Original Research

Green Tea Extract Along with Rumen Protected Choline Improves Immune Status by Modulating Oxidative Stress 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/day) and in T3- RPC + GTE (55+3g/day) along with basal diet. The treatment was given 30 days before calving to 60 days after calving. No significant (P≥0.05) difference was observed among the groups for somatic cell count. The concentration of total immunoglobulin concentration increased significantly (P≤0.01) in T1, T2 and T3 as compared to control. Serum level of TBARS (P<0.01) *and TAC reduced significantly (P≤0.05) in T1, T2 and T3 as compared to control from 7th day to 30th day of parturition. In conclusion, feeding of RPC and GTE in combination improved immune status and alleviated oxidative stress during transition period in Karan Fries cows.*

Key words: Green Tea Extract, Karan Fries Cows, Rumen Protected Choline, Somatic Cell, TAC TBARS, Total Immunoglobulin, Transition

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Introduction

Besides the metabolic stress, the liver of early lactating cows is exposed to diverse inflammatory challenges, like microbial components, proinflammatory cytokines and reactive oxygen species, as a result of infectious diseases, such as mastitis, endometritis, but also subacute rumen acidosis and abomasal displacement (Zebeli and Metzler-Zebeli, 2012), which frequently occur during parturition and/or the onset of lactation. So, supplementation of antioxidant with anti-inflammatory action, with basal feed formulation might reduce the stress of liver in transition cows.

Choline, a component of phospholipid and methyl donor, takes parts an important role in VLDL synthesis and thereby fat export from liver (Yao and Vance, 1988). Green tea extract is known to be a potent anti-oxidant and anti-inflammatory agent which helps in reducing inflammation and stress in liver around parturition (Winkler *et al*., 2015) in dairy cows. Lower lipid oxidation in response to green tea extract may be due to the free radical scavenging capacity of catechin components, especially epigallocatechin-3-gallate (EGCG) (Kondo *et al*., 2004). Indeed, its (EGCG) ability to neutralize free radicals is 100 times greater than that of vitamin C and 25 times higher than that of vitamin E (Graham, 1992).

Very few studies have been reported till yet, regarding the effect of RPC and GTE on immune function and oxidative stress in transition dairy cows. So, the present study has been designed to study the effect of supplementation of rumen protected choline (RPC) and green tea extract (GTE) on immune status and oxidative stress.

Materials and Methods

This study was conducted in the Livestock Research Centre (LRC) unit of National Dairy Research Institute (NDRI), Karnal, India. The RPC was purchased from Kemin Animal Nutrition, India, which was prepared by spray freeze drying technology, in the form of encapsulation with fatty acids. The green tea extract (GTE) was purchased from Sarthak Herbs, Karnal. Ethical permission was granted for the experiment by the Institutional Animal Ethical Committee (IAEC) of Indian Council of Agricultural Research-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.

Thirty-two pregnant dairy cows were selected from the herd with most probable production ability (MPPA) of around 4000 L milk production, in their second to fourth lactation stage and maintained at LRC, NDRI, Karnal. They were fed basal diet constituting of concentrate mixture, green fodder (sorghum, maize, oats, sugar graze) and dry roughage (wheat straw) as per NRC, 2001.The experimental groups were divided as follows-

Control (C) - basal diet without supplementation,
Treatment 1 (T1) - basal diet with RPC (55grams/day),
Treatment 2 (T2) - basal diet with GTE (3grams/day),
Treatment 3 (T3) - basal diet with RPC (55grams/day) + GTE (3grams/day)

The experiment was started around 37 days before expected date of parturition and given adaption period of seven days. The total duration of experiment was 90 days. The treatment was given 30 days before calving to 60 days after calving.

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Milk Somatic Cell Count

Animals were machine milked thrice a day i.e. 05.00 h, 11.30 h and 18.00 h up to 60 days postpartum. Milk samples were collected at each milking and proportionately pooled to represent milk sample of that animal at weekly interval. Milk somatic cells were estimated on weekly interval by Lactoscan Automatic Analyser (New Dairy Engineering and trading Co. Pvt. Ltd., Bulgaria) according to the manufacturer's protocol.

Analysis of Blood Samples for Biochemical Analysis

Blood Collection

Blood samples were collected at weekly intervals, starting from thirty days prior to calving up to 60 days after calving. The blood samples from individual animal were collected by jugular vein puncture into vacutainer tubes, 16 X 100 mm (Becton Dickinson, Rutherford, NJ). The serum was separated by centrifugation of the blood samples at 2400 rpm for 15 min. and stored in vials at -20 °C.

Estimation of Total Immunoglobulin Concentration

Total immunoglobulin was estimated in serum samples by using Bovine Total Immunoglobulin (TIG) ELISA kit (Cat. No. MBS006013) purchased from MyBiosource, California, San Diego (USA).

Estimation of TBARS

Lipid peroxidation in plasma was estimated by method of Niehaus and Samuelsson (1968) modified by Kaushal and Kansal (2012). In this method malondialdehyde and other thiobarbituric acid reactive substance (TBARS) was measured by their reactivity with Thiobarbituric acid (TBA) in acidic condition to generate a pink colour chromophore which was read at 535 nm.

Estimation of TAC

Total antioxidant capacity was estimated in serum samples by using Total Antioxidant Capacity (T-AOC) Assay Kit (ABTS method) (Cat. No. MBS2548432) purchased from MyBiosource, California, San Diego (USA).

Statistical Analysis

Statistical analysis of data was carried out to find the mean ± SE. One-way analysis of variance (ANOVA) techniques (By using GRAPHPAD PRISM software) was adapted to find out significant difference between groups and day of treatments and to interpret the effect of dietary treatment on various parameters.

Result and Discussion

Effect of RPC and GTE on Somatic Cell Count (SCC)

The milk SCC ranged from 2.49 ± 0.12 ($*10^5$) to 1.60 ± 0.11($*10^5$) in control, 2.29 ± 0.15 ($*10^5$) to 1.57 ± 0.09 ($*10^5$) in T1, 2.21 ± 0.19 ($*10^5$) to 1.54 ± 0.13 ($*10^5$) in T2 and 2.18 ± 0.14 ($*10^5$) to 1.43 ± 0.13 ($*10^5$)

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in T3 from first week to ninth weeks of lactation (Fig. 1). There was no significant difference in milk somatic cell count and the data were of normal range indicating immunity and intact self-defense power during the transition period.

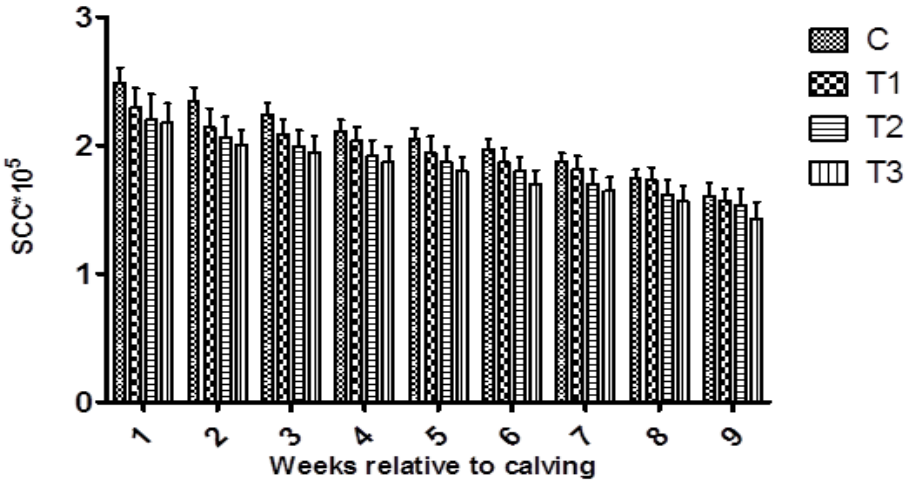

Fig. 1: Effect of RPC and GTE supplementation on milk somatic cell count (SCC*10^5) of KF cows

Somatic cell count is a proper method for measuring the quality of milk. Pirestani *et al*. (2011) observed that supplementing both choline (60 gm/daily/cow) and L–carnitine (50 gm/daily/cow) had a positive effect on milk SCC. Under stress conditions (such as labor, lactation) or diseases, the SCC in the milk increased due to reduced immunity, wear and tear of tissues and inflammation. Methyl groups are required for building polyamides, boosting immune system, tissue repair and helps in reducing inflammation in the body (Bindel *et al.*, 2000). Addition of choline in the diet might have provided more methyl groups to the transmethylation reaction that helped in building polyamides, repair tissues and increasing udder immune system and subsequently decreased SCC.

Although there were scanty reports on effect of GTE on SCC, many researches showed that GTE has immune-stimulating and immune-modulating effects (Monobe *et al*., 2008; Pae and Wu, 2013) indicating a positive effect on self-defense power of the animals which might have helped in decreasing the milk SSC in the treatment groups.

Effect of RPC and GTE on Total Immunoglobulin

The mean ± SE values of plasma immunoglobulin ranged from 38.18 ± 0.31 to 30.95 ± 0.75 (mg/mL) in control, 39.05 ± 0.68 to 34.38 ± 0.48 (mg/mL) in T1, 39.23 ± 0. 39 to 34.58 ± 0.78 (mg/mL) in T2 and 39.26 ± 1.20 to 35.05 ± 0.84 (mg/mL) in T3 (Table 1). Our results are in line with earlier workers (Pinotti *et al*., 2004; Sheikh *et al*., 2014) who also marked significant increase ($P \leq 0.05$) in total immunoglobulin

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concentration in RPC treated cows. Choline supplementation improved vitamin E and plasma folate status in transition dairy animals, thereby have a positive effect on immune system (Chatterjee *et al*., 2003). Sun *et al*. (2016) observed elevated plasma interleukin 2 (IL-2) concentration and the CD4+/CD8+ T lymphocyte ratio in peripheral blood (P<0.05) of RPC treated multiparous transition cows.

Table 1: Effect of RPC and GTE supplementation on total Immunoglobulin (mg/mL) of KF cows

DAYS	C	T 1	T 2	T 3
-30	38.18 ± 0. 31	39.05 ± 0. 68	39.23 ± 0. 39	39.26 ± 1. 20
-15	33.86 ± 0. 84	35.40 ± 1. 03	35.48 ± 0. 55	36.02 ± 1. 55
-7	30.59 ± 0. 80	32.35 ± 0. 93	33.33 ± 0. 52	33.81 ± 1. 50
0*	27.61 ^{a} ± 0. 55	30.82 ^{b} ± 1. 08	30.29 ^{b} ± 0. 41	30.98 ^{b} ± 1. 11
7**	24.25 ^{a} ± 0. 63	29.08 ^{b} ± 0. 59	29.50 ^{b} ± 0. 59	30.13 ^{b} ± 1. 13
15**	26.88 ^{a} ± 0. 87	31.30 ^{b} ± 0. 98	31.78 ^{b} ± 0. 72	32.87 ^{b} ± 0. 78
30**	28.04 ^{a} ± 0. 64	33.06 ^{b} ± 0. 87	33.06 ^{b} ± 0. 78	34.30 ^{b} ± 0. 58
45**	29.72 ^{a} ± 0. 56	33.38 ^{b} ± 0. 95	34.11 ^{b} ± 0. 95	35.05 ^{b} ± 0. 52
60**	30.95 ^{a} ± 0. 75	34.38 ^{b} ± 0. 48	34.58 ^{b} ± 0. 78	35.05 ^{b} ± 0. 84

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

Total immunoglobulin status improved (P≤0.05) in the GTE supplemented group (T2). It might be due to the fact that, GTE is rich in polyphenols (e.g., catechin, epicatechin, epigallocatechin, and their gallates). There are evidences that, catechins, the anti-oxidant compound present in green tea may improve immune system of the individual as demonstrated in several *in vitro* models (Wong *et al*., 2011; Saleh *et al*., 2014). Zhu *et al*. (1999) showed that green tea and its major components ameliorate immune dysfunction in mice bearing Lewis lung carcinoma and treated with the carcinogen. Recent studies suggest that EGCG can vary the strength of both the innate and adaptive defensive abilities of the immune system (Min *et al*., 2015). In T3, total immunoglobulin status also improved (P≤0.05) as compared to control. It might be due to, both RPC and GTE in combination enhanced the defense system of transition KF cows by the above described phenomenon.

Effect of RPC and GTE on Oxidative Stress

TBARS

The mean ± SE values of plasma TBARS levels (Table 2) indicating that, TBARS level was significantly reduced by supplementing RPC and GTE than control animals. Sun *et al*. (2016), reported elevated blood antioxidant status, as indicated by total antioxidant capacity (TAOC), glutathione and reduced the plasma malondialdehyde (MDA) level (P<0.05) in RPC treated multiparous transition cows. The effects of dietary inclusion of green tea on TBARS reduction have also been reported in pig (Ko *et al*., 2008; Hossain *et al*., 2012) and broiler meat (Yang *et al*., 2003). Whereas, Winkler *et al*. (2015) supplemented 0.175g of plant

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product consisting of 95% green tea and 5% curcuma extract per kg DM of product and found that cows supplemented with polyphenols had no influence on systemic antioxidant status.

Table 2: Effect of RPC and GTE supplementation on TBARS (µmol/mL) of KF cows

DAYS	C	T1	T2	T3
-30	1.78 ± 0.03	1.69± 0.03	1.71± 0.04	1.72± 0.05
-15	1.93± 0.08	1.82± 0.03	1.84± 0.05	1.79± 0.05
-7	2.21± 0.08	2.13± 0.06	2.08± 0.06	2.12± 0.14
0	2.62± 0.06	2.53± 0.06	2.43± 0.08	2.39± 0.08
7**	2.91^{b} ± 0.07	2.93^{b}± 0.09	2.69^{ab}± 0.09	2.57^{a}± 0.07
15**	3.41^{b} ± 0.08	3.01^{a}± 0.08	2.98^{a} ± 0.16	2.82^{a} ± 0.08
30**	3.56^{b}± 0.06	3.39^{b}± 0.07	3.08^{a}± 0.10	2.99^{a}± 0.07

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

The period of transition between late pregnancy and early lactation is associated with lipid and protein metabolic changes (Castillo *et al*., 2006). The energy reduction in the first weeks after parturition results in increased fat mobilization, which is related to the generation of lipid peroxides and reactive oxygen species (ROS). These ROS are normally neutralized by sufficient antioxidant levels of living organisms. Imbalance between the production of ROS and the defense ability of biological systems to scavenge these reactive intermediates causes oxidative stress (Trevisan *et al*., 2001; Konvičná *et al*., 2015). In the present study, the highest TBARS of dairy cows was determined in the first week after parturition. Similarly, to our results, many authors have also recorded a tendency to transient increase of lipid peroxidation in dairy cows in the same period after calving (Mudron and Konvicna, 2006; Saleh *et al*., 2007; Sharma *et al*., 2011). So, supplementing a potent antioxidant *i.e*. green tea extract helped the animals to face the oxidative stress and neutralizing the ROS generated during the process by inducing antioxidant enzymes.

Effect of RPC and GTE on Total Antioxidant Capacity (TAC)

There was a significant (P≤0.05) increase in total antioxidant capacity from 7 days to 30 days after parturition (Table 3). There was a significantly (P≤0.05) higher level of TAC in T2 and T3 group than control and T1. Salman *et al*. (2017) found a significantly higher (P≤0.05) serum TAC value in RPC supplemented group than control, 3 weeks before parturition. But, no significant difference was observed for TAC values on first or third week of lactation. Sun *et al*. (2016) also reported elevated blood antioxidant status in RPC treated transition cows. Winkler *et al*. (2015) supplemented 0.175g of plant product consisting of 95% green tea and 5% curcuma extract per kg DM of product and found that cows supplemented with polyphenols had no influence on systemic antioxidant status. There are many reports indicating antioxidant capacity of green tea (Hajimahmoodi *et al*., 2008; Shannon *et al*., 2017).

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Table 3: Effect of RPC and GTE supplementation on TAC (µmol/ml) of KF cows

DAYS	C	T 1	T 2	T 3
-7	223.45 ± 6.36	220.77 ± 7.18	232.01 ± 8.22	235.98 ± 8.09
0	202.09 ± 6.00	211.69 ± 7.45	225.51 ± 8.67	228.15 ± 8.26
7*	187.59^{a} ± 8.48	202.98^{ab}± 7.39	218.63^{b} ± 9.29	220.33^{b} ± 7.65
15*	202.34^{a} ± 8.24	214.06^{ab} ±6.99	234.89^{b} ± 7.04	233.05^{b} ± 8.16
30*	216.43^{a} ± 6.59	222.75^{a} ± 6.98	245.85^{b} ± 7.67	243.93^{b} ± 7.92

*Mean bearing different superscripts between the drugs treatments differ significantly; Data represented as mean ± SE * P<0.05)*

Although literatures are deficient in explaining TAC level in dairy animals but, in mice and other laboratory animals it was found to be a good antioxidant (Koutelidakis *et al*., 2014; Sharifzadeh *et al*., 2017). The major catechin present in green tea is epigallocatechin-3-gallate (EGCG, more than 50% of the total polyphenolic structure), which is believed to be responsible for the anti-oxidant capacity of GTE (Wong *et al*., 2011; Salah *et al*., 2014).

Conclusion

Significant treatment differences were observed in parameters like total immunoglobulin, TBARS, TAC with supplementation of RPC and GTE in KF cows. This improvement in immune status along with alleviation of oxidative stress might be attributable to green tea components as well as RPC, which helped the transition animals to face the critical challenges during their transition period.

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