance of betaine supplemented group.

The overall mean plasma cortisol concentrations were significantly ($P<0.05$) lower in cows of treatment group as compared to control group (Table 7). Cows which were taken for our experiment were transition cows. Therefore, cows of both groups had experienced gestational stress, parturition stress and lactation stress besides heat stress. During stress conditions, adrenal glands become more active under influence of acute release of ACTH from pituitary gland. The concentrations of plasma cortisol

increased under such stressful situations (Lopez-Diaz and Bosu 1992). Melendez *et al.* (2003) observed that cows developing lameness after parturition showed high levels of stress metabolites (ACTH, cortisol) which may favor development of cystic follicles or persistent large follicles. Amweg *et al.* (2013) found that ACTH stimulation caused induction of high local follicular cortisol production, which played a significant role in pathogenesis of cystic ovarian disease. In an *in-vitro* culture of bovine follicular walls, it is found that ACTH causes secretion of cortisol from cultured tissues (Amweg *et al.* 2011). Therefore, these results consistently revealed that ACTH release during stressful situations like heat stress causes endogenous cortisol production at local follicular levels and may involve in development of cystic follicles. Our results showed that in betaine supplemented cows, the concentrations of cortisol in blood plasma were significantly lower ($P < 0.05$) indicating the stress mitigating action of betaine and thus protecting cows from the risk of development of follicular cyst. Although it was reported that betaine is involved in eliminating various stressful situations triggered by various stressors like heat stress, high salinity stress, etc. at cellular level (Craig 2004).

It can be concluded that supplementation of 50 g of betaine/cow/day reduces the incidence of follicular cyst along with reduction in plasma NEFA and cortisol levels. Therefore, betaine supplementation in dairy cows helps in improving reproductive efficiency in dairy cows particularly during stressful situation of hot-humid condition. This is

Table 6. Correlation coefficient between DMI, NEFA and body weight

Parameters	Control		Treatment	
	Value of <i>r</i>	Level of significance	Value of <i>r</i>	Level of significance
DMI and NEFA	-0.070	ns	-0.312	ns
Body weight and NEFA	0.265	ns	0.173	ns

ns, nonsignificant.

Table 7. Fortnightly mean $\pm$ SE plasma cortisol level (ng/ml) of KF cows

Days	Control	Treatment
-21	6.69 $\pm$ 1.04	6.45 $\pm$ 1.12
-7	7.13 ^b $\pm$ 1.40	4.35 ^a $\pm$ 0.32
0	9.58 $\pm$ 1.10	8.16 $\pm$ 0.59
7	6.90 ^b $\pm$ 1.05	4.81 ^a $\pm$ 0.36
21	6.47 ^b $\pm$ 1.12	3.78 ^a $\pm$ 0.69
35	5.91 ^b $\pm$ 1.22	3.52 ^a $\pm$ 0.44
49	5.58 ^b $\pm$ 1.06	3.28 ^a $\pm$ 0.33
Mean $\pm$ S.E	6.89 ^b $\pm$ 0.49	4.91 ^a $\pm$ 0.68

^{a,b}Means having different superscripts within a row differ significantly ($P < 0.05$)

perhaps the first report elucidating the effect of dietary betaine in reducing the incidence of follicular cyst in association with plasma NEFA and cortisol levels in postpartum KF cows during hot-humid season. However, a large sample size is still needed to validate our results.

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