RESEARCH ARTICLE

Effect of Peripartum Supplementation of Rumen Protected Choline and Rumen Protected Fat on Energy Status, Insulin, IGF-1 and Postpartum Fertility in Gir Cows

Miteshkumar R. Chavda[1*], Harish H. Savsani[2], Mahesh R. Gadariya[3], Karsan B. Vala[4,] Arjan J. Dhami[5], Vijay K. Karangiya[6]

Abstract

The experiment was carried out on 24 advanced pregnant Gir cows in their first to third lactation to study the effects of supplementing rumen protected choline (RPC) and rumen protected fat (RPF) alone and in combination on their blood glucose, plasma insulin and IGF-1 profile, and postpartum fertility. The cows were divided equally into four treatment groups (n = 6 each), T1 (control), T2 (RPC), T3 (RPF) and T4 (RPC and RPF) on the basis of parity, body weight and previous lactation yield. In T1 group, cows were fed with basal diet to meet their nutrient requirement as per Indian Council of Agricultural Research (ICAR) (2013) feeding standards. In T2, each cow was supplemented with RPC @ 45 g/day, in T3 with RPF @ 80 g/d and in T4 with RPC @ 45 g/day + RPF @ 80 g/d along with basal diet of T1, starting from 30 days before expected date of calving to 60 days postpartum. Concentrations of blood glucose, plasma insulin and IGF-1 studied at monthly interval revealed drop in values around day 30 and 60 postpartum with significant differences in both the hormones. Supplementation of RPC and RPF alone or in combination had no significant effect on blood glucose and plasma insulin levels, overall or at any period, except on day 60 postpartum where glucose was significantly higher in RPF group. Plasma concentration of IGF-1 was significantly ($p < 0.001$) lower in T3 and T4 and non-significantly in T2 as compared to control overall and at prepartum and on the day of calving. It was also lowest on day 30 postpartum and fluctuated non-significant between -30, 0 and +60 days in all four groups. First heat postpartum appeared significantly earlier in all three supplementation groups as compared to control (46.17 ± 3.89 to 53.60 ± 4.92 vs.81.67 ± 3.87 days), however reduction in service period and number of services per conception was statistically non-significant. Combined supplement of RPC and RPF had no additional advantage in the study. It can be concluded that RPC or RPF alone can be supplemented in the ration of periparturient Gir cows to improve their energy status and postpartum fertility.

Keywords: Energy status, Gir cows, Plasma insulin, Postpartum fertility, Rumen protected choline, Rumen protected fat.

Ind J Vet Sci and Biotech (2022): 10.21887/ijvsbt.18.1.7

Introduction

The negative energy balance (NEB) within a few weeks before calving and in early lactation is the major factor causing increased incidence of postpartum metabolic diseases, reduced milk production and impaired reproductive performance. Though NEB is an important characteristic of transition cows and is a normal adaptive mechanism in high yielding dairy animals (Wankhade *et al.,* 2017), the cows try to adapt with NEB by mobilizing adipose tissue reserve through lipolysis, as a result of which more non-esterified fatty acids (NEFAs) are drained towards liver (Drackley *et al.,* 2014). Resumption of ovarian activity is reported to be initiated during NEB (Beam and Butler, 1999), but in early lactation NEB has subsequent adverse effects on fertility due to delayed postpartum ovarian activity and poor conception rates (Wathes *et al.,* 2007), apart from affecting peak milk yield and overall lactation yield.

Choline is involved in the metabolism of fatty acids in the liver and plays an important role in very low density lipoprotein synthesis and thereby contributes to fat export from the liver (Acharya *et al.,* 2019[b]). Most of feedstuffs for dairy cattle contain free choline and phosphatidyl choline, but its bioavailability from these feedstuffs is very low

[1]Polytechnic in Animal Husbandry, College of Veterinary Science & A.H., Kamdhenu University, Junagadh, Gujarat, India
[2]Department of Animal Nutrition, College of Veterinary Science & A.H., Junagadh,Kamdhenu University, Gujarat, India
[3]Bull Mother Farm, Junagadh Agricultural University, Amreli, Gujarat, India
[4]Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science & A.H., Junagadh, Kamdhenu University, Gujarat, India
[5]Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science & A.H., Anand, Kamdhenu University, Gujarat, India
[6]Cattle Breeding Farm, Junagadh Agricultural University, Junagadh, Gujarat, India

Corresponding Author: Miteshkumar R. Chavda, Polytechnic in Animal Husbandry, College of Veterinary Science & A.H., Kamdhenu University, Junagadh, Gujarat, India, e-mail: drmrchavda@gmail.com

How to cite this article: Chavda MR, Savsani HH, Gadariya MR, Vala KB, Dhami AJ, Karangiya VK (2022). Effect of Peripartum Supplementation of Rumen Protected Choline and Rumen Protected Fat on Energy Status, Insulin, IGF-1 and Postpartum Fertility in Gir Cows. Ind J Vet Sci and Biotech, 18(1): 34-38.

Source of support: Nil

Conflict of interest: None.

Submitted: 23/09/2021 **Accepted:** 29/12/2021 **Published:** 10/01/2022

in ruminants (<30%) because of extensive degradation by rumen micro-organisms (Sharma and Erdman, 1989). As dietary choline gets degraded rapidly in the rumen, it must be supplemented in the protected form (Elek *et al.,* 2008). Supplementing RPF to high producing lactating cows can enhance energy density of ration and energy intake in early lactation without compromising rumen cellulolytic bacterial activity (Thakur and Shelke, 2010) and reduces the deleterious effect of NEB during early lactation (Ganjkhanlou *et al.,* 2009). Supplementation of bypass fat before parturition could improve lactation performance as well as lower the metabolic problems (Duske *et al.,* 2009). Considering the role of rumen protected choline and fat in the diet of transitional dairy cows, the present study was aimed to evaluate the effect of peripartum supplementation of RPC and rumen protected fat (RPF) alone and in combination on energy status, metabolic hormones and postpartum fertility in Gir cows.

Materials and Methods

Selection and Management of Animals

This study was carried out during the period from August, 2020 to May, 2021 at the Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh in collaboration with Cattle Breeding Farm, Junagadh Agricultural University, Junagadh (India), following ethical approval by the Institutional Animal Ethics Committee, vide No. JAU/JVC/ IAEC/LA/64/2020. In all 24 advanced pregnant Gir cows were selected in their first to third lactation. They were divided into four equal treatment groups comprising six animals in each on the basis of their parity, body weight and previous lactation yield, *viz.,* T1 (control), T2 (RPC), T3 (RPF) and T4 (RPC and RPF). In T1 group, cows were fed with basal diet of 250 g maize bhardo, 10 kg green sorghum, mature pasture grass hay *ad lib.*, and compound cattle feed and cotton seed cake to meet their nutrient requirement as per ICAR (2013) feeding standards. Additionally in T2 group, each cow was supplemented with RPC @ 45 g/day, in T3 with RPF @ 80 g/d and in T4 with RPC @ 45 g/day + RPF @ 80 g/d along with above basal diet, starting from 30 days before expected date of calving to 60 days postpartum. All the cows were maintained in well ventilated hygienic sheds and *ad lib.* wholesome drinking water was made available to them. RPC and RPF were purchased from Kemin Industries South Asia Pvt. Ltd.

Blood Sampling, Lab Assay and Postpartum Events

Blood samples were collected from jugular vein in heparinised vacutainers on days -30, 0, 30, and 60 of calving from all the cows. The plasma was separated immediately by centrifugation of samples and stored at -20°C with a drop of merthiolate until analyzed. The blood glucose level was determined in fresh blood samples with glucometer using Dr. Morepen Gluco-One BG-03 blood glucose strips and Meter (Morpen Laboratories Limited, Delhi). Plasma Insulin and IGF-1 concentrations were determined by standard enzyme linked immune sorbent assay technique using Bovine Insulin and Bovine IGF-1 ELISA kits of MybioSource, Inc., USA as per the manufacturer's instruction.

The occurrence of first estrus and fertile estrus postpartum and numbers of services per conception were recorded for each cow. Cows showing estrus 60 days after calving were inseminated and pregnancy was confirmed 45 days after last AI.

Statistical Analyses

The data on different blood parameters were analyzed by two way analysis of variance (ANOVA) for treatment and period effects and on reproductive traits by one way ANOVA for treatment effect (Snedecor and Cochran, 1994). Pair-wise mean differences between groups were compared by Tukey's post-hoc test for significance at $p < 0.05$.

Results and Discussion

Effect on Blood Glucose Levels

The mean values of monthly blood glucose concentrations (mg/dL) recorded in cows of different nutrients supplemented groups varied non-significantly between groups at all periods, except on day 60 postpartum, where the blood glucose level of group T3 supplemented with RPF was significantly ($p<0.05$) highest and that of group T2 supplemented with RPC was lower than other two groups due to different energy status of two nutrients used (Table 1; Fig. 1). The levels were lowest on day 30 postpartum in all the groups, except T4. Further the level was highest on the day of calving in T2 group supplemented with RPC and on day 60 postpartum in T3 group supplemented with RPF. The overall mean value of T3 group was non-significantly higher than other groups. The mean blood glucose levels in different treatment groups fluctuated non-significantly without any specific trend during different peripartum periods. Overall, the values were found to be within the normal range of 45 to 75 mg/dl in all groups and periods. The reason may be a high metabolic rate of utilization of glucose and homeostatic mechanism of animal body that did not allow appreciable change in glucose level. Similar non-significant difference in blood glucose concentration was reported earlier in cows supplemented with RPC @ 55 g/day during peripartum period (Acharya *et al.,* 2019[b]) or @ 50 and 100 g/d of RPC before and after calving, respectively (Leiva *et al.,* 2015). Similar results were also reported by many workers following RPC supplementation at various dose rates during peripartum in dairy cows (Zom *et al.,* 2011; Amrutkar *et al.,* 2015). On the other hands, in several studies plasma glucose concentration had increased significantly in the dairy cows supplemented with RPC during peripartum period (Gupta *et al.,* 2018; Anonymous, 2020).

The present results of RPF supplementation were comparable with the observations of Tyagi *et al.* (2010), who observed non-significant difference in blood glucose concentration in periparturient crossbred cows receiving bypass fat (2.5 % of dry matter intake) for 40 days pre-partum till 90 days postpartum. However, Waghmare *et al.* (2016) and Nirwan *et al.* (2019) observed significant increase in blood glucose level in the cows supplemented with RPF during postpartum and peripartum period, respectively.

Plasma Insulin Level

The mean plasma insulin levels across the groups revealed non-significant effect of treatments (Table 1, Fig. 1). However, the insulin levels were found to be reduced significantly ($p < 0.05$) on day 30 postpartum in T1 and T4 groups, and it fluctuated insignificantly between days 30 and 60 postpartum in all group, as compared to prepartum values. Further, the plasma insulin levels dropped on the day of calving in all groups, but the drop was comparatively more in T3 and T4 group than T1 and T2 group, which might be due to beneficial effect of RPF supplement on this hormone. Overall plasma insulin levels were apparently higher in T1 and T2 groups as compared to T3 and T4 groups. Supplementation of both RPC and RPF alone or in combination did not show any beneficial effect on plasma insulin concentration in periparturient Gir cows. Similar lack of significant effect on plasma insulin concentration with RPC supplementation @ 50 g/day (Chung *et al.,* 2009) in lactating Holstein cows and bypass fat (2.5 % of dry matter intake) 40 days pre-partum till 90 days postpartum (Tyagi *et al.,* 2010) has been reported earlier. However, Leiva *et al.* (2015) and Acharya *et al.* (2019[b]) reported significantly higher blood insulin level in the dairy cows supplemented with RPC during peripartum periods. Singh *et al.* (2014) also reported non-significant effect of prill fat supplementation @ 75 g/day in lactating crossbred cows on plasma insulin level. However, Garnsworthy *et al.* (2008) reported significantly decreased plasma insulin concentration in dairy cows supplemented with bypass fat exceeding 15 g/kg of dry matter.

Plasma Insulin-like Growth Factor-1 (IGF-1)

The mean values of plasma IGF-1 were found to be significantly ($p<0.001$) lower in groups T3 and T4, and non-significantly lower in T2 as compared to control. The significant differences were observed during prepartum period and on the day of calving among four groups, the values being lower in all three treatment groups than in control (Table 1, Fig. 1). Plasma IGF-1 levels decreased significantly ($p<0.001$) on day 30th postpartum as compared to values of prepartum and on the day of calving in all groups, except in group T3, where the differences were non-significant. Further, the IGF-1 levels fluctuated non-significantly between -30, 0 and +60 days in three supplement groups T2, T3 and T4. Leiva *et al.* (2015) reported non-significant effect, while Acharya *et al.* (2019[b]) reported significantly higher plasma IGF-1 level in cows supplemented with RPC @ 55 g/day during peripartum period. We observed significant decrease in plasma IGF-1 level in the cows supplemented with RPF and RPF+RPC

Table 1: Effect of rumen protected choline (RPC) and rumen protected fat (RPF) supplementation peripartum on monthly blood glucose, plasma insulin and IGF-1 levels in Gir cows (Mean ± SE)

		Dietary treatment groups			
Parameter studied	*Peripartum Days*	*T1*	*T2*	*T3*	*T4*
Blood glucose (mg/dL)	-30	58.50 ± 1.84	56.17^{AB} ± 4.38	52.67 ± 6.47	56.00 ± 8.27
	0	55.67 ± 1.12	67.83^{B} ± 6.78	61.83 ± 8.03	53.33 ± 4.34
	30	48.00 ± 5.97	43.17^{A} ± 3.88	50.00 ± 8.03	51.67 ± 2.23
	60*	50.33^{ab} ± 6.86	43.00^{aA} ± 5.06	68.17^{b} ± 2.33	48.17^{ab} ± 3.77
	Overall	53.13 ± 2.34	52.54 ± 2.10	58.17 ± 3.69	52.29 ± 2.56
Plasma insulin (pmol/L)	-30	40.71^{B} ± 7.28	36.59^{B} ± 3.94	34.57^{B} ± 4.37	34.64^{B} ± 3.31
	0	35.91^{B} ± 4.07	34.18^{AB} ± 3.59	22.43^{AB} ± 2.20	22.85^{AB} ± 3.21
	30	19.95^{A} ± 3.37	26.40^{AB} ± 4.67	21.43^{AB} ± 3.23	20.45^{A} ± 1.72
	60	20.85^{A} ± 1.60	22.96^{A} ± 3.11	20.17^{A} ± 1.84	25.07^{AB} ± 2.86
	Overall	29.35 ± 2.65	30.03 ± 2.90	24.65 ± 1.71	25.75 ± 1.71
Plasma IGF-1 (ng/mL)	-30*	16.50^{bC} ± 0.82	13.61^{abB} ± 0.74	10.65^{aAB} ± 1.12	13.08^{aB} ± 1.26
	0*	16.00^{bcB} ± 0.65	14.47^{bB} ± 1.18	11.17^{aAB} ± 0.26	11.58^{aB} ± 0.87
	30	8.70^{A} ± 0.49	9.38^{A} ± 1.39	9.03^{A} ± 0.30	8.11^{A} ± 0.84
	60	13.06^{B} ± 0.90	11.30^{AB} ± 0.99	12.80^{B} ± 0.64	10.06^{AB} ± 0.29
	Overall***	13.56^{b} ± 0.20	12.19^{ab} ± 0.88	10.91^{a} ± 0.12	10.71^{a} ± 0.51

T1 Control; T2 RPC; T3 RPF, T4 RPC + RPF, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for group differences.
Means bearing different lower case superscripts (a, b) within the row for groups, and upper case superscripts (A, B) within the column for periods/days differ significantly ($p < 0.05$) for a parameter.

Table 2: Effect of peripartum RPC and RPF supplementation on reproductive traits of Gir cows (Mean ± SE)

	Dietary treatment groups			
Particulars	*T1*	*T2*	*T3*	*T4*
Days to first observed heat*	$81.67^{b} \pm 3.87$	$51.17^{a} \pm 4.00$	$46.17^{a} \pm 3.89$	$53.50^{a} \pm 4.92$
Service period	155.00 ± 17.17	125.33 ±12.21	126.50 ±15.95	138.50 ± 6.05
No. of services/conception	2.50 ± 0.50	1.83 ± 0.17	2.00 ± 0.26	2.00 ± 0.26

T1 Control; T2 RPC; T3 RPF, T4 RPC + RPF. *$p < 0.001$ between groups.
Means bearing uncommon superscripts (a, b) within the row differ significantly ($p < 0.05$).

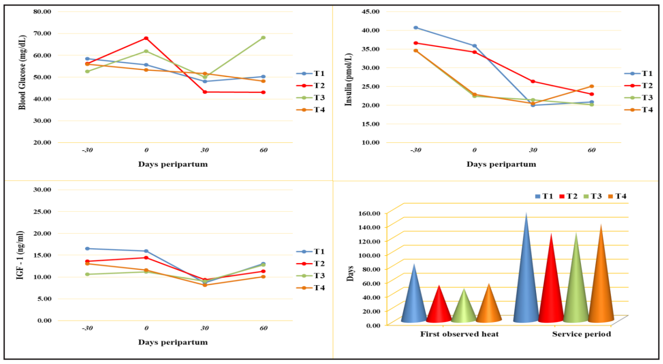

Fig. 1: Effect of peripartum RPC and RPF supplementation on monthly blood glucose, plasma insulin, plasma IGF-1 levels and reproductive traits of Gir cows

as compared to control or RPC alone. On the contrary, Garnsworthy *et al.* (2008) observed no significant effect of various levels of bypass fat on plasma IGF-1 concentration in lactating dairy cows.

Days to First Postpartum Heat

Commencement of cyclicity was significantly earlier ($p < 0.001$) in all the nutrients supplemented Gir cows as compared to control (Table 2 and Fig. 1), and it was found to be similar among three supplemented groups. Onset of postpartum cyclicity is related with the process of involution of uterus. It was reported to be enhanced significantly earlier by Pirestani and Aghakhani (2018) and non-significantly by Acharya *et al.* (2019[a]) in cows fed with RPC. Conversely, in many studies it was observed that supplementation of RPC during peripartum period did not influence cyclicity (Amrutkar *et al.,* 2015; Anonymous, 2020). The significant effect of peripartum RPF supplementation on commencement of cylicity observed in present study was in agreement with findings of Dhami *et al.* (2017), who observed significantly reduced time for commencement of cyclicity in peripartum and postpartum RPF supplemented cows as compared to control. In contrast, Nirwan *et al.* (2019) observed no significant difference in peripartum RPF supplemented group for this trait.

Service Period and Number of Services per Conception

Service period was non-significantly shorter with numerically reduced number of services per conception (Table 2; Fig. 1) in all the nutrients supplemented groups than that of control, suggesting beneficial effect of RPC and RPF supplementation in transition cows. Our results were in agreement with Pirestani and Aghakhani (2018), who also observed non-significant improvement in service period with reduced AI/conception in the cows receiving RPC in peripartum periods. However, in some studies, the service period and AI/conception were reported to be improved significantly due to supplementation of RPC (Amrutkar *et al.*, 2015; Acharya *et al.*, 2019[a]; Anonymous, 2020) and RPF (Dhami *et al.*, 2017; Nirwan *et al.*, 2019) to dairy cows during peripartum period.

Conclusions

Based on the results, it can be concluded that peripartum supplementation of both RPC and RPF alone or in combination does not have significant effect on concentration of plasma glucose and insulin in Gir cows. However, concentration of IGF-1 decreases non-significantly by RPC alone, and significantly by RPF alone and in combination with RPC also as compared to control. Supplementation of both RPC and RPF alone or in combination improves postpartum reproductive performance by reducing days to first observed heat, service period and number of services per conception.

Acknowledgement

Authors thank the Principal and Dean, College of Veterinary Science & AH, Junagadh and the Research Scientist, Cattle Breeding Farm, Junagadh Agricultural University, Junagadh for the facilities and cooperation extended for this work. The financial assistant by ICAR, New Delhi to Dr. A.J. Dhami, PI at AAU, Anand, through “AICRP on nutritional and physiological interventions for enhancing reproductive performance of animals” is also gratefully acknowledged.

References

Acharya, P., Lathwal, S.S., Baithalu, R., Patnaik, N., Thul, M.R., & Moharana, B. (2019[a]). Supplementing rumen-protected choline with green tea extract improves reproductive performances in transition Karan Fries cows. *Indian Journal of Animal Research, 54*(4), 452-455.

Acharya, P., Lathwal, S.S., Patnaik, N.M., & Moharana, B. (2019[b]). Rumen protected choline along with green tea extract maintain glucose homeostasis in transition Karan Fries cows. *Indian Journal of Animal Nutrition*, *36*(3), 276-280.

Amrutkar, S.A., Pawar, S.P., Thakur, S.S., Kewalramani, N.J., & Mahesh, M.S. (2015). Dietary supplementation of rumen-protected methionine, lysine and choline improves lactation performance and blood metabolic profile of Karan-Fries cows. *Agricultural Research*, *4*(4), 396-404.

Anonymous. (2020). The effect of feeding protected choline on milk and production efficiency in dairy cows. *In:* Research Report (2019-20), 18th Meeting of Agricultural Research Subcommittee on Animal Production and Fisheries Subcommittee, College of Veterinary Science, Anand Agricultural University, Anand, Gujarat. pp. 298-303.

Beam, S.W., & Butler, W.R. (1999). Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. *Journal of Reproduction and Fertility, Supplement*, *54*, 411-424.

Chung, Y.H., Brown, N.E., Martinez, C.M., Cassidy, T.W., & Varga, G.A. (2009). Effects of rumen-protected choline and dry propylene glycol on feed intake and blood parameters for Holstein dairy cows in early lactation. *Journal of Dairy Science*, *92*(6), 2729-2736.

Dhami, A.J., Theodore, V.K., Panchal, M.T., Hadiya, K.K., Lunagariya, P.M., & Sarvaiya, N.P. (2017). Effects of peripartum nutritional supplementation on postpartum fertility and blood biochemical and steroid hormone profile in crossbred cows. *Indian Journal of Animal Research*, *51*(5), 821-826.

Drackley, J.K., Wallace, R.L., Graugnard, D., Vasquez, J., Richards, B.F., & Loor, J.J. (2014). Visceral adipose tissue mass in non-lactating dairy cows fed diets differing in energy density. *Journal of Dairy Science, 97*(6), 3420-3430.

Duske, K., Hammon, H.M., Langhof, A.K., Bellmann, O., Losand, B., Nürnberg, K., Nurnberg, G., Sauerwein, H., Seyfert, H.M., & Metges, C.C. (2009). Metabolism and lactation performance in dairy cows fed a diet containing rumen-protected fat during the last twelve weeks of gestation. *Journal of Dairy Science, 92*(4), 1670-1684.

Elek, P., Newbold, J.R., Gall, T., Wagner, L., & Husventh, F. (2008). Effects of rumen protected choline supplementation on milk production and choline supply of peri-parturient dairy cows. *Animal*, *2*(11), 1595-1601.

Ganjkhanlou, M., Rezayazdi, K., Ghorbani, G.R., Banadaky, M.D., Morraveg, H., & Yang, W.Z. (2009). Effects of protected fat supplements on production of early lactation Holstein cows. *Animal Feed Science and Technology, 154*(3-4), 276-283.

Garnsworthy, P.C., Lock, A., Mann, G.E., Sinclair, K.D., & Webb, R. (2008). Nutrition, metabolism, and fertility in dairy cows: 2. Dietary fatty acids and ovarian function. *Journal of Dairy Science*, *91*(10), 3824-3833.

Gupta, D.K., Grewal, R.S., Kaur, S., & Lamba, J.S. (2018). Effect of prepartum rumen protected choline and energy supplementation on the postpartum milk production and metabolic profile of crossbred dairy cows. *International Journal of Current Microbiology and Applied Sciences*, *7*(4), 230-235.

ICAR. (2013). *Nutrient Requirements of Cattle and Buffaloes*. Indian Council of Agricultural Research, New Delhi, India.

Leiva, T., Cooke, R.F., Brandao, A.P., Marques, R.S., & Vasconcelos, J.L.M. (2015). Effects of rumen-protected choline supplementation on metabolic and performance responses of transition dairy cows. *Journal of Animal Science, 93*(4), 1896-1904.

Nirwan, S.S., Mehta, J.S., Kumar, A., Kumar, P., Kumar, A., & Singh, V. (2019). Effects of bypass fat on postpartum reproductive performance in dairy cattle. *Indian Journal of Dairy Science, 72*(2), 194-200.

Pirestani, A., & Aghakhani, M. (2018). The effects of rumen-protected choline and L-carnitine supplementation in the transition period on reproduction, production, and some metabolic diseases of dairy cattle. *Journal of Applied Animal Research, 46*(1), 435-440.

Sharma, B., & Erdman, R. (1989). *In vitro* degradation of choline from selected feedstuffs and choline supplements. *Journal of Dairy Science, 72*(10), 2772–2776.

Singh, M., Sehgal, J.P., Roy, A.K., Pandita, S., & Rajesh, G. (2014). Effect of prill fat supplementation on hormones, milk production and energy metabolites during mid lactation in crossbred cows. *Veterinary World, 7*(6), 384-388.

Snedecor, G.W., & Cochran, W.G. (1994). *Statistical Methods*. 8th edn. Oxford and IBH Publishing House, New Delhi, India, pp. 312-317.

Thakur, S.S., & Shelke, S.K. (2010). Effect of supplementing bypass fat prepared from soybean acid oil on milk yield and nutrient utilization in Murrah buffaloes. *Indian Journal of Animal Sciences, 80*(4), 354-357.

Tyagi, N., Thakur, S.S., & Shelke, S.K. (2010). Effect of bypass fat supplementation on productive and reproductive performance in crossbred cows. *Tropical Animal Health and Production*, *42*(8), 1749-1755.

Waghmare, S.P., Meshram, R.B., Dakshinkar, N.P., Pajai, K.S., & Siddiqui, M.F. M.F. (2016). Effect of supplementation of bypass fat on biochemical profile in dairy cows. *Asian Journal of Animal Sciences*, *11*(2), 111-114.

Wankhade, P.R., Manimaran, A., Kumaresan, A., Jeyakumar, S., Ramesha, K.P., Sejian, V., Rajendran, D., & Varghese, M.R. (2017). Metabolic and immunological changes in transition dairy cows: A review. *Veterinary World, 10*(11), 1367-1377.

Wathes, D.C., Fenwick, M., Cheng, Z., Bourne, N., Llewellyn, S., Morris, D.G., Kenny, D., Murphy, J., & Fitzpatrick, R. (2007). Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. *Theriogenology*, *68*(5), 232-241.

Zom, R.L.G., Van Baal, J., Goselink, R.M.A., Bakker, J.A., De Veth, M.J., & Van Vuuren, A.M. (2011). Effect of rumen-protected choline on performance, blood metabolites, and hepatic triacylglycerols of periparturient dairy cattle. *Journal of Dairy Science, 94*(8), 4016-4027.