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Research Article

Impact of Phytogenic Feed Additive on Milk Production and Milk Components in Holstein Friesian Cross Breed Dairy Cows Under Field Conditions

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Abstract | Choline is a supplement that can have an incredible effect in production and wellbeing of dairy cows, yet its prerequisites have not been clearly characterized. Therefore, the present study was designed to assess the impact of supplementing phytogenic feed additive (PTF; a natural replacer of synthetic choline chloride) on milk production, milk quality and biochemical parameters. A total of 35 (15 nos. – control group and 20 nos. – PTF group) early lactating dairy cows weighing average of 450 kg were randomly distributed to two groups *viz* G1 (control) and G2 (supplemented with PTF) based on average milk yield. The freshly prepared mixture of PTF (40 g/cow/day) in concentrate feed and water was fed to the dairy cows for four consecutive weeks. The effectiveness of PTF was evaluated by assessing the parameters like milk yield (L), 4% fat corrected milk (4% FCM), milk fat (%), blood beta-hydroxybutyrate (BHB) (mmol/L), and serum cholesterol & triglycerides (mg/dL), and liver marker enzymes (IU/L) *viz.* aspartate aminotransferase (AST), alanine transaminase (ALT), and gamma-glutamyl transferase (GGT). Results revealed that 4% FCM was numerically improved on week 1 (0.11), week 2 (0.46), week 3 (0.56), and week 4 (0.54) as compared to baseline in PTF supplemented group. Milk fat (%) was also significantly ($p < 0.001$) improved on weeks 2, 3 & 4 as compared to baseline in PTF supplemented group. Serum total cholesterol ($p < 0.001$) and triglycerides ($p < 0.05$) levels were significantly increased after 4 weeks of PTF supplementation. In conclusion, our study demonstrated that PTF has potential to imitate the biological functions of choline chloride, and hence, PTF could be recommended for the administration in the ration of dairy cows for the enhancement of milk quality and overall health status of dairy cows.

Keywords | Phytogenic feed additive, Choline, Milk fat, Milk quality, Liver

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INTRODUCTION

Choline is a supplement that can have an incredible effect in production and wellbeing of dairy cows, yet its prerequisites have not been clearly characterized (NRC, 2001). Choline is a crucial supplement for animals since it plays a critical role in lipotropic function as well as involves in various metabolic processes including the development and support of cell structure and formation of acetylcholine (Berchielli et al., 2011). In mammals, the main endogenous pathway for choline biosynthesis is the methylation of phosphatidylethanolamine to phosphatidylcholine by phosphatidylethanolamine N-methyltransferase coupled to phosphatidylcholine degradation (Li et al., 2005).

Studies regarding ruminally protected choline (RPC) have demonstrated the way that milk production and wellbeing of dairy animals can be improved, and the occurrence of fatty liver issues decreased when RPC is given during late transition and early postpartum periods (Piepenbrink and Overton, 2003; Lima et al., 2012). Notwithstanding the logical proof accumulated in a few literatures review articles, RPC is excluded from many dairy milk units on the grounds that the relative profitability shows up little when investigated exclusively from milk yield (Pinotti et al., 2010; Jayaprakash et al., 2016).

Another issue with RPC is that products available in the market varies in their choline content and rumen degradability (Kung et al., 2003; Brusemeister and Sudekum. 2006). Moreover, up to 61% of choline chloride arriving at the duodenum is consumed (De Veth et al., 2016). Therefore, researchers have searched for a natural product which can bypass the rumen degradability but with choline-like function in ruminants. Hence, we designed the present study with the main aim to evaluate the phytogenic feed additive (PTF) in the ration of dairy cows and to study its effect on milk production, milk quality and biochemical parameters.

MATERIALS AND METHODS

Phytogenic Feed Additive (PTF)

Kolin Plus™ (a natural replacer of synthetic choline chloride) is a proprietary PTF developed by M/s. Natural Remedies Private Limited, Bengaluru, India, containing *Acacia nilotica* and *Curcuma longa* plant parts.

Study Subjects

A total of 35 dairy cows of Holstein Friesian cross (HFx) breed weighing average of 450 kg were selected from five different farms at Davangere district, Karnataka state, India, were randomly distributed into two groups *viz* G1 (served as control) and G2 (supplemented with PTF) based on average milk yield of animals. The average lactation age was 2.5 & 2.6 months for animals in G1 and G2, respectively. The regular feed *viz.* concentrated feed (approximately 10 kg/day/cow) and roughages (approximately 34 kg/day/cow) was offered. The drinking water was made available *ad libitum.* The study protocol was prepared and approved by Institutional Animal Ethics Committee (Natural Remedies Private Limited).

Study Details

40 g PTF powder was thoroughly mixed with concentrate feed and supplemented once daily to the lactating dairy cows for four consecutive weeks. When PTF is being administered to the dairy cows, concurrent administration with other herbal/synthetic choline-based feed additives were not followed.

Study Parameters

Milk Production

Daily milk yield (L) of individual lactating cows was recorded in the morning and evening and calculated the 4% fat corrected milk (FCM) using the formula = (0.4 x milk yield (L)) + (0.15 x milk yield (L) x fat yield (%)) (Gaines, 1928). The impact of PTF supplementation on milk production was assessed one week before (baseline) supplementation of PTF and thereafter every day after oral supplementation of PTF for 28 consecutive days. The data of milk fat (%) was assessed before (baseline) and after oral supplementation of PTF on every week for four consecutive weeks.

Sample Collection

The blood sample (approximately 5 ml) was collected in a vacutainer with clot activator *via* venipuncture of the jugular vein on day 0 (baseline) and 28 days after administration of PTF (after supplementation). The serum was separated by centrifugation at 4000 rpm for 5 mins and stored at -20°C until analysis of serum biochemical parameters.

Beta-Hydroxybutyrate (BHB)

The blood BHB (mmol/L) was measured using portable electronic meter (BHB Check Plus, Porta Check, Inc., USA) on day 0 (baseline) and 28 days after administration of PTF (after supplementation).

Biochemical Parameters

The serum cholesterol, triglycerides (mg/dL), and liver marker enzymes *viz.* aspartate aminotransferase (AST) (IU/L), alanine transaminase (ALT) (IU/L), and gamma-glutamyl transferase (GGT) (IU/L) was measured using International Federation of Clinical Chemistry (IFCC) method (Central Lab, Bangalore, India).

Statistical Analysis

The mean data of milk yield (L) and milk fat (%) were subjected to repeated measures one-way ANOVA followed by Dunnett's multiple comparison *post-hoc* test to draw the comparison between baseline and during the supplementation period *i.e.,* week 1, week 2, week 3, and week 4. The data (mean ± SEM) of blood BHB (mmol/L), serum cholesterol, triglycerides (mg/dL), and liver marker enzymes *viz.* AST (IU/L), ALT (IU/L), and GGT (IU/L) was subjected to paired t-test to draw a comparison between baseline and after 4 weeks supplementation effects. $p \leq 0.05$ was considered as statistically significant. The statistical analysis was performed using SPSS software Version.21.0; SPSS Inc., Chicago, IL, USA.

Table 1: Effect of PTF on milk production and milk quality parameters of dairy cows

Group	Baseline	Week 1	Week 2	Week 3	Week 4
	Milk Yield (L)				
G1-Control (n=15)	10.41	10.63	10.64	10.58	10.59
G2-PTF (n=20)	10.97	11.01	11.06	11.09	11.04
	4% Fat Corrected Milk				
G1-Control (n=15)	11.07	11.33	11.37	11.34	11.35
G2-PTF (n=20)	11.50	11.60	11.96	12.05	12.03
	Milk Fat (%)				
G1-Control (n=15)	4.42	4.44	4.46	4.48	4.48
G2-PTF (n=20)	4.32	4.36	***4.54	***4.58	***4.60

PTF – Phytogenic feed additive; Values were expressed as Mean; ***p<0.001 was considered as significant when compared to baseline

Table 2: Effect of PTF on biochemical parameters in dairy cows

Parameters	G1-Control		G2-PTF	
	Baseline	After Supplementation	Baseline	After Supplementation
BHB (mmol/L)	0.86 ± 0.09	0.87 ± 0.09	0.82 ± 0.07	0.85 ± 0.06
Cholesterol (mg/dL)	124.38 ± 11.18	140.23 ± 8.94	103.00 ± 8.79	***153.62 ± 12.39
Triglycerides (mg/dL)	18.30 ± 1.83	17.92 ± 1.22	13.94 ± 1.33	*18.08 ± 0.98
AST (IU/L)	56.00 ± 2.84	58.62 ± 3.42	57.08 ± 3.32	65.08 ± 3.56
ALT (IU/L)	22.00 ± 1.09	22.23 ± 1.77	21.15 ± 1.43	23.15 ± 2.19
GGT (IU/L)	21.54 ± 2.17	28.85 ± 6.52	22.31 ± 2.47	21.69 ± 1.25

PTF – Phytogenic feed additive; BHB - Beta-hydroxybutyrate; AST - Aspartate aminotransferase; ALT - Alanine transaminase, GGT - Gamma-glutamyl transferase; Values were expressed as Mean ± SEM; n=13-15; *p<0.05 and ***p<0.001was considered as significant when compared to baseline

RESULTS AND DISCUSSION

Choline helps to balance the metabolic demand in rumi nants during the change from pregnancy to lactation, as this transition period creates negative energy balance in animals because of high metabolic demand by the mammary gland (Drackley, 1999; Pinotti et al., 2005). Various research investigators depicted that choline supplementation in the feed of high yielding dairy cows are fundamental to keep up with milk yield and quality, since choline deficiency might be a restricting element for milk production (Pinotti et al., 2002; Baldi and Pinotti, 2006). Literature reports revealed that assessments for 30 to 90 days are substantial to find responses in dairy cows (Pinotti et al., 2005; Pinotti et al., 2002; Ardalan et al., 2010; Davidson et al., 2008; Leiva et al., 2015). However, lack of sufficient *de novo synthesis* as well as the extensive ruminal degradation of choline (Pinotti et al., 2005) made the researchers to identify the alternative sources which can mimic the function of choline in ruminants. Consequently, the chance of further enhancement of milk production and milk components in dairy cows by expanding the duodenal progression of choline and methionine with herbal products ought to be assessed. Additionally, herbal additives are of interest on the grounds that might work on animals' wellbeing and milk production. However, not all the mechanisms of action of phytochemicals are known and it is important to identify those and their appropriate doses to use them safely in different species (Frankic et al., 2009). Hence, in the present study we mainly aimed to evaluate the effectiveness of PTF addition in the ration of dairy cows for 28 consecutive days.

In our study, milk fat (%) was significantly ($p < 0.001$) improved as early as from two consecutive weeks administration of PTF as compared to baseline. 4% FCM was numerically improved on week 1 (0.11), week 2 (0.46), week 3 (0.56), and week 4 (0.54) as compared to baseline in PTF supplemented group (Table 1). These findings were in accordance with Sun et al. (2016) wherein administration of choline and methionine in the diet of dairy cows enhanced milk production and milk fat (%) and protein (%).

The results of serum biochemical parameters following supplementation of PTF was represented in Table 2. Results revealed that serum total cholesterol ($p < 0.001$) and triglycerides ($p < 0.05$) levels were significantly increased after 4 weeks PTF supplementation. The increased concentration of triglycerides in the after supplementation period could be ascribed to the lipotropic action of choline since it is a lipotropic component that can maintain the harmony

between fat and protein synthesis in dairy cows (Sales et al., 2010). Choline is also essential for the transport and digestion of lipid and cholesterol (Zeisel and Da Costa, 2009), which would make sense of increased concentration of serum cholesterol in after supplementation period in our study. The mechanism by which hepatic cholesterol synthesis is stimulated by choline in ruminants is not fully understood, but in other species, choline is required to synthesize the phosphatidylcholine portion in the very low-density lipoprotein (Yao and Vance, 1988).

It was understood from literature reports that the herbal ingredients like *Acacia nilotica* and *Curcuma longa* of PTF are a rich source of polyphenols and curcuminoids, respectively, and have potential to imitate the hepatoprotective activity of choline (Selvam et al., 2018). This statement was further supported by Narayanan et al. (2013). In concurrence with the literature reports in our study there was no significant change in serum AST, ALT & GGT levels after PTF supplementation indicating hepatoprotection in dairy cows supplemented with PTF. No change in BHB (mmol/L) levels following addition of PTF in the ration of dairy cows in our study suggested that PTF administration at the dose level of 40g/cow/day did not alter the rumen microbiota (Mentschel et al., 2001).

There was substantial evidence available in the literature that PTF imitate the biological activities of synthetic choline chloride in broilers. Selvam et al. (2018) reported PTF capableness of imitating the biological activities of synthetic choline chloride *in-vivo* in avian experimental model. In this experiment PTF replaced the function of 1 kg/ton of synthetic choline chloride at 400 g/ton inclusion rate in broiler diets; this was reflected by the improved growth performance and feed conversion ratio. Moreover, D'Souza et al. (2019) further demonstrated the choline chloride imitating activities of PTF through genomic studies; wherein supplementation of PTF at 400 g/ton of feed positively influenced target genes of muscle growth promotion, which favors' the productive phenotypic response in broiler chickens fed with choline deficient diet. Hence, the current study demonstrated that PTF could improve the milk quality through the mobilization of triglycerides and cholesterol from liver to the serum in dairy cows.

CONCLUSION

In conclusion, administration of dairy cows' ration with phytogenic feed additive at the dose level of 40 g/cow/day for four consecutive weeks resulted in significant improvement in milk fat (%). Furthermore, addition of PTF in the ration of dairy cows caused increase in the serum cholesterol and triglycerides levels. Hence, PTF could be recommended to include in the ration of dairy cattle for the enhancement of milk quality and overall health status of dairy cows. However, further research investigations are recommended to deduce the optimal dose and duration of PTF administration which could also help in augmentation of milk production in dairy cows.

DATA AVAILABILITY

The raw data that support this study will be shared upon reasonable request to the corresponding author.

CONFLICTS OF INTEREST

None.

DECLARATION OF FUNDING

No specific funding received for this research work.

NOVELTY STATEMENT

Choline helps to balance the metabolic demand in ruminants during the change from pregnancy to lactation, as this transition period creates negative energy balance in animals because of high metabolic demand by the mammary gland. Even though many researchers were worked on the choline and rumen protected choline (RPC) supplementation, there is a scarce of literature citing the cholinomimetic effect of herbal supplements in ruminants. Moreover, there is plenty of RPC products available in the market but having variable choline content and rumen degradability makes them less efficacious and less profitable to the dairy farmers. Evaluation of phytogenic feed additive as a natural, viable and cheap alternate to RPC is addressed in the present study through its effect on milk production (milk yield, milk fat), hepatic function (AST, ALT, GGT) and fat metabolism (triglycerides, cholesterol). Hence, the main objective of this study is to find out the impact of herbal feed additive on milk production and fat metabolism in dairy cows at early lactation.

AUTHOR CONTRIBUTION

Prashanth D'Souza, Saravanakumar and Suresh participated in design the experiments. Suresh and Saravanakumar performed the data interpretation. Suresh involved in sample collection. Saravanakumar wrote the manuscript. Prashanth D'Souza and Suresh reviewed the manuscript.

REFERENCES

Ardalan M, Rezayazdi K, Dehghan-Banadaky M (2010). Effect of rumen-protected choline and methionine on physiological

and metabolic disorders and reproductive indices of dairy cows. J. Anim. Physiol. Anim. Nutrit. 94:259-265. https://doi.org/10.1111/j.1439-0396.2009.00966.x

Baldi A, Pinotti L (2006). Choline metabolism in high-producing dairy cows: Metabolic and nutritional basis. Canadian J. Anim. Sci. 86:207-212. https://doi.org/10.4141/A05-061

Berchielli TT, Pires AV, Oliveira SG (2011). Ruminant Nutrition. (In: Jaboticabal, S.P. (Ed; 2nd ed) pp.616. (FUNEP)

Brusemeister F, Sudekum KH (2006). Rumen-protected choline for dairy cows: the in-situ evaluation of a commercial source and literature evaluation of effects on performance and interactions between methionine and choline metabolism. Anim. Res.. 55:93-104. https://doi.org/10.1051/animres:2006002

D'Souza P, Marimuthu S, Kaninathan A, Selvam R (2019). Modulatory effect of Kolin Plus TM, a polyherbal formulation on muscle growth development in choline deficient diet model of broilers: a genomic analysis using microarray. Int. J. Scient. Rep. **5**:344-350. https://doi.org/10.18203/issn.2454-2156.IntJSciRep20195298

Davidson S, Hopkins BA, Odle J, Brownie C, Fellner V, Whitlow LW (2008). Supplementing limited methionine diets with rumen-protected methionine, betaine, and choline in early lactation Holstein cows. J. Dairy Sci. 91:1552-1559. https://doi.org/10.3168/jds.2007-0721

De Veth MJ, Artegoitia VM, Campagna SR, Lapierre H, Harte F, Girard CL (2016). Choline absorption and evaluation of bioavailability markers when supplementing choline to lactating dairy cows. J. Dairy Sci. 99**:**9732-9744. https://doi.org/ 10.3168/jds.2016-11382

Drackley JK (1999). Biology of dairy cows during the transition period: The final frontier?. J. Dairy Sci. 82:2259-2273. https://doi.org/10.3168/jds.S0022-0302(99)75474-3

Frankic T, Voljč M, Salobir J, Rezar V (2009). Use of herbs and spices and their extracts in animal nutrition. Acta Agricult. Slovenica. 94:95-102.

Gaines, WL (1928). The energy basis of measuring milk yield in dairy cows. University of Lllinois. Agriculture Experiment Station. Bulletin No. 308.

Jayaprakash G, Sathiyabarathi M, Robert MA, Tamilmani T (2016). Rumen-protected choline: A significance effect on dairy cattle nutrition. Vet. World. 9:837. https://doi.org/10.14202/vetworld.2016.837-841

Kung L, Putnam DE, Garrett JE (2003). Comparison of commercially available rumen-stable choline products. J. Dairy Sci. 86:275. https://doi.org/10.3168/jds.S0022-0302(06)72530-9

Leiva T, Cooke RF, Brandao AP, Marques RS, Vasconcelos JL (2015). Effects of rumen-protected choline supplementation on metabolic and performance responses of transition dairy cows. J. Anim. Sci. 93:1896-1904. https://doi.org/10.2527/jas2014-8606

Li Z, Agellon LB, Vance DE (2005). Phosphatidylcholine homeostasis and liver failure. J. Biolog. Chem. 280:37798-3802. https://doi.org/10.1074/jbc.M508575200

Lima FS, Sá Filho MF, Greco LF, Santos JE (2012). Effects of feeding rumen-protected choline on incidence of diseases and reproduction of dairy cows. Vet. J. 193:140-145. https://doi.org/ 10.1016/j.tvjl.2011.09.019

Mentschel J, Leiser R, Mulling C, Pfarrer C, Claus R (2001). Butyric acid stimulates rumen mucosa development in the calf mainly by a reduction of apoptosis. Archiv. Anim. Nutrit. **55.** 85-102. https://doi.org/10.1080/17450390109386185

Narayanan K, Kunnathur MS, Chandrasekaran G (2013). Protective effect of Acacia nilotica (L.) against acetaminophen-induced hepatocellular damage in Wistar rats. Adv. Pharmacolog. Sci. 987692. https://doi.org/10.1155/2013/987692

National Research Council (2001). Nutrient requirements of dairy cattle. (7th Ed) pp. 519. (National Academies Press).

Piepenbrink MS, Overton TR (2003). Liver metabolism and production of cows fed increasing amounts of rumen-protected choline during the periparturient period. J. Dairy Sci. 86:1722-33. https://doi.org/10.3168/jds.S0022-0302(03)73758-8

Pinotti L, Baldi A, Dell'Orto V (2002). Comparative mammalian choline metabolism with emphasis on the high-yielding dairy cow. Nutrit. Res. Rev. 15:315-32. https://doi.org/10.1079/NRR200247

Pinotti L, Campagnoli A, Dell'Orto V, Baldi A (2005) Choline: Is there a need in the lactating dairy cow?. Livest. Prod. Sci. 98:149-152.

Pinotti L, Polidori C, Campagnoli A, Dell'Orto V, Baldi A (2010). A meta-analysis of the effects of rumen protected choline supplementation on milk production in dairy cows. Energy and protein metabolism and nutrition. Euro. Assoc. Anim. Prod. 127:321-322.

Sales J, Homolka P, Koukolova V (2010). Effect of dietary rumen-protected choline on milk production of dairy cows: A meta-analysis. J. Dairy Sci. 93:3746-54. https://doi.org/10.3168/jds.2010-3106

Selvam R, Saravanakumar M, Suresh S, Chandrasekeran CV, Prashanth DS (2018). Evaluation of polyherbal formulation and synthetic choline chloride on choline deficiency model in broilers: implications on zootechnical parameters, serum biochemistry and liver histopathology. Asian-Australasian J. Anim. Sci. 31**:**1795-1806. https://doi.org/10.5713/ajas.18.0018

Sun F, Cao Y, Cai C, Li S, Yu C, Yao J (2016). Regulation of nutritional metabolism in transition dairy cows: energy homeostasis and health in response to post-ruminal choline and methionine. PloS One. 11:0160659. https://doi.org/10.1371/journal.pone.0160659

Yao ZM, Vance DE (1988). The active synthesis of phosphatidylcholine is required for very low-density lipoprotein secretion from rat hepatocytes. J. Biolog. Chem. 263:2998-3004. https://doi.org/10.1016/S0021-9258(18)69166-5

Zeisel SH, Da Costa KA (2009). Choline: an essential nutrient for public health. Nutrit. Rev. 67:615-623. https://doi.org/10.1111/j.1753-4887.2009.00246.x