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Supplementing Rumen Protected Choline with Green Tea Extract Improves Reproductive Performances in Transition Karan Fries Cows

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ABSTRACT

The present experiment was carried out on thirty two pregnant Karan Fries (KF) cows. In control group, cows were fed basal diet. In T1 each cow was fed rumen protected choline (RPC) (55g/day), in T2 - green tea extract (GTE) (3g/d) and in T3-RPC + GTE (55+3)g/day along with basal diet. The duration of experiment was 30 days before calving to 60 days after parturition. Animals were evaluated on their reproductive performances. Diameter of cervix, uterine horn and service period reduced significantly ($p \leq 0.01$) in the treatment groups compared to control group. Conception rate was highest for T3 (75%) followed by T2 (62.5%), T1 (62.5%) and C (50%). There were lesser incidences of reproductive disorders in treatment groups. In conclusion, feeding of RPC and GTE in combination improved reproductive performance during transition period in Karan Fries cows.

Key words: Green tea extract, Reproductive performances, Rumen protected choline.

INTRODUCTION

The transition period, roughly stretching from 3 weeks before to 3 weeks after parturition (Cetin *et al*., 2018), is a difficult period for high-yielding dairy cows and is characterized by a high incidence of metabolic, infectious, and reproductive disorders (Goff and Horst, 1997).

Green tea (*Camellia sinensis* L.) of the family 'Theaceae' is one of the most popular beverages in the world. The beneficial properties of green tea are due to the abundant polyphenolic compounds ("catechins") that it contains. The green-tea catechins include catechin (C), (2)-epicatechin (EC), (2)-epigallocatechin (EGC), (2)-epicatechin-3-gallate (ECG) and (2)-epigallocatechin-3-gallate (EGCG). Out of these, EGCG is a predominant catechin, and has several biological and pharmacological properties (Hodgson *et al*., 2013). Green tea extract is known to be a potent anti oxidant and anti inflammatory agent which helps in reducing inflammation and stress in liver in dairy cows (Winkler *et al*., 2015). After analyzing several literatures, choline has the characteristics to reduce fatty liver condition and due to its presence at biological structures, it might help in recovery of reproductive tracts after parturition. As of green tea is concern, its potent antioxidant might help the animals to recover from critical transition period and prevent from fatty liver. With this aim, the present study has been designed to study the 'effect of supplementation of rumen protected choline (RPC) and green tea extract (GTE) on reproductive performance during transition period in Karan Fries cows.

MATERIALS AND METHODS

The study was conducted in the experimental cattle shed of National Dairy Research Institute (NDRI), Karnal, India which is located at 29° 42″ 20 sec N and 76° 58″ 52.5 sec E at an altitude of 834 feet ASML. The sample analysis was done in Feed Processing and Quality Control Laboratory and Precision Instrument Laboratory of Dairy Cattle Nutrition Division, NDRI, Karnal. The RPC, in the form of encapsulation with fatty acids and prepared by spray freeze drying technology, was purchased from Kemin animal nutrition, India. The green tea extract (GTE) was purchased from Sarthak Herbs, Karnal, India.

The experiment was approved by the Institutional Animal Ethical Committee (IAEC) of Indian Council of Agricultural Research (ICAR) – National Dairy Research Institute (NDRI) constituted as per article 13 of the CPCSEA rules, laid down by the Govt. of India (Regd no-1705/GO/al/13 CPCSEA) dated 3/7/2013. All the ethical guidelines were followed during the course of the experiment.

Thirty two pregnant dairy cows in second to fourth lactation with most probable production ability (MPPA) of around 4000 L milk production were selected from the herd,

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maintained at Cattle Yard, NDRI, Karnal. They were randomly divided into four (4) groups based on their MPPA and lactation number. Their requirements were fulfilled by feeding concentrate mixture, green fodder (sorghum, maize, oats, sugar graze) and dry roughage (wheat straw) based on National Research Council (NRC, 2001), recommendations.

The experimental groups and supplementation of RPC and GTE were as follows.

Groups	Feeding schedule
Control	diet without supplementation
Treatment 1	Basal diet with Rumen Protected Choline (RPC) (55 gram/day)
Treatment 2	Basal diet with Green Tea Extract (GTE) (3 gram/day)
Treatment 3	Basal diet with RPC (55 gram/day) + GTE (3 gram/day)

The animals were put on the experiment around 37 days before expected date of parturition and given adaptation period of seven days. The duration of experiment was 30 days before calving to 60 days after parturition. Total duration of the experiment was 90 days.

To see the effect of RPC and GTE on reproductive traits of KF cows, the following parameters were considered- a) days to first observed heat b) service period c) number of services per conception d) conception rate e) pregnancy rate. Trans rectal ultrasonography (Prosound, Akola Ltd., Japan) was used to determine the diameter of cervix, uterine horn, number of follicles at 10, 20, 30, 40 and 50 days interval after parturition. The diameter of follicle greater than 10 mm was considered as large follicle, between (5-9) mm as medium follicle and between (2-4) mm as small follicle. The occurrence of reproductive disorders like retention of placenta (ROP), still birth, dystocia, uterine infections (metritis, endometritis, pyometra) and any other changes associated with calving were recorded. For this, the help of experts in the field of veterinary medicine and veterinary gynecology in the Livestock research centre (LRC), NDRI was taken for disease diagnosis and treatment.

Analysis of data was carried out using one way analysis of variance (ANOVA) techniques (using GRAPHPAD PRISM software) to interpret the effect of dietary treatments on various parameters and presented as mean ± SE. Values of $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

The days to first observed heat i.e., commencement of cyclicity was comparatively ($p \leq 0.05$) lower in T3 treated cows than that of control (Table 1). Pandurang *et al*. (2012) also noted, commencement of cyclicity was comparatively lower (62.44 d) in RPC treated cows than that of control (65.13 d). Conversely, Lima *et al*. (2007) observed that, supplementation of RPC did not influence cyclicity. The average values of service period was decreased significantly ($p \leq 0.01$) in T1, T2 and T3 in comparison to control. Our result is in agreement with previous workers (Pirestani *et al*., 2011 and Pandurang *et al*., 2012) who also observed reduction in service period. No difference was found for 'number of services per conception' across the groups. This is in agreement with Lima *et al*. (2007) and Pandurang *et al*. (2012) who observed no significant difference in RPC supplemented group for this trait. However, other workers (Ardalan *et al*., 2009 and Pirestani *et al*., 2011) observed decrease ($p \leq 0.05$) in number of services required for conception in RPC supplemented group. The conception rate varied from 50 percent in control to 62.5 percent in T1 and T2 and highest rate was marked for T3 (75 percent). Pregnancy rate was higher in T3 group than control (40% vs. 65%) (Table 1). This is in agreement with Lima *et al*. (2007) and Pandurang *et al*. (2012) who observed no significant difference in RPC supplemented group for this trait. However, other workers (Ardalan *et al*., 2009 and Pirestani *et al*., 2011) observed decrease ($p \leq 0.05$) in number of services required for conception in RPC supplemented group. Roth *et al*. (2008) showed that *in vivo* administration of the antioxidant EGCG improves developmental competence and the quality of the embryos that develop from oocytes in mice. In another study, cows receiving embryos treated with 15 mM GTP had higher pregnancy rates on day 30 (34.8% vs. 28.6%) and day 60 (34.8% vs. 23.9%) than those receiving control embryos ($P < 0.05$) and hence improved pregnancy rates (Wang *et al*., 2013). There are no reports available regarding combined effect of RPC and GTE on the above mentioned reproductive traits in dairy cows. Phosphatidylcholine, being an integral part of cell membrane is very much essential for the repair of damaged reproductive tract and choline is required for the synthesis of phosphatidylcholine. So, RPC might be required for repair of tissues and helps in enhancing reproductive performance.

Table 1: Effect of RPC and GTE supplementation on Reproductive traits of KF cows.

Parameters	C	T1	T2	T3
Days to first observed heat*	101.50^{b} ± 8.08	93.63^{b} ± 3.27	86.75^{ab} ± 6.26	76.13^{a} ± 2.45
Service period* (days)	144.25^{c} ± 3.18	115.63^{b} ± 3.27	108.76^{ab} ± 6.17	97.38^{a} ± 2.43
No of services/conception	2.50 ± 0.19	2.25 ± 0.16	2.13 ± 0.13	2.13 ± 0.23
Conception rate	50%	62.5%	62.5%	75%
Pregancy rate	40%	52.5%	52.5%	65%

Mean bearing different superscripts between the treatments differ significantly.

Data represented as mean ± SE (*P<0.05).

Starting from 20th day to 50th day, the treatments induced reduction in the size of the cervix ($p \leq 0.05$) and uterine horn ($p \leq 0.01$) significantly than control (Table 2, 3). No study has been conducted to know the effect of RPC or GTE on status of cervix, uterine horn and follicles. The diameter of cervix significantly reduced ($p \leq 0.05$) in T3 than control. Improved humeral immunity in T3 due to combined action of RPC and GTE might have provided greater protection against uterine infection and hence, faster recovery and early involution in T3 than control. The diameter of uterine horn significantly reduced ($p \leq 0.01$) in T3 than control by 50th days of parturition. It might be due to both RPC and GTE in combination provided strong immune boosting and antioxidant profile that helped in retaining the

Table 2: Effect of RPC and GTE supplementation on Cervix Diameter (cm) of KF cows.

Days	C	T1	T2	T3
10	5.80 ± 0.46	5.88 ± 0.10	5.58 ± 0.08	5.71 ± 0.08
20**	5.19^{c} ± 0.06	4.98^{bc} ± 0.11	4.73^{a} ± 0.06	4.76^{ab} ± 0.06
30*	4.39^{b} ± 0.15	4.11^{ab} ± 0.13	4.06^{ab} ± 0.03	3.85^{a} ± 0.09
40*	3.85^{b} ± 0.11	3.50^{a} ± 0.14	3.53^{a} ± 0.08	3.34^{a} ± 0.08
50*	3.71^{b} ± 0.15	3.40^{ab} ± 0.12	3.33^{a} ± 0.08	3.14^{a} ± 0.09

Mean bearing different superscripts between the treatments differ significantly.
Data represented as mean ± SE (**P<0.01, *P<0.05).

Table 3: Effect of RPC and GTE supplementation on Uterine Horn of KF cows.

Days	C	T1	T2	T3
10	5.69 ± 0.08	5.75 ± 0.07	5.66 ± 0.07	5.58 ± 0.14
20**	5.03^{b} ± 0.08	4.61^{a} ± 0.12	4.66^{a} ± 0.08	4.45^{a} ± 0.09
30*	3.8^{b} ± 0.17	3.68^{b} ± 0.17	3.55^{ab} ± 0.13	3.18^{a} ± 0.10
40**	2.95^{b} ± 0.07	2.74^{b} ± 0.12	2.69^{b} ± 0.11	2.35^{a} ± 0.07
50**	2.49^{c} ± 0.12	2.26^{bc} ± 0.12	2.15^{b} ± 0.09	1.81^{a} ± 0.07

Mean bearing different superscripts between the treatments differ significantly.
Data represented as mean ± SE (**P<0.01, *P<0.05).

Table 4.1: Effect of RPC and GTE supplementation on Large follicle number of KF cows.

Days	C	T1	T2	T3
10	0.38 ± 0.26	0.50 ± 0.38	0.75 ± 0.31	0.88 ± 0.48
20	0.75 ± 0.31	0.63 ± 0.26	0.88 ± 0.29	0.75 ± 0.31
30	0.50 ± 0.38	0.88 ± 0.39	0.75 ± 0.41	1.00 ± 0.50
40	0.75 ± 0.31	0.75 ± 0.37	0.63 ± 0.38	1.00 ± 0.42
50	1.13 ± 0.29	1.25 ± 0.25	1.38 ± 0.26	1.50 ± 0.27

Table 4.2: Effect of RPC and GTE supplementation on Medium follicle number of KF cows.

Days	C	T1	T2	T3
10	0.63 ± 0.26	0.63 ± 0.26	0.75 ± 0.25	0.63 ± 0.26
20	1.00 ± 0.33	1.00 ± 0.33	1.13 ± 0.35	1.25 ± 0.45
30	1.13 ± 0.29	1.13 ± 0.39	1.25 ± 0.41	1.38 ± 0.46
40*	1.00^{a} ± 0.33	1.50^{ab} ± 0.27	1.50^{ab} ± 0.33	2.38^{b} ± 0.38
50*	1.25^{a} ± 0.31	1.88^{ab} ± 0.23	1.75^{ab} ± 0.31	2.50^{b} ± 0.19

Table 4.3: Effect of RPC and GTE supplementation on Small follicle number of KF cow.

Days	C	T1	T2	T3
10	0.50 ± 0.27	0.75 ± 0.31	0.63 ± 0.26	0.88 ± 0.29
20	1.00 ± 0.53	1.25 ± 0.77	1.38 ± 0.49	1.25 ± 0.37
30	1.13 ± 0.67	1.38 ± 0.38	1.38 ± 0.38	1.50 ± 0.38
40*	0.88^{a} ± 0.44	1.88^{ab} ± 0.48	1.75^{ab} ± 0.37	2.88^{b} ± 0.44
50*	1.25^{a} ± 0.41	2.38^{ab} ± 0.49	2.50^{ab} ± 0.27	3.63^{b} ± 0.82

Mean bearing different superscripts between the treatments differ significantly.
Data represented as mean ± SE (**P<0.01, *P<0.05).

Table 5: Effect of RPC and GTE supplementation on incidence of reproductive disorders of KF cows.

Incidence	C	T1	T2	T3
ROP	3	1	1	0
Still Birth	1	0	0	0
Dystocia	1	0	0	0
Uterine infections	5	4	2	2

plasticity and normal anatomy of horn without allowing any infection to persist. These helped the animal in reducing the uterine horn diameter in T3 group.

From beginning of the study period up to 30 days after parturition, no significant ($p \geq 0.05$) difference were seen across the groups, but, after 40 days of parturition, small and medium follicle numbers were significantly ($p \leq 0.05$) higher in T 3 group than control group but no difference was seen in case of large follicle numbers (Table 4.1; 4.2; 4.3). Choline deficiency caused decreased hormones production (FSH and LH) because of the necessity of choline in cell membrane structures (Evans *et al.* 2006). Feeding 240g of RPC/cow/d, from parturition to day 42 in milk was associated with increased ovarian follicle growth and earlier resumption of ovulation (Shahsavari, 2012). Early onset of cyclicity is also related with lower incidences of reproductive disorder and improved energy balance (Vries and Veerkamp, 2000).

We considered retention of placenta (ROP), still birth, dystocia and uterine infection (metritis, endometritis, pyometra) as reproductive disorders in our study. During the study, we found three cases of ROP, one still birth, one case of dystocia and five cases of uterine infections in control animals (Table 5). One case of ROP and four cases of uterine infections were found in T1. In T2, one case of ROP and two cases of uterine infections were observed. Not a single case of still birth and dystocia were noted in T2 and T3. Only two cases of uterine infection were marked in T3 without any other complications. A recent study showed that feeding of 60 g of RPC/cow/d, from 21 d prepartum to 21 d postpartum was associated with less incidences of endometritis, lower number of stillbirths and more cyclic cows (Furken and Hoedemaker, 2014). Negative energy balance and compromised immune status around parturition leads to uterine infection in dairy cows. So, lower incidences of reproductive disorders in the treatment groups might be due to improved metabolic status and enhancement of antioxidant and immune system.

CONCLUSION

Rumen protected choline (RPC) may be beneficial to improve reproductive performances. This improvement may be more when green tea extract is used in combination with choline supplementation by giving protection against oxidative challenge faced by the animals at the critical transition phase.

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