Original Research

Supplementing Rumen Protected Choline with Green Tea Extract Exerts Hepatoprotective Effect in Transition Karan Fries Cows by Modulating Serum Biochemistry

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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 treatment was given 30 days before calving to 60 days after calving. The plasma non-esterfied fatty acids (NEFA) concentration reduced significantly ($p \leq 0.01$) in T1, T2 and T3 than control from the day of parturition up to the end of experiment. The cows supplemented with RPC and GTE showed a trend towards decreased plasma beta- hydroxy butyric acid (BHBA) ($p \leq 0.01$) than control. No significant ($p \geq 0.05$) difference was observed across the groups for plasma cholesterol and blood urea nitrogen (BUN) concentration. Plasma aspartate amino transferase (AST) concentration reduced significantly ($p \leq 0.05$) in T1, T2 and T3 as compared to control from 0th day to 15th day of parturition. The concentration of plasma triacylglycerol (TAG) and very low-density lipoprotein (VLDL) increased significantly ($p \leq 0.01$) in T1 and T3 as compared to control. In conclusion, feeding of RPC and GTE in combination improved hepatic performances during transition period in Karan Fries cows.

Key words: BHBA, Green Tea Extract, NEFA, Rumen Protected Choline, Transition Karan Fries Cows

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Introduction

The transition period between late pregnancy and early lactation (also called the periparturient period) surely is the most critical time of the lactation cycle. 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.

It is clear from the above facts that liver is the primary organ which comes under stress during periparturient phase. It leads to fatty liver condition and ketosis, which has a carryover effect on production and reproduction (Grummer, 1995). If the stress on liver can be reduced at this high time, that will affect overall production in a positive way. 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 antioxidant and anti-inflammatory agent which helps in reducing inflammation and stress in liver around parturition (Winkler *et al*., 2015) in dairy cows. So, the present study has been aimed to explore the effect of supplementation of rumen protected choline (RPC) and green tea extract (GTE) on hepatic stress in transition cows.

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 (Reg. 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 (ICAR Feeding standard),

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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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 was collected by jugular vein puncture into heparinized vacutainer tubes, 16 X 100 mm (Becton Dickinson, Rutherford, NJ). The plasma was separated by centrifugation of the blood samples at 2400 rpm for 15 min. and stored in vials at -20 °C.

Estimation of NEFA Concentration

NEFA was estimated in serum samples by using bovine non esterified fatty acid ELISA kit purchased from Bioassay Technology Laboratory, Yanghu Dist. Shanghai, China (Cat. No. E0021Bo).

Estimation of Plasma Biochemical Metabolites

Plasma was analysed for BHBA (BioVision's β-HB assay kit), cholesterol, (Recombigen Laboratories Pvt. Ltd., India), BUN (Erba Manheim Urea assay kit), AST (Recombigen Laboratories Pvt. Ltd., India), and TAG (GPO-PAP liquid Gold Kit). VLDL was calculated from the value of triglycerides as per the formula given by Friedewald *et al.* (1972).

$$\text{VLDL (mg/dl)} = \text{Triglycerides (mg/dl)} \div 5$$

Statistical Analysis

Data were represented as mean ± SE. One-way analysis of variance (ANOVA) (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.

Results and Discussion

Effect of RPC and GTE on NEFA (µmol/L) and BHBA (µmol/L) Concentration

The mean ± SE values of plasma NEFA, which is marker of energy balance and lipid mobilization, ranged from 157.49 ± 5.85 to 211.37 ± 7.40 µmol/L in control, 157.71 ± 8.13 to 171.39 ± 6.49 µmol/L in T1, 162.68 ± 2.55 to 169.12 ± 2.68 µmol/L in T2 and 161.59 ± 3.46 to 166.46 ± 5.90 µmol/L in T3 from 30 days pre partum to 60 days' post-partum (Fig. 1). The peak of NEFA was noted on the very day of calving, which was typical for peri parturient dairy animals. It was due to higher mobilization of adipose tissue in response to negative energy balance of animal body around parturition. Gradually the level of NEFA decreased in all four groups up to the 60th day of lactation. The plasma NEFA concentration reduced significantly ($p \leq 0.01$) in T1, T2 and T3 than control from the day of parturition up to the end of experiment. No significant difference was noted between T1 and T2 or T1 and T3.

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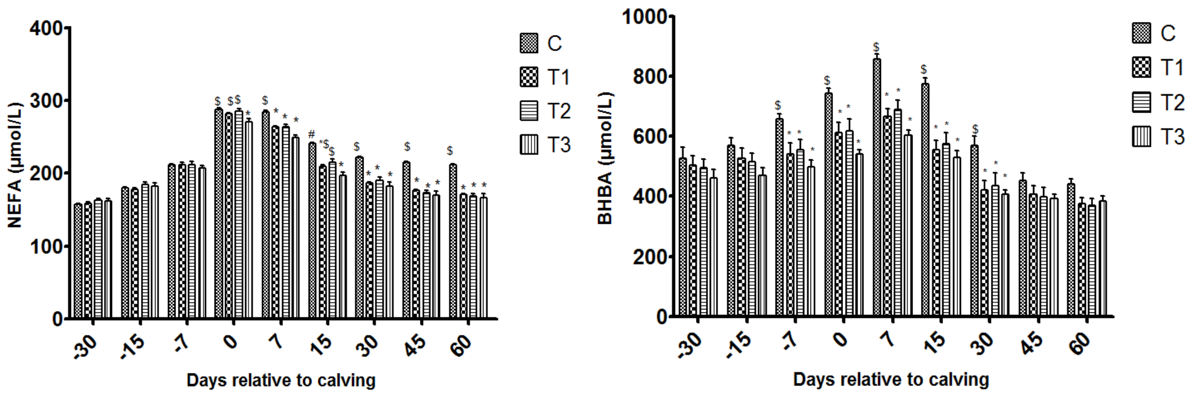

Fig. 1: Effect of RPC and GTE supplementation on- a) NEFA (µmol/L); b) BHBA (µmol/L) of KF cows ($p \leq 0.01$). Mean bearing different symbols between the treatments differ significantly.

The beneficial effect of RPC on plasma NEFA concentration, as observed in the present study, corroborate to the findings, reported by Pinotti *et al*. (2003). In contrary to this, Hartwell *et al*. (2000) and Leiva *et al.* (2015) observed no effect of RPC on plasma NEFA concentration. During transition phase, more NEFA are mobilized to liver from peripheral adipose tissue to compensate the negative energy balance. But, the time, liver exceeds the capacity to oxidize NEFA, there is re-esterification and it gets converted to TAG in liver prior to being packed inside VLDL and then exported. Choline is required for phophatidylcholine synthesis, an active component of VLDL, plays a major role in TAG-VLDL secretion from liver (Piepenbrink and Overton, 2003). There by, RPC helps in reducing plasma NEFA concentration in liver. The present study is in agreement with those of Winkler *et al*. (2015) who observed significant reduction ($p \leq 0.01$) in plasma NEFA level in cows which were given plant product consisting of GTE (95%) and curcuma extract (5 %). GTE is a potent anti-inflammatory reagent which helps in reduction of inflammation and stress in liver around parturition (Winkler *et al*., 2015). As a result of which, when both combined, helped in optimizing NEFA concentration in plasma.

BHBA, which is an indicator of rate of ketogenesis, increased from 30-day pre partum to 7 days' post-partum and then decreased gradually up to the end of the experiment. However, we found significant ($p \leq 0.01$) difference between groups from 7 days before parturition up to 30 days after parturition. All the treatments were able to significantly ($p \leq 0.01$) lower BHBA concentration up to a normal level. The findings of the present study corroborate to those of Zahra *et al*. (2006) who declared a decreased concentration of serum BHBA ($p \leq 0.01$) in HF cows, who were given 56 g of RPC per day. However, many previous researchers could not find any significant difference in BHBA concentration in RPC supplemented groups (Hartwell *et al*., 2000; Piepenbrink and Overton, 2003; Zom *et al*., 2011). In T2, the plasma BHBA concentration was significantly lower than control ($p \leq 0.01$), though there was no difference was found between T1 and T2. The findings in the present experiment are at variance with those of Winkler

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et al. (2015) who found no difference in BHBA concentration in green tea supplemented group. It indicated that both RPC and GTE acted synergistically on liver and altered the rate of ketogenesis which helped in improving the liver status.

Effect of RPC and GTE Supplementation on Cholesterol (mg/dl) and BUN

No significant difference was observed across the groups for plasma cholesterol and BUN concentration. Similar findings were also provided by Pinotti *et al*. (2004) where 20 g RPC did not produce any effect in plasma cholesterol concentration.

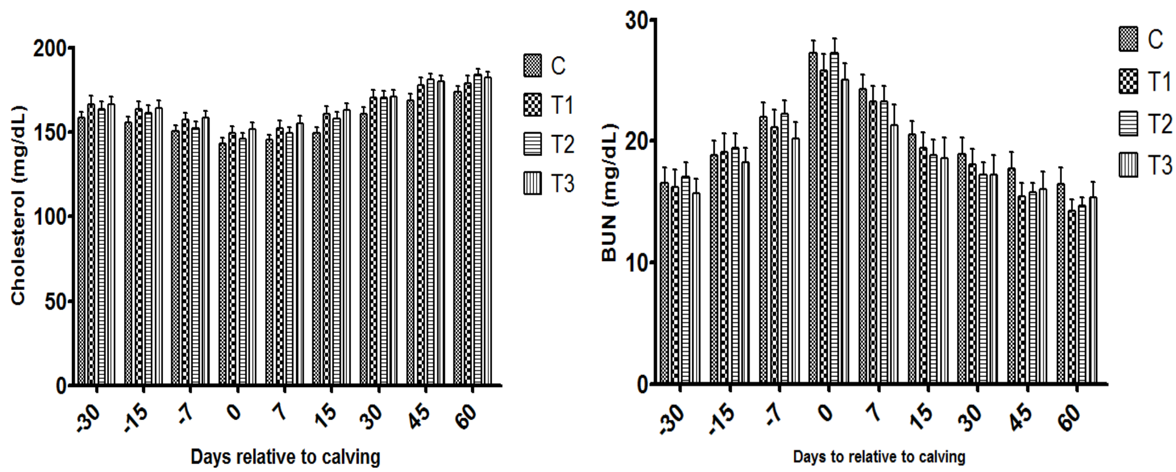

Fig. 2: Effect of RPC and GTE supplementation on- a) Cholesterol (mg/dl); b) BUN of KF cows

No significant difference was observed across the groups for plasma BUN concentration. Similar findings were also drawn by and Zahra *et al*. (2006); Pinotti *et al*. (2008); Ardalan *et al*. (2011) and Rahmani *et al*. (2014) in RPC treated group. Although, no treatment effect was found for plasma BUN across the groups in our study, it was found that BUN level was higher at the day of parturition and early lactation, which decreased subsequently with advancement of days. The reason might be, during early lactation, body fat is primarily mobilized, nevertheless some amount of body protein also gets mobilized which increases the plasma urea concentration. There is accumulation of TAG in liver in early lactation which results in higher plasma urea level in body of cow due to inhibition of ureagenesis (Zhu *et al*., 2000). Numerically higher values of BUN in control group indicated lesser efficient utilization of dietary nitrogen for microbial protein synthesis due to higher ammonia concentration. There is no literature available regarding the effect of GTE on BUN concentration in dairy animals. It was also found from our study that both the treatments did not have any adverse effect on renal function and renal toxicity as BUN is a marker for good renal function.

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Effect of RPC and GTE Supplementation on AST (IU/lit)

Plasma AST concentration reduced significantly ($p \leq 0.05$) in T1, T2 and T3 as compared to control from 0th day to 15th day of parturition. But no significant difference was found between T1 and T2 or T1and T 3 or T 2 and T 3. Our result is in agreement with Sheikh *et al*. (2014), who observed lower concentration ($p \leq 0.05$) of AST in RPC treated group. In contrast, some other workers (Zahra *et al*., 2006; Rahmani *et al*., 2012; Rahmani *et al*., 2014) reported that AST concentration was not affected by choline supplementation in cows. AST is a marker for hepatocellular integrity, which presence in plasma refers to any insult on hepatic cells and mitochondria. The decrease in AST concentration in treatment groups in our study might be attributed to the improved hepatic status of transition KF cows.

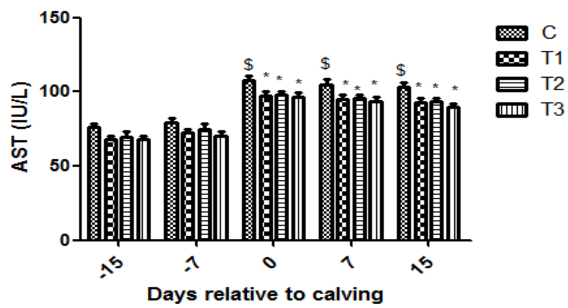

Fig. 3: Effect of RPC and GTE supplementation on AST (IU/lit) of KF cows ($p \leq 0.05$). Mean bearing different symbols between the treatments differ significantly.

Effect of RPC and GTE Supplementation on TAG (mg/dL) and VLDL (mg/dL)

TAG concentration differed significantly ($p \leq 0.01$) in T1, T2 and T3 than control from 7th day of post-partum up to 60th day of post-partum. No significant difference was observed across T1, T2 and T3. In our study, the mean ± SE values of plasma VLDL varied from 3.80 ± 0.09 to 2.18 ± 0.02 mg/dL, 3.96 ± 0.11 to 2.36 ± 0.05 mg/dL, 3.88 ± 0.08 to 2.30 ± 0.05 mg/dL and 4.00 ± 0.09 to 2.37 ± 0.06 mg/dL in C, T1, T2 and T3 respectively. Significant difference ($p \leq 0.01$) was found among control and treatment groups, whereas, no effect was obtained across treatment groups.

TAG increases in the liver around parturition due to re esterification of plasma NEFA and this TAG requires VLDL as a carrier to be exported from liver. Previous studies (Piepenbrink and Overton, 2003; Cooke *et al*., 2007) reported that periparturient dairy cows are choline deficient, which negatively affects liver status. In our study, supplementation of RPC during transition period might have helped in synthesis of phosphatidylcholine, a precursor of VLDL synthesis and increased export of TAG as VLDL. Pandurang *et al*. (2012) and Sun *et al*. (2016) also reported increased concentration of VLDL in RPC treated cows during transition phase. However, no reports are available regarding the effect of GTE on VLDL concentration.

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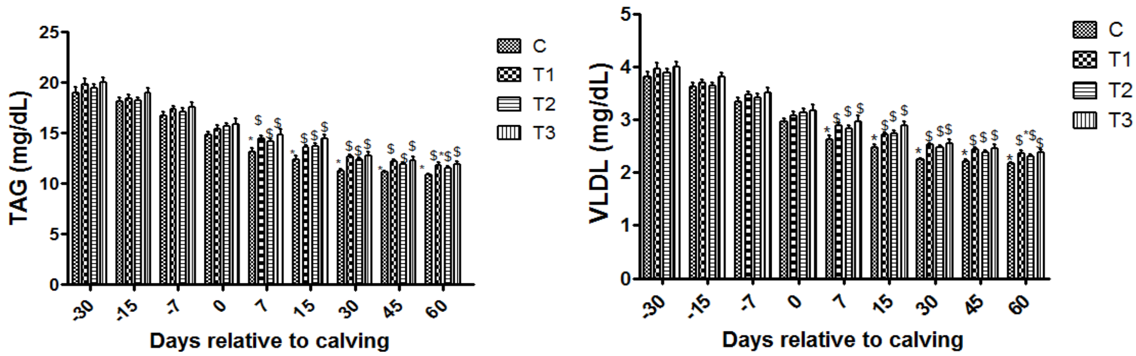

Fig. 4: Effect of RPC and GTE supplementation on- a) TAG (mg/dL); b) VLDL (mg/dL) of KF cows ($p \leq 0.01$). Mean bearing different symbols between the treatments differ significantly.

Conclusion

Significant treatment differences were observed in metabolic parameters like BHBA, NEFA, AST, TAG and VLDL with supplementation of RPC and GTE. So, rumen protected choline (RPC) may be beneficial to provide hepato-protective effect and prevent occurrence of fatty liver condition in transition cows. 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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