International Journal of Agriculture Sciences
ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 14, Issue 7, 2022, pp.-11510-11513.
Available online at https://bioinfopublication.org/pages/jouarchive.php?id=BPJ0000217

Research Article

PRODUCTION RESPONSES OF TRANSITION DAIRY COWS TO DIFFERENT DIETARY PROTEIN LEVELS

S.S. NAIR*[1], K. ALLY[1], V. DILDEEP[1], J.J. MATHEW[1] AND V.L. GLEEJA[2]

[1]Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy, 680651, Kerala Veterinary and Animal Sciences University, Pookode, 673576, India
[2]Department of Statistics, College of Veterinary and Animal Sciences, Mannuthy, 680651, Kerala Veterinary and Animal Sciences University, Pookode, 673576, Kerala, India
*Corresponding Author: Email - sreepvsnair93@gmail.com

Received: July 08, 2022; Revised: July 26, 2022; Accepted: July 27, 2022; Published: July 30, 2022

Abstract: Twenty cross bred cows, three weeks prior to their expected date of calving were selected to find the influence of different dietary protein levels on production parameters, from University Livestock Farm and Fodder Research and Development Scheme (ULF & FRDS), Kerala Veterinary and Animal Sciences University, Kerala. Before calving, the animals were randomly allotted to 2 dietary treatments – T_1 (12 per cent CP (crude protein) TMR (Total Mixed Ration)) and T_2 (14 per cent CP TMR). After calving, half of the animals in T_1 group were allotted to T_3 group (16 per cent CP TMR) and remaining half to T_4 (18 per cent CP TMR). And half of the animals in T_2 were allotted to T_3 and remaining to T_4. The feeding trial was carried out for a period from 3 weeks pre-partum to 45 days postpartum. Results revealed that the average body weight and dry matter intake (DMI) of lactating cows maintained on the combination of dietary treatments were found to have no significant difference between the groups. The digestibility of crude fibre was higher and significantly different ($p<0.05$) for groups receiving treatments T_2T_3 (51.11±1.82). Nitrogen free extract (73.50±0.31 per cent) was higher and significantly different ($p<0.05$) for groups receiving treatments T_1T_4 (72.66±0.75) and T_2T_4 (73.17±0.472) when compared with the other groups. DMI postpartum was maintained throughout the experimental period without any marked difference between the treatment groups. From the results, it can be concluded that for medium producing animals, TMR containing low CP level pre-partum and high CP level postpartum will be beneficial in meeting the energy requirements during the transition period.

Keywords: *Total mixed ration, Dry matter intake, Transition period, Dietary protein, Body weight*

Citation: S.S. Nair, *et al*., (2022) Production Responses of Transition Dairy Cows to Different Dietary Protein Levels. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 14, Issue 7, pp.- 11510-11513.

Academic Editor / Reviewer: Dr Chethan K P, Dr Aakash S Alothumattam

Introduction

The transition period in dairy cows is defined as the time period from three weeks prior to parturition to three weeks after parturition. Animals in this period require nutrients for maintenance, foetal growth and production [1]. As a result of foetal development in late pregnancy, the nutrient demand increases especially glucose and amino acids [2]. The substantial milk yield acceleration, seen particularly in the last week pre-partum and in the first 2 weeks of lactation, which reflects the dramatic increase in nutrient demand at the onset of lactation. The nutrient requirement in late pregnancy, and particularly the nutrient demand for lactation, calls for a coordination of the physiological processes in different tissues resulting in metabolic changes that try to ensure that cows reach their maximum genetic potential for milk yield.

The DMI will be peaking at 10-14 weeks postpartum while milk production peaks at 5-8 weeks, leading to negative energy balance during the postpartum period [3]. 75 per cent of metabolic diseases in dairy herds occurred within the first month of lactation and the maximum risk being the first ten days following calving [4, 5]. In transition period, primiparous cows require a higher dietary protein concentration at 14 to 16 per cent of total DM, than the multiparous cows at 12 per cent. Negative energy balance (NEB) caused by severe increment in nutrient demand for milk production coupled with greater decrease (about 30 per cent) in dry matter intake (DMI) is the biggest challenge faced by transition cows [6]. Calving stress and endocrine changes that occur during early stages of the lactation are two main reasons for the reduction of DMI [7].

The average daily maintenance requirements for a dairy cow are 490 kJ of metabolisable energy (ME) and 293 kJ of net energy for lactation (NEL) per kg $LW^{0.75}$. Consequently, the daily energy requirements for maintenance of a stall-fed dairy cow weighing 650 kg are around 37.7 MJ NEL.

On the whole, it is estimated that a gestating cow has a daily demand of 360 g of metabolisable protein and 12-20 MJ of NEL [2]. The demands of energy of gestating cows reach 1.3 to 1.5 times the maintenance requirements by the end of gestation. In transition period, cows consume less than what they require, resulting in the negative energy balance which is predominant in the first week postpartum [8].

Production parameters during this period can be used as indicators for health status of the animal. In such a scenario, the production responses to different dietary protein levels in transition cows and interaction of dietary protein levels in pre and postpartum crossbred cows were evaluated in the study.

Materials and Methods

This study was conducted at the University Livestock Farm and Fodder Research Station (ULF & FRDS), College of Veterinary and Animal Sciences, Kerala, India.

Preparation of total mixed rations

Four types of total mixed rations were formulated using commonly used ingredients along with paddy straw as roughage source. The four experimental rations were: pre-partum diet-T_1 and T_2 (12 and 14 per cent CP TMR respectively), post-partum diet-T_3 and T_4 (16 and 18 per cent CP TMR respectively). These rations were iso-caloric (60 per cent TDN). Their chemical composition is presented in the [Table-1].

Feeding trial

Twenty dairy cows, around 325-340 kg body weight with 11-12 L of average milk production in previous lactation, three weeks prior to their expected date of calving

were selected and divided into two groups in a completely randomised block design and were then randomly allotted to dietary treatments, T_1 and T_2. After calving five animals from each group were allotted to T_3 and the remaining five to T_4 making four experimental groups. The feeding trial was carried out for a period of 3 weeks before the expected date of calving and 45 days after calving. All the experimental animals were fed as per standards [9].

Table-1 *Ingredient composition of paddy straw based TMR offered to experimental animals maintained on four dietary treatments*

Ingredients	T_1(kg)	T_2(kg)	T_3(kg)	T_4(kg)
Maize	27	20	20	14
Rice Polish	9	10	9	10
Tapioca Starch waste	4	4	4	2
De-oiled rice bran	7	10	8	12
CGF	10	13	9	15
Coconut Cake	5	5	9	9
Alfalfa	5.5	8.5	14	15
Straw	31	28	24	20
Calcite	0	0	1.5	1.5
Salt	0.5	0.5	0.5	0.5
Mineral Mixture	1	1	1	1
Total	100*	100*	100*	100*

**To every 100 kg of complete feed, 10g of Vitamin AD3E supplement (containing 10,00,000 I.U of Vitamin A, 2,00,000I.U of Vitamin D3 and 1,00,000 I.U of Vitamin E) was added*

Table-2 *Chemical composition of the total mixed rations[1] fed to the experimental cows on DM basis, %*

Parameter	Dietary treatments			
	T_1	T_2	T_3	T_4
Dry matter	90.01±0.58	90.08±0.48	88.92±0.46	88.94±0.68
Crude protein	11.82±0.13	13.71±0.17	16.11±0.18	18.21±0.14
Ether extract	3.62±0.15	3.55±0.18	3.10±0.16	3.26±0.12
Crude fibre	15.21±0.24	13.69±0.28	12.87±0.21	10.43±0.33
Total ash	8.99±0.26	10.09±0.36	11.60±0.41	12.61±0.38
Nitrogen free extract	60.36±0.16	58.96±0.14	55.32±0.11	55.49±0.15
Acid insoluble ash	4.25±0.06	4.13±0.05	5.14±0.08	4.77±0.11
Calcium	0.63±0.11	0.62±0.14	0.82±0.13	0.85±0.17
Phosphorus	0.35±0.13	0.41±0.16	0.47±0.15	0.51±0.14
NDF	48.65±0.28	47.36±0.25	47.65±0.31	45.21±0.29
ADF	28.21±0.14	26.48±0.16	26.56±0.17	24.25±0.15

[1]Values expressed on DM basis, average of six values

Body Weight

The animals were weighed on 14th, 7th day before the expected date of calving, on the day of calving and at weekly intervals up to 45 days postpartum.

Dry Matter Intake

The animals were fed ad libitum with respective TMRs at 2% of body weight during pre-partum period and at 3 % of body weight after calving. The animals were fed twice a day, in the morning and afternoon at 5 AM (before milking) and 1 PM, respectively. Feed residue was weighed throughout the feeding trial. Samples of fee residue were taken daily for analysing the moisture content and the daily dry matter intake. The data on daily dry matter intake was recorded throughout the entire experimental period.

Digestion Trial

A digestibility trial for five days duration was carried out at the end of feeding trial by total collection method. Representative samples of feed offered were taken daily during the trial period for proximate analysis. Dry matter content of the feed offered as well as residue was determined daily. Feed samples were collected in double lined polythene bags and stored for analysing proximate principles as per standard procedure [10]. Later, the dung voided by each animal was collected quantitatively uncontaminated with urine, feed residue or dirt as and when they were voided in individual containers. The entire quantity of dung voided by each animal during 24 hours were weighed separately at 9 A.M. every day and mixed thoroughly and representative samples at the rate of one per cent of total quantity were taken. The samples thus collected were stored in double lined polythene bags and stored in deep freezer (-20ºC) for further analysis. At the end of the trial, samples of dung collected for the five consecutive days from each animal were thawed and pooled. Then representative samples were taken after thorough mixing for chemical analysis. The moisture and CP in faecal samples were estimated using fresh samples. Balance samples were dried and ground for rest of the analysis as per standard procedure [10].

Statistical Analysis

Data obtained on the various parameters during the course of the experiment were analysed statistically [11] by analysis of variance (ANOVA) technique, using the software Statistical Product and Service Solutions (SPSS) version 24.0.

Results and Discussion

The chemical composition of the total mixed rations used for the feeding trial are given in the [Table-2]. The four total mixed rations T_1, T_2, T_3 and T_4 had 11.82 ± 0.13, 13.71 ± 0.17, 16.11 ± 0.18 and 18.21 ± 0.14 per cent of crude protein, respectively, on dry matter basis.

Consolidated data on weekly average daily DM intake of experimental animals maintained before and after calving are shown in [Table-3] and [Table-4] respectively. DM intake of experimental animals maintained on T_1 and T_2, two weeks before calving were 8.11±0.01 and 8.16±0.04 kg respectively. DM intake of experimental animals received treatment combinations T_1T_3, T_1T_4, T_2T_3 and T_2T_4 one week after calving were 8.38±0.45, 8.95±0.34, 8.16±0.2 and 9.06±0.23 kg respectively and after six weeks they were found to be 11.44±0.73, 12.07±0.48, 12.41±0.75 and 13.35±0.16 kg. Statistical analysis of the data revealed that there was no significant difference ($p>0.05$) in the average DM intake of cows fed on different dietary treatments. Similarly, DMI were not different among the four treatment groups (11-12 kg) on feeding different percent of dietary crude protein (CP) concentration and amount of rumen un-degradable protein (RUP) pre-partum and the extent of DMI depression was similar in all groups (30 per cent) beginning 7 to 10 d pre-partum [12]. They also observed that post-partum DMI was higher (21.8 kg/day) for the animals fed with 12 per cent CP and 26 per cent RUP than the other treatment groups (18-19 kg).

Table-3 *Weekly average daily DMI[1] of dairy cows maintained on two experimental diets before calving, kg*

Week	Dietary treatments		P value
	T_1	T_2	
2 weeks before calving	8.11±0.01	8.16±0.04	0.230
1week before calving	7.39±0.13	7.36±0.18	0.088

[1]Mean values are based on ten replicates with SE

Table-4 *Weekly average daily DMI[1] of dairy cows maintained on four Treatment combinations after calving, kg*

Week	Treatment combinations				P value
	T_1T_3	T_1T_4	T_2T_3	T_2T_4	
1	8.38±0.45	8.95±0.34	8.16±0.2	9.06±0.23	0.547
2	10.07±0.75	10.77±0.22	10.38±0.58	11.35±0.56	0.453
3	11.12±0.8	11.95±0.15	11.75±0.68	12.81±0.38	0.563
4	11.32±0.77	12.36±0.35	12.42±0.6	13.32±0.18	0.325
5	11.39±0.77	12.24±0.43	12.68±0.51	13.45±0.21	0.435
6	11.44±0.73	12.07±0.48	12.41±0.75	13.35±0.16	0.534

[1]Mean values are based on five replicates with SE

The average daily DMI of experimental lactating cows maintained on dietary treatments T_1 (NDF: 25.88per cent), T_2 (NDF: 30.03 per cent) and T_3 (NDF: 35.59 per cent) were 13.55±0.55, 13.62±0.31 and 13.01±0.60 kg, respectively, DMI per kg metabolic body size of experimental lactating cows were 0.15±0.02, 0.17±0.01 and 0.17±0.01 kg, respectively and DMI per 100 kg body weight of lactating cows were 3.80±0.18, 4.05±0.28 and 3.99±0.06 kg, respectively during early lactation [13]. An average daily DMI of 11.35, 10.68, 10.75 and 11.65 kg for the experimental animals maintained on varying proportion of concentrates and legumes during early lactation [14].

In the present study, DMI showed a decrease in last week of gestation and gradually increased after calving. Similarly, a study on animal and dietary factors affecting feed intake during the pre-fresh transition period in Holsteins indicated that DMI decreased 32 per cent during the final 3 week of gestation, and 89 per cent of that decline occurred during the final week of gestation [15].

For cows fed with TMR, DMI was increased from 9.6 kg one week prior to calving to 14 kg, 17 kg, 19 kg and 22 kg, on 11th, 21st, 33rd and 83rd days after calving [16]. DMI was increased from 13 kg on the day of calving to 20 kg, two weeks after calving for primiparous animals [17].

Table-5 *Weekly average body weight of experimental animals maintained on two treatments, before calving, kg*

Week[1]	Dietary treatments		P value
	T_1	T_2	
2 weeks before calving	385.20±8.72	420.80±7.92	0.72
1 week before calving	387.00±9.32	422.80±7.80	0.61
On the day of calving	360.10±9.49	397.00±8.06	0.77

[1]*Mean values are based on ten replicates with SE*

Table-6 *Weekly average body weight[1] of experimental animals maintained on four treatment combinations after calving, kg*

Week	Treatment combinations				P value
	T_1T_3	T_1T_4	T_2T_3	T_2T_4	
1	351.6±13.17	349±13.17	377±13.17	403.2±13.17	0.381
2	344.21±14.12	345.22±13.69	369.40±13.47	397.15±13.22	0.342
3	342.15±12.89	344.45±12.44	369.4±12.34	393.6±12.11	0.424
4	345.74±13.12	343.85±13.06	369.42±13.65	395.42±13.44	0.332
5	346.26±12.96	344.63±12.88	370.69±12.76	396.82±12.77	0.321
6	349.20±13.11	345.41±13.26	372.11±13.35	396.21±13.27	0.322

[1]*Mean values are based on five replicates with SE*

The average body weight of experimental animals maintained on four total mixed rations, recorded at weekly intervals, before and after calving are shown in [Table-5] and [Table-6] respectively. The average initial body weight of experimental animals maintained on two total mixed rations T_1 and T_2 were 385.20±8.72 and 420.80±7.92 kg and were found to be 360.10±9.49 and 397.00±8.06 kg on the day of calving. The average body weights of experimental animals fed on four total mixed ration combinations T_1T_3, T_1T4, T_2T_3 and T_2T_4 were 351.6±13.17, 349±13.17, 377±13.17 and 403.2±13.17 one week after calving and were found to be 349.20±13.11, 345.41±13.26, 372.11±13.35 and 396.21±13.27, six weeks after calving. Similarly, the BW change were not affected by CP levels in the pre-partum diet on evaluation of the effects of shortening the close-up period (from 21 to 10 d) combined with feeding different MP (7.9, 10.1 and 11.8 per cent on DM) levels on performance and metabolic status of multiparous Holstein cows [18].

The digestibility coefficient of nutrients of four total mixed ration combinations T_1T_3, T_1T4, T_2T_3 and T_2T_4 are presented in [Table-7]. The dry matter digestibility per cent of four total mixed ration combinations T_1T3, T_1T_4, T_2T_3 and T_2T_4 were 60.82±1.22, 62.84±0.98, 61.41±0.75 and 62.92±0.46 per cent respectively. Statistical analysis of the data revealed that there was no significant difference in the digestibility of DM in total mixed rations fed to cows maintained on different treatments. The digestibility coefficient of DM in lactating cross bred cows fed on berseem based TMRs containing varying levels of protein and energy (14 per cent CP plus 65 per cent TDN, 12 per cent CP plus 60 per cent TDN and 10 per cent CP plus 55 per cent TDN were similar and the values ranged from 66.76 to 72.17 per cent [19].

Table-7 *Digestibility coefficient of nutrients in experimental diet fed to dairy cows, %*

Parameter[1]	Treatment combinations				P value
	T_1T_3	T_1T_4	T_2T_3	T_2T_4	
Dry matter	60.82±1.22	62.84±0.98	61.41±0.75	62.92±0.46	0.292
Crude protein	59.51±0.73	61.56±1.33	62.31±2.1	62.77±1.38	0.774
Crude fiber	48.06±3.86^{ab}	41.29±3.42^{b}	51.11±1.82^{a}	40.08±1.93^{b}	0.434
Ether extract	86.86±1.6	88.17±2.09	87.86±1.01	86.24±0.79	0.045
NFE	69.9±1.31^{ab}	72.66±0.75^{a}	68.86±1.35^{b}	73.17±0.47^{a}	0.026
NDF	56.78±2.52	55.74±1.17	56.33±1.04	56.36±0.79	0.971
ADF	42.39±2.43	41.01±2.11	41.48±1.05	39.93±1.5	0.820

[1]*Mean values are based on five replicates with SE*

Mean ± SE of different treatment having different alphabets (a-b) as superscripts differs significantly with in a row at p<0.05

The Crude protein digestibility per cent of four total mixed ration combinations T_1T_3, T_1T_4, T_2T_3 and T_2T_4 were 59.51±0.73, 61.56±1.33, 62.31±2.1 and 62.77±1.38 per cent respectively. Statistical analysis of the data revealed that there was no significant difference in the digestibility of Crude Protein in total mixed rations fed to cows maintained on different treatments. Similar CP digestibility (59 to 60 per cent) for ration containing 17 per cent CP and 35 per cent UDP was also observed in early lactating crossbred cows [20].

The Ether Extract digestibility per cent of four total mixed ration combinations T_1T_3, T_1T_4, T_2T_3 and T_2T_4 were 86.86±1.6, 88.17±2.09, 87.86±1.01 and 86.24±0.79 per cent respectively. Statistical analysis of the data revealed that there was no significant difference ($p>0.05$) in the digestibility of Ether Extract in total mixed rations fed to cows maintained on different treatments. Similar values were reported on a study in cross-bred cattle and reported that the digestibility of EE in the group fed on complete rations with and without green fodder were similar to those fed on a conventional ration and the values ranged from 84.58 to 86.11 per cent [21]. Studies in lactating Holstein cows by feeding them with complete diets containing corn-milling coproducts and the digestibility coefficient of EE was similar in all the added groups as well as a control group without added coproducts, with the values being in the range of 83.80 to 86.30 per cent [22].

The Crude Fibre digestibility per cent of four total mixed ration combinations T_1T_3, T_1T_4, T_2T_3 and T_2T_4 were 48.06±3.861, 41.29±3.42, 51.11±1.82 and 40.08±1.93 per cent respectively. Treatments applied after calving is creating a significant difference between mean CF. Mean CF for T_3 is higher than T_4 and they are statistically significantly different ($p<0.05$). Similarly, the digestibility coefficient of CF in lactating cross-bred cows fed on berseem based TMRs containing 10 per cent CP and 55 per cent TDN and 14 per cent CP and 65 per cent TDN, were significantly higher than those fed on 12 per cent CP and 60 per cent TDN and the values in the three mentioned groups ranged from 64.13 to 72.06 per cent [19].

The Nitrogen Free Extract digestibility per cent of four total mixed ration combinations T_1T_3, T_1T_4, T_2T_3 and T_2T_4 were 69.9±1.3112, 72.66±0.752, 68.86±1.351 and 73.17±0.472 per cent respectively. The average of digestibility coefficients for NFE of treatments T_1 and T_2 before calving are 71.283 and 71.018, respectively and that of T_3 and T_4 after calving are 69.383 and 72.917 respectively. No significant difference was noticed in NFE digestibility between T_1 and T_2. Treatments applied after calving is creating a significant difference between mean NFE. Mean NFE for T_4 is higher than T_3 and they are statistically different ($p<0.05$). NFE digestibility of groups that received T_3 after calving were similar [23] and the values range from 66 to 67 per cent.

The Neutral detergent fibre digestibility per cent of four total mixed ration combinations T_1T_3, T_1T_4, T_2T_3 and T_2T_4 were 56.78±2.52, 55.74±1.17, 56.33±1.04 and 56.36±0.79 per cent respectively. Statistical analysis of the data revealed that there was no significant difference ($p>0.05$) in the digestibility of Neutral detergent fibre in total mixed rations fed to cows maintained on different treatments. The Acid detergent fibre digestibility per cent of four total mixed ration combinations T_1T_3, T_1T_4, T_2T_3 and T_2T_4 were 42.39±2.43, 41.01±2.11, 41.48±1.05 and 39.93±1.5 %, respectively. Statistical analysis of the data revealed that there was no significant difference ($p>0.05$) in the digestibility of Acid detergent fibre in total mixed rations fed to cows maintained on different treatments. Similarly, ADF (480 g/kg) and NDF (570 g/kg) digestibility were found to be similar for the groups fed with high protein level (133 g/kg DM) with no calcium salts of fatty acid, high protein level with calcium salts of fatty acids, low protein level with no calcium salts of fatty acids and low protein level with calcium salts of fatty acids [24].

Conclusion

The average body weight, dry matter intake (DMI) and milk production of lactating cows maintained on the combination of dietary treatments were found to have no significant difference between the groups. The digestibility of crude fibre was higher and significantly different for groups receiving treatments T_2T_3 (51.11±1.82). Nitrogen free extract (73.50±0.31 per cent) was higher and significantly different for groups receiving treatments T_1T_4 (72.66±0.75) and T_2T_4 (73.17±0.472) when compared with the other groups. The dry matter, crude protein, ether extract, neutral detergent fibre and acid detergent fibre digestibility were similar between treatment groups. DMI postpartum and milk production were maintained throughout the experimental period without any marked difference between the treatment groups. There was no occurrence of metabolic diseases in any of the experimental animals, indicating all the dietary treatments were sufficient to meet the requirement of the animals. From the results, it can be concluded that for medium producing animals, TMR containing low CP level pre-partum and high CP level postpartum will be beneficial in meeting the energy requirement and maintaining the milk production with improved milk protein level.

Application of research: Study of production responses of transition dairy cows to different dietary protein levels

Research Category: Veterinary and Animal Sciences

Acknowledgement / Funding: Authors are thankful to Department of Animal Nutrition; Department of Statistics, College of Veterinary and Animal Sciences, Mannuthy, 680651, Kerala Veterinary and Animal Sciences University, Pookode, 673576, Kerala, India

****Research Guide or Chairperson of research: Dr K. Ally**
University: Kerala Veterinary and Animal Sciences University, Pookode, 673576, Kerala, India
Research project name or number: MVSc Thesis

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: University Livestock Farm and Fodder Research Station (ULF & FRDS)

Cultivar / Variety / Breed name: Dairy cows

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

References

[1] Grummer R.R. (1995) *Journal of Animal Science*, 73, 2820-2833.
[2] Bell A.W. (1995) *Journal of Animal Science*, 73(9), 2804-2819.
[3] Butler W.R. and Smith R.D. (1989) *Journal of Dairy Science*, 72(3), 767-783.
[4] Ingvartsen K.L., Dewhurst R.J. and Friggens N.C. (2003) *Livestock Production Science*, 83(2-3), 277-308.
[5] LeBlanc S.J. (2006) *In Proceedings of the 26th World Biuatrics Congress, Nice*, 460-472.
[6] Horst R.L., Goff J.P. and Reinhardt T.A. (1994) *Journal of Dairy Science*, 77(7), 1936-1951.
[7] LeBlanc S. (2013) *In Book of Abstracts, 15th Conference on Production Diseases in Farm Animals; Dalin G., Ed.; Swedish University of Agricultural Science (SLU), Uppsala, Sweden,* 62-65.
[8] Beever D.E. (2006) *Animal Reproduction Science*, 96(3-4), 212-226.
[9] ICAR [Indian Council of Agricultural Research] (2013) *Nutrient requirements of Animals-cattle and buffalo. (3rd Ed.). Indian Council of Agricultural Research, New Delhi*, 59.
[10] AOAC [Association of Official Analytical Chemists] (2016) *Official methods of analysis. (20th Ed.). Association of Official Analytical Chemists, Washington DC*, 1885.
[11] Snedecor G.W. and Cochran W.G. (1994) *Statistical Methods (8th Ed.). The Iowa state university press, Ames, Iowa, USA*. 503.
[12] Greenfield R.B., Cecava M.J., Johnson T.R. and Donkin S.S. (2000) *Journal of Dairy Science*, 83(4), 703-710.
[13] Chacko B. (2015) *PhD Thesis. Kerala Veterinary and Animal Sciences University, Pookode*, 250.
[14] Jayaprakash G. (2016) *M.V.Sc. Thesis. Kerala Veterinary and Animal Sciences University, Pookode*, 82.
[15] Hayirli A., Grummer R.R., Nordheim E.V. and Crump P.M. (2002) *Journal of Dairy Science*, 85(12), 3430-3443.
[16] Reynolds C.K., Aikman P.C., Lupoli B., Humphries D.J. and Beever D.E. (2003) *Journal of Dairy Science*, 86(4), 1201-1217.
[17] Neave H.W., Lomb J., Von Keyserlingk M.A.G., Behnam-Shabahang A. and Weary D.M. (2017) *Journal of Dairy Science*, 100(1), 548-561.
[18] Farahani T.A., Amanlou H. and Kazemi-Bonchenari M. (2017) *Journal of Dairy Science*, 100(8), 6199-6217.
[19] Girdhar N. and Balaraman N. (2005) *Indian Journal of Animal sciences*, 75, 47-51.
[20] Dominic G. (2013) *M.V.Sc. Thesis. Kerala Veterinary and Animal Sciences University, Pookode*, 95.
[21] Gupta R.B., Tiwari D.P. and Kumar A.N.I.L. (2006) *Indian Journal of Animal Sciences, India*, 76(1), 55-60.
[22] Kelzer J.M., Kononoff P.J., Gehman A.M., Tedeschi L.O., Karges K. and Gibson M.L. (2009) *Journal of Dairy Science*, 92(10), 5120-5132.
[23] Vijayaraghavendran S. (2019) *M.V.Sc. Thesis. Kerala Veterinary and Animal Sciences University, Pookode*, 82.
[24] Yang W.Z. and Beauchemin K.A. (2005) *Journal of Dairy Science*, 88(3), 1090-1098.