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Rumen Protected Choline along with Green Tea Extract Maintain Glucose Homeostasis in Transition Karan Fries Cows

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ABSTRACT

The aim of this study was to determine the effect of supplementation of rumen protected choline (RPC) and green tea extract (GTE) on glucose homeostasis in transition cows. Thirty-two pregnant Karan Fries (KF) cows were randomly allocated into three groups on the basis of most probable production ability (MPPA) and parity. In control group, cows were fed basal diet. In T_1 group, each cow was fed 55g of RPC/day along with basal diet; cows in T_2 group were fed on basal diet supplemented with 3g of GTE/day and in T_3 group, 55 g of RPC and 3 g of GTE/day along with basal diet was fed. The duration of experiment was 30 days before calving to 60 days after parturition. Non significant difference was observed across the groups for plasma glucose concentration. The concentration of insulin and IGF-1 increased significantly ($P \leq 0.01$) in T_1, T_2 and T_3 as compared to control in days' dependant manner around parturition. In conclusion, feeding of RPC and GTE in combination maintained glucose homeostasis during transition period in Karan Fries cows.

Key words: Green tea extract, Glucose, Insulin, IGF-1, Rumen protected choline, Transition Karan Fries cows

INTRODUCTION

High-yielding dairy cows enter a state of negative energy balance (NEB) around calving when the energy demand for maintenance and lactation exceeds that of dietary energy intake (Bauman and Currie, 1980). Around 46 % of maternal glucose taken up by the uterus is utilised by the foetus (Bell, 1995). Additionally, a cow producing 30 kg of milk per day uses at least 2 kg of blood glucose to synthesize lactose (Bell, 1995). So, to maintain the heavy glucose demand, the liver synthesizes all of this glucose from propionate and amino acids. Again, just after calving the energy requirement of body increases approximately 2.5 times (Grummer, 1995). As cows begin to use large amount of energy to drive milk production, a lag occurs in DMI, and they find themselves physically unable to increase DMI enough to meet their needs (Grummer, 1995). It is well known that fat accumulation by the liver, during the transition period, inhibits liver glucose production (Grummer, 1995). Choline, a component of phospholipid and methyl donor, takes part an important role in VLDL synthesis and thereby fat transport from liver. Dietary supply of choline in post parturient dairy cows may be inadequate, even though choline can be synthesized by the animals (Pires and Grummer, 2008). Methionine and lysine are the 2 most limiting AA for milk production in dairy cattle (NRC, 2001). The demand for choline as a methyl donor is probably the main factor determining how rapidly choline deficiency induces a disease state (Zeisel *et al.* 1991). Therefore, supplementation of choline could spare a portion of methionine needed to meet daily choline needs, which would leave a larger supply of methionine for milk production.

Green tea contains polyphenols (Major catechin present in green tea is epigallocatechin-3-gallate [EGCG]), tannins and caffeine (Khan and Mukhtar, 2019). Some of these components have been shown to enhance the basal and insulin-stimulated glucose uptake of adipocytes (Wu *et al.,* 2004) and inhibit intestinal glucose uptake by inhibiting the sodium-dependent glucose transporter of intestinal epithelial cells (Kobayashi *et al.,* 2000). Very few studies have been reported till date, regarding the effect of RPC and GTE on glucose and insulin correlation in transition dairy cows. So, the present study has been designed to study the effect of supplementation of RPC and GTE on glucose homeostasis in transition KF 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

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purchased from Kemin animal nutrition, India, which was prepared by spray freeze drying technology, in the form of encapsulation with fatty acids. The 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 KF cows (30 days before calving) were selected from the herd with MPPA of around 4000 l milk production, in their second to fourth lactation stage. Nutrient requirement of experimental cows were met by feeding basal diet consisted of concentrate mixture, green fodder and wheat straw as per the guidelines of NRC (2001). Ingredients and nutrients composition is depicted in Table 1.

In control group, cows were fed basal diet. In T_1 group, each cow was fed 55g of RPC/day along with basal diet; In T_2 group, each cow was fed on basal diet supplemented with 3g of GTE/day and in T_3 group, 55 g of RPC and 3 g of GTE/day along with basal diet was fed. The treatment was given 30 days before calving to 60 days after calving. The blood samples were collected at weekly intervals by jugular vein puncture into vacuutainer tubes (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 until further analysis. Estimation of blood glucose was done by using kit purchased from Recombigen Laboratories Pvt. Ltd. New Delhi, India. Insulin was estimated in serum samples by using Bovine Insulin ELISA kit (Cat. No: E0015Bo, Bioassay Teachnology Laboratory, Shanghai, China). IGF-1 was estimated in serum samples by using Bovine Insulin-like Growth Factors 1 ELISA kit (Cat. No. E0016Bo, Bioassay Technology Laboratory, Yanghu Dist. Shanghai, China).

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

The mean plasma glucose concentration in experimental KF cows during transition period is presented in Table 2. No significant difference was found

Table 1. Physical and chemical composition of concentrate mixture

Ingredients	**Parts (%)**
Maize grain	33
Groundnut Cake (GNC)	21
Mustard Cake	12
Wheat Bran	20
Deoiled Rice Bran (DORB)	11
Mineral Mixture	2
Common Salt	1
Total	100

Nutrient percent	**Concentratem mixture**	**Sorghum**	**Maize**	**Oats**	**Sugar graze**	**Wheat straw**
DM	89.87	24.61	14.90	24.29	26.33	90.11
OM	92.57	91.41	92.60	90.81	90.66	91.43
CP	19.93	9.54	9.82	9.80	11.11	2.93
EE	3.75	1.85	2.1	1.44	2.10	1.35
NDF	25.63	58.75	50.45	64.42	59.55	78.40
ADF	15.42	33.63	28.45	40.61	36.91	51.20
Ash	7.29	8.55	7.25	9.13	9.34	8.52

Table 2. Effect of RPC and GTE supplementation on serum glucose concentration (mg/dl)

Days of calving	Group				SEM	P-value
	C	T_1	T_2	T_3		
Days	**C**	**T_1**	**T_2**	**T_3**		
-30	58.00±2.42	59.37±2.15	60.62±2.48	60.50±1.47		
-15	55.87±2.35	56.50±2.16	58.32±2.23	57.25±1.67		
-7	53.12±2.25	54.37±2.00	55.10±1.96	53.00±1.98		
0	48.25±2.13	50.75±1.84	48.04±1.54	49.25±1.50		
7	45.87±1.69	47.75±1.53	47.04±2.09	48.25±1.49		
15	48.62±1.91	50.50±1.99	50.42±1.77	53.00±1.55		
30	51.87±1.52	52.75±1.88	53.02±1.38	54.50±1.40		
45	53.62±1.34	55.00±1.81	53.98±1.33	56.25±1.11		
60	55.25±1.11	56.00±1.60	56.63±1.23	57.37±0.99		

across the groups for plasma glucose concentration. Similar results were also obtained by many workers (Chung *et al.,* 2009; Zom *et al.,* 2011; Leiva *et al.,* 2015) in RPC supplemented transition cows. However, Sheikh *et al.* (2014) found significant increase ($P \leq 0.05$) in plasma glucose concentration in transition KF cows when they were fed 60 g of RPC per day. Various reports documented that green tea supplementation lowers blood glucose level (Tsuneki *et al.,* 2004). In T_3 group numerically higher concentration of glucose in spite of supplementation of GTE indicates overshadowing effect of RPC on GTE's glucose lowering ability, that enabled maintenance of a balance gluconeogenic state in transition cows to meet the high energy demand.

Significant difference ($P \leq 0.05$) was noted between control and treatment groups from seven days before parturition up to day of parturition and significant ($P \leq 0.01$) difference was seen from 15 days after parturition up to 30th days of parturition, whereas no significant difference was recorded between T_1, T_2 or T_2, T_3 or T_1, T_3 in plasma insulin level (Table 3).

Leiva *et al.,* (2015) reported significantly higher ($P \leq 0.01$) concentration of insulin in transition HF cows, those were given 50 and 100 g of RPC before and after calving. In early lactation, there is decrease in insulin concentration which is considered as a part of energy metabolism, allowing an animal to mobilize the fat deposit. In one study, EGCG dose-dependently improved insulin resistance in NAFLD mice not only by reducing body weight but also through enhancing the insulin clearance by hepatic insulin degrading enzyme (IDE) (Gan *et al.,* 2015). So to meet the energy demand,

Table 3. Effect of RPC and GTE supplementation on insulin (pmol/L) of KF cows

DAYS	C	T_1	T_2	T_3
-30	17.98±0.75	20.61±0.84	19.59±0.71	20.28±0.49
-15	17.32±0.73	19.82±0.75	18.64±0.71	19.63±0.46
-7*	16.46^{a}±0.69	18.73^{b}±0.66	17.94^{ab}±0.66	19.13^{b}±0.49
0*	14.75^{a}±0.62	17.01^{b}±0.71	16.05^{ab}±0.70	17.23^{b}±0.52
7	14.61±0.657	17.29±0.71	15.37±1.90	17.19±0.57
15**	15.33^{a}±0.60	18.49^{b}±0.76	16.66^{ab}±0.65	18.03^{b}±0.39
30**	16.08^{a}±0.47	18.50^{b}±0.70	17.40^{ab}±0.62	19.07^{b}±0.49
45	17.18±0.51	18.35±0.45	18.15±0.59	18.99±0.68
60	17.65±0.44	18.96±0.59	18.48±0.52	19.64±0.57

^{a,b} Mean bearing different superscripts in a row differ significantly ($P<0.05$)

Table 4. Effect of RPC and GTE supplementation on IGF-1 (ng/ml) of KF cows

DAYS	C	T_1	T_2	T_3
-30	126.07±5.79	130.28±6.69	131.29±7.27	134.39±7.54
-15	114.44±5.79	120.26±6.29	119.27±7.58	126.16±7.72
-7	104.50±5.35	112.01±6.26	109.49±7.86	121.56±7.44
0*	70.23^{a}±3.75	87.01^{ab}±5.89	86.32^{ab}±6.43	98.06^{b}±7.55
7**	58.79^{a}±3.48	74.03^{ab}±5.37	71.37^{ab}±6.36	90.93^{b}±7.48
15**	67.45^{a}±3.07	90.56^{b}±6.23	86.61^{b}±6.09	111.33^{c}±7.14
30**	90.12^{a}±4.78	112.68^{b}±5.36	104.75^{ab}±6.05	134.73^{c}±7.31
45**	95.77^{a}±4.26	115.32^{b}±5.36	108.64^{ab}±6.00	136.87^{c}±7.81
60**	99.64^{a}±4.38	122.49^{b}±5.20	113.27^{ab}±6.17	141.29^{c}±7.61

^{a,b} Mean bearing different superscripts in a row differ significantly (P<0.05)

glucose need to be utilized by cells and increase in concentration of insulin by RPC and GTE helped the cells to achieve that successfully to avoid NEB.

From day of parturition to 60 days of parturition, all the treatments significantly (P≤0.05) increased IGF-1 concentration from control (Table 4). In T_1, IGF-1 concentration was significantly higher (P≤0.01) than control from 15th day post-partum to 60th day of post-partum in our study. However, Leiva *et al*. (2015) detected no treatment effect (P≥0.68) for IGF-1 in RPC treated cows during transition period. Wimmer *et al*. (2015) showed that flavonoid Epigallocatechin 3-gallate (EGCG), found in the popular beverage green tea demonstrated similar effects as the endogenous hormones IGF-1 and insulin in the ability to suppress action of the atrophy-promoting transcription factor.

Cows under NEB have limited hepatic expression of GH receptor 1A triggered by low circulating concentrations of insulin (Butler *et al.,* 2003, 2004). This phenomenon uncouples the growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis which reduces the synthesis of IGF-1 by the liver. Reduced concentrations of IGF-1 in blood have been associated with diminished follicle sensitivity to LH, growth and steroidogenesis (Lucy *et al.,* 1992; Butler *et al.,* 2004). Conversely, the increase in circulating concentrations of insulin as energy balance improves seems to be one of the signals to re-establish GH receptor expression in the liver and restore IGF-1 synthesis in dairy cows (Butler *et al.,* 2003).

CONCLUSION

Insulin and IGF-1 are not only involved in energy homeostasis, but also involved in growth and maturation of follicles as well as in maintaining the healthy reproductive tract. Hence, both RPC and GTE could be supplemented in the diet of transition cows to enhance the plasma concentration of insulin and IGF -1, which have a stimulatory effect on production and reproduction of animals.

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