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COLOSTRUM PRODUCTION, CALF BIRTH WEIGHT, AND POSTPARTUM OVARIAN FOLLICULAR ACTIVITY OF DAIRY COWS FED RESTRICTED DIET WITH DIFFERENT PROTEIN LEVELS DURING THE PREPARTUM PERIOD

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ABSTRACT

Prepartum carry-over nutritional effects on postpartum reproductive performance result from complex interactions of many dietary variables, most importantly energy and protein. Thirty-six multiparous Holstein cows were blocked at −21 day relative to expected calving and randomly assigned one of the four treatments until calving: high (HMP) or low dietary metabolizable protein (LMP) with ad libitum (AFI) or restricted feed intake (RFI). The supplies of MP were 65 and 90 g/kg of DM for LMP and HMP diets, whereas intake was controlled to achieve 100 and 160% of NRC (2001) energy requirements for RFI and AFI groups, respectively. All the cows were fed a similar lactation diet after calving. Calving ease score and total number of ovarian follicles tended to increase in RFI versus AFI cows. The interaction between MP × intake indicated that calf birth weight tended to increase in RFI versus AFI cows when fed HMP versus LMP diet during the prepartum period. The day of first heat, calving to conception interval, and services per conception were not affected by prepartum dietary treatments. In conclusion, RFI versus AFI cows had easier calving, increased number of ovarian follicles, and higher calf birth weight, whereas HMP versus LMP diet increased size of small follicles and tended to increase size of large follicles.

Keywords: Periparturient cow, restricted energy, ovarian follicle, reproductive performance, calf birth weight

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INTRODUCTION

The 6 to 8 week period centered on parturition is known as transition or periparturient period (Drackley *et al.,* 2005). Although certain regulatory mechanisms remained to be explained, few fundamental factors facilitating reproductive activities have become obvious (Zollinger and Hansen, 2003). One of the earliest factors recognized is the deep influence of nutritional strategies during this period. Metabolic disturbances resulting from nutrient imbalance during transition may have negative direct or indirect influence on subsequent reproduction (Chapinal *et al.,* 2012). Different nutritional strategies have been proposed to facilitate metabolic and physiological adaptations from gestation to lactation including controlled energy (Cardoso *et al.,* 2020) and increased protein supplies (Kokkonen, 2014). However, due to limited research, results on postpartum reproductive performance in response to prepartum dietary changes are inconsistent, with studies showing either positive (Chagas *et al.,* 2006), negative (Keady *et al.,* 2001), or no effect (Pushpakumara *et al.,* 2003). Furthermore, studies on colostrum production and calf birth weight in response to prepartum dietary changes are conducted mostly in beef cows (Olson *et al.,* 1981; Hough *et al.,* 1990; McGee *et al.,* 2006). However, these results are poorly applicable in dairy cows due to different metabolic changes during the periparturient period compared with beef cows (Mann *et al.,* 2016).

Carryover effects of prepartum nutrition are commonly related to the changes in dietary energy supplies. To support the gravid uterus growth, energy requirements of dairy cows increases by 20% during the last month of gestation (Moe *et al.,* 1972). Increased fetal development and calf birth weight in prepartum overfed beef cows are reported previously (Spitzer *et al.,* 1995; Stalker *et al.,* 2006). Contrarily, restricted energy during the last few weeks of gestation is reported to improve postpartum health status of dairy cows (Janovick *et al.,* 2011). Moreover, improved calving ease is repeatedly observed by farmers with prepartum low dietary energy (Beever, 2006), whereas prepartum high dietary energy increased calving difficulty (Zollinger and Hansen, 2003). Postpartum resumption of ovarian activity is closely related to the prepartum feeding management (Cavestany *et al.,* 2009). Prepartum high energy supply and subsequent metabolic stress during the postpartum phase is considered one of the reasons of delayed resumption of ovarian activity (Castro *et al.,* 2012). Apart from energy, changes in the dietary protein supply have possible effects on ovarian functions (Butler, 2001). Maintenance of maternal protein reserves is important for reproduction (Bell *et al.,* 2000) as postpartum deficiency of metabolizable protein (MP) may decrease the fertility and ovarian activity during the postpartum period (Drackley and Cardoso, 2014).

Although several studies indicated indirect signs of improvement in reproductive performance with controlled prepartum dietary energy (Cardoso *et al.,* 2013), most of the studies focused on postpartum dry matter intake (DMI), milk production, and incidence of health disorders (Dann *et al.,* 2005; Janovick and Drackley, 2010; Graugnard *et al.,* 2012). As per authors' knowledge, no study observed the effects and interaction of prepartum dietary MP and feed intake on colostrum production, colostrum quality, calf birth weight, ovarian follicular activity, and postpartum reproductive performance of dairy cows. Therefore, the objective of this study was to investigate the effects of low and high dietary MP fed with restricted versus ad libitum feed intake during the prepartum period on colostrum production, calf birth weight, and postpartum reproductive performance of multiparous Holstein dairy cows.

MATERIALS AND METHODS

Cows, Experimental Design, and Treatments: This experiment was conducted at University of Veterinary and Animal Sciences, Ravi Campus, Pattoki, Pakistan. The complete details of enrolled cows, animal management, and treatment diets were described by Akhtar *et al.* (2021). In brief, thirty-six multiparous Holstein cows were enrolled in this study. Cows were dried off 60 day before expected date of parturition and enrolled based on parity, previous lactation yield, and expected date of calving. Cows were kept in individual pens in semi-open concrete floor shed with sand bedding. The enrolled cows received four dietary treatments in a 2 × 2 factorial arrangement during the close-up period. Treatments were high (HMP; 90 g/kg of MP) and low metabolizable protein (LMP; 65 g/kg of MP) diets for ad libitum feed intake [AFI; 160% of NRC (2001) energy requirements] or restricted feed intake [RFI; 100% of NRC (2001) energy requirements]. Treatment diets were offered from −21 day relative to expected calving and cows remained on their respective treatments through calving. The restriction of energy intake was accomplished by limiting the DMI (by 20%) in RFI group relative to the DMI predicted by the NRC (2001) model. From calving through 63 days in milk, all the cows received a similar lactation diet for ad libitum intake. Both pre- and postpartum diets were mixed daily and offered as total mixed ration. The cows were milked thrice daily and had free access to fresh drinking water round the clock.

Measurements and Sample Analysis: The birth of each calf was carefully observed and calves were removed and weighed on digital weighing balance before colostrum feeding approximately 15-20 min after calving. Calves were not allowed to suckle their dams and cows were milked within 45 min after calving. Cows were milked through automatic milking parlor (GEA WestfaliaSurge GmbH, Germany) and colostrum was measured and sampled at the first milking. Colostrum IgG content was estimated using Brix value of refractometer. Duplicate sample of colostrum was used for the analysis of composition within an hour after parturition for fat, protein, and lactose contents with the help of Lactoscan-S milk analyzer (Milkotronic Ltd. Bulgaria).

Reproductive Performance and Ultrasonography: Calving ease was scored using a 5-point scale (1 = no calving problem, 2 = minor problem, 3 = needed assistance, 4 = considerable force, 5 = caesarian; Park *et al.,* 2002). Time for placenta expulsion was time between parturition and expulsion of placenta for each calving. Starting from week 2 postpartum, all cows were rectally palpated weekly to examine the cows for changes in uterine horns and to access the activity on both ovaries (number of follicles, size of follicles, and number of corpus luteum). This examination was done with the use of ultrasound scanner Honda-1500 (7.5 MHz linear array transducer; Honda Electronics, Tokyo, Japan). Once the fecal material was removed, lubricated ultrasound probe was inserted into the rectum to cover the transducer for better contact and images, and probe was moved along the dorsal surface of uterine horns. Probe was moved laterally to be positioned adjacent to each ovary for examination. By moving the probe along its surface, each ovary was scanned in several positions. Follicle diameter was calculated as the average length and width of antrum. All follicles $\geq$ 3 mm were counted. For the analyses, ovarian follicles were organized into three classes based on diameter: small (< 5.0 mm), medium (5.0 to 10.0 mm), and large (> 10.0 mm; Badiei *et al.,* 2014).

Calculations and Statistical Analysis: Fat and protein corrected colostrum was calculated from the following equation of Barros *et al.* (2017): colostrum yield (kg) × [(0.1226 × fat %) + (0.0776 × protein %) + 0.2534]. Milk N (MkN) = protein content/6.38 and milk energy (MkE) = [0.00929 × fat yield (g)] + [0.00563 × protein yield (g)] + 0.00395 × lactose yield (g)] following Barros *et al.* (2017). Energy conversion efficiency was calculated from energy corrected colostrum ÷ energy intake (Mäntysaari *et al.,* 2019). All cows were included in the statistical analysis. Calving day and postpartum data were analyzed separately to avoid problem with fitting covariate structure. Data were analyzed using the

GLIMMIX procedure of SAS University Edition (SAS Institute Inc. Cary, NC). The statistical model included the fixed effects of MP and intake levels, time relative to calving, and random effect of cow within a treatment as error term. For variables measured over time (number of follicles, size of follicles, and diameter of uterine horns), repeated measures analysis was done and week was considered as a repeated measure. Covariance structure with the lowest Akaike's information criterion was used (Littell *et al.,* 1998). The data of calving day (colostrum production and calf birth weight), reproductive performance, and largest follicle size were analyzed using the same model without the repeated measures statement using the GLIMMIX procedure of SAS University Edition (SAS Institute Inc. Cary, NC). The model used for calf birth weight also included calf sex and semen used to rule out their possible effect on calf birth weight. Results were declared significant at $P < 0.05$ and tendency toward significance at $0.05 < P \leq 0.10$ using Tukey's multiple range test.

RESULTS

Colostrum Production and Composition: Effects of prepartum dietary treatments on colostrum production, composition, and IgG content are presented in Table 1. Actual colostrum and fat and protein corrected colostrum production tended to increase in cows fed HMP diet compared with those fed LMP diet during the prepartum period (P=0.10 and P=0.08, respectively). Prepartum feed intake had no effect on colostrum yield (P>0.10). Colostrum fat, protein, lactose, and IgG contents and colostrum density were not affected by prepartum dietary MP level and feed intake (P>0.10). A trend in MP × I interaction indicated that colostrum fat content increased in RFI cows compared with AFI cows when fed LMP diet but not when fed HMP diet (P=0.07).

Table 1. Effects of prepartum dietary treatments on colostrum production.

Item	Close-up period diet[1]							
	HMP		LMP			*P*-value[2]		
	AFI	RFI	AFI	RFI	SEM	MP	I	MP × I
Colostrum yield (kg)								
Actual	3.53	3.70	2.30	2.27	0.821	0.10	0.92	0.90
Fat and protein corrected	4.13	4.44	2.30	2.98	0.944	0.08	0.58	0.83
Component (%)								
Fat	2.58	1.85	1.83	3.65	0.726	0.45	0.44	0.07
Protein	7.32	8.26	7.83	8.10	0.595	0.76	0.31	0.57
Lactose	10.9	11.6	11.6	12.1	0.967	0.50	0.56	0.90
Component (g)								
Fat	92.9	75.8	39.8	79.8	26.57	0.33	0.65	0.26
Protein	251	294	187	191	70.8	0.22	0.73	0.78
Lactose	382	418	273	283	106.6	0.24	0.82	0.90
Colostrum density	79.4	87.1	79.4	83.8	5.47	0.76	0.27	0.75
Colostrum IgG (mg/ml)	56.3	61.1	58.0	56.5	4.22	0.71	0.69	0.45

[1]HMP = high metabolizable protein = 90 g/kg MP and LMP = low metabolizable protein = 65 g/kg MP diets for AFI = ad libitum feed intake to achieve NE_L intake in excess (~160%) of NRC (2001) recommendation and RFI = restricted feed intake to restrict NE_L intake to 100% of NRC (2001) recommendation during the close-up period.
[2]MP = metabolizable protein; I = ad libitum or restricted feed intake; MP × I = interaction between MP and I.

Calf Birth Weight and Colostrum Production Efficiency: Effects of prepartum dietary treatments on calf birth weight and colostrum production efficiencies are presented in Table 2. Birth weight of the calves tended to increase in RFI cows compared with AFI cows (P=0.06). A trend in dietary MP × I interaction indicated that calf birth weight tended to increase in RFI cows compared with AFI cows when fed HMP diet but not when fed LMP diet during the prepartum period (P=0.06). Efficiency of actual colostrum production tended to increase in HMP versus LMP cows, whereas fat and protein corrected colostrum production efficiency tended to increase in both HMP (P=0.09) and RFI cows (P=0.07). Efficiencies of N (P=0.10) and energy conversion (P=0.06) tended to increase in RFI versus AFI cows. A trend in MP × I interaction indicated that MkN: MkE decreased in RFI versus AFI cows when fed LMP diet but not when fed HMP diet during the prepartum period (P=0.06).

Reproductive Performance: Effects of prepartum dietary treatments on reproductive performance of dairy cows are presented in Table 3. Calving ease score tended to decrease in RFI cows compared with AFI cows (P=0.09). Other reproductive parameters including time for placenta expulsion, postpartum first heat, calving to first service, calving to conception, and services per conception were not affected by prepartum dietary MP level (P>0.10) and feed intake (P>0.10).

Table 2. Effects of prepartum dietary treatments on calf birth weight and colostrum production efficiency.

Item, g/d	Close-up period diet[1]							
	HMP		LMP			*P*-value[2]		
	AFI	RFI	AFI	RFI	SEM	MP	I	MP × I
Calf birth weight (kg)	34.3	39.6	36.3	36.3	1.37	0.64	0.06	0.06
Colostrum production efficiencies								
Actual colostrum (%)	0.27	0.40	0.17	0.25	0.078	0.09	0.16	0.72
Fat and protein corrected (%)	0.32	0.49	0.17	0.33	0.090	0.08	0.07	0.95
N efficiency (%)	13.1	21.7	13.6	21.4	4.92	0.97	0.10	0.93
Energy conversion efficiency	0.03	0.05	0.02	0.04	0.009	0.16	0.06	0.94
Milk N: Milk Energy (g/Mcal)	11.0	11.8	12.1	10.4	0.65	0.81	0.51	0.06

[1]HMP = high metabolizable protein = 90 g/kg MP and LMP = low metabolizable protein = 65 g/kg MP diets for AFI = ad libitum feed intake to achieve NE_L intake in excess (~160%) of NRC (2001) recommendation and RFI = restricted feed intake to restrict NE_L intake to 100% of NRC (2001) recommendation during the close-up period.
[2]MP = metabolizable protein; I = ad libitum or restricted feed intake; MP × I = interaction between MP and I.

Table 3. Effects of prepartum dietary treatments on reproductive performance of dairy cows.

Item	Close-up period diet[1]							
	HMP		LMP			*P*-value[2]		
	AFI	RFI	AFI	RFI	SEM	MP	I	MP × I
Calving ease score	2.22	1.25	1.62	1.37	0.364	0.51	0.09	0.32
Time for placenta expulsion (h)	7.55	6.77	5.62	6.37	2.205	0.59	0.99	0.72
First heat (d)	40.1	46.3	44.1	33.6	7.21	0.53	0.75	0.23
Calving to 1st service[3] (d)	82.0	67.7	85.0	76.7	10.89	0.45	0.16	0.70
Calving to conception[3] (d)	98.0	107	115	126	34.2	0.48	0.68	0.96
Services per conception[3]	1.60	2.33	2.00	2.00	0.866	0.95	0.56	0.56
Animal conceived (%)	55.6	66.7	22.2	66.7	-	-	-	-

[1]HMP = high metabolizable protein = 90 g/kg MP and LMP = low metabolizable protein = 65 g/kg MP diets for AFI = ad libitum feed intake to achieve NE_L intake in excess (~160%) of NRC (2001) recommendation and RFI = restricted feed intake to restrict NE_L intake to 100% of NRC (2001) recommendation during the close-up period.
[2]MP = metabolizable protein; I = ad libitum or restricted feed intake; MP × I = interaction between MP and I.
[3]For cows pregnant by 150 DIM.

Diameter of the Largest Follicle: Effects of prepartum dietary treatments on diameters of the largest follicle on different days during the postpartum period are presented in Table 4. The diameter of the largest follicle increased on day 49 with HMP diet by 61% compared with the LMP cows (P=0.05). Prepartum feed intake had no effect on postpartum diameter of the largest follicle (P>0.10). A trend in interaction between prepartum dietary MP × I indicated that diameter of the largest follicle decreased in RFI cows on day 35 (P=0.07) and increased on day 63 (P=0.08) when fed LMP diet but no such change was observed in cows fed HMP diet.

Ultrasonography Measurements: Results of ultrasonography measurements are presented in Table 5. Number of large follicles on right ovary and diameter of small follicles on left ovary increased by 71.4 and 7.38%, respectively in cows fed HMP diet compared with those fed LMP diet (P=0.05). Diameter of all follicles on right ovary tended to increase (P=0.06) and number of large follicles on left ovary tended to decrease (P=0.06) in cows fed HMP versus LMP diet. Total number of follicles, size of follicles, and number of corpus luteum on both ovaries were not affected by prepartum dietary MP level (P>0.10). Number of all follicles and small follicles on right ovary increased in RFI cows compared with AFI cows (P<0.05). Total number of follicles on both ovaries tended to increase in RFI cows compared with AFI cows (P=0.09). An interaction of MP × I indicated that number of medium sized follicles on right ovary increased in RFI cows when fed HMP diet and increased in AFI cows when fed LMP diet (P=0.01).

Table 4. Effects of prepartum dietary treatments on postpartum diameter of largest follicle (mm).

Item	Close-up period diet[1]							
	HMP		LMP			*P*-value[2]		
	AFI	RFI	AFI	RFI	SEM	MP	I	MP × I
Day 21	10.2	10.9	7.86	11.8	2.281	0.73	0.28	0.45
Day 28	13.2	13.7	8.94	12.9	2.185	0.23	0.29	0.43
Day 35	9.13	10.6	10.8	6.35	1.703	0.42	0.36	0.07
Day 49	11.0	12.8	8.77	6.00	3.176	0.05	0.83	0.30
Day 63	10.7	8.70	7.82	12.1	1.835	0.88	0.52	0.08

[1]HMP = high metabolizable protein = 90 g/kg MP and LMP = low metabolizable protein = 65 g/kg MP diets for AFI = ad libitum feed intake to achieve NE_L intake in excess (~160%) of NRC (2001) recommendation and RFI = restricted feed intake to restrict NE_L intake to 100% of NRC (2001) recommendation during the close-up period.
[2]MP = metabolizable protein; I = ad libitum or restricted feed intake; MP × I = interaction between MP and I.

Table 5. Effects of prepartum dietary treatments on postpartum ovarian follicular activity of dairy cows.

Item[3]	Close-up period diet[1]							
	HMP		LMP			*P*-value[2]		
	AFI	RFI	AFI	RFI	SEM	MP	I	MP × I
Right ovary								
All follicles (no.)	1.35	2.28	1.56	1.94	0.319	0.84	0.04	0.36
Small follicles (no.)	0.62	1.55	0.84	1.45	0.331	0.85	0.02	0.60
Medium follicles (no.)	0.18	0.33	0.48	0.16	0.101	0.55	0.40	0.01
Large follicles (no.)	0.56	0.40	0.24	0.32	0.104	0.05	0.71	0.23
All follicles diameter (mm)	10.3	9.28	7.75	7.81	1.120	0.06	0.63	0.58
Small follicles diameter (mm)	3.49	3.62	3.54	3.52	0.094	0.70	0.43	0.32
Medium follicles diameter (mm)	8.91	7.80	7.99	8.46	0.667	0.81	0.57	0.16
Large follicles diameter (mm)	12.3	14.5	14.2	13.4	1.41	0.70	0.56	0.19
Left ovary								
All follicles (no.)	1.85	1.88	1.46	1.70	0.373	0.43	0.70	0.77
Small follicles (no.)	1.50	1.45	0.95	1.17	0.359	0.24	0.80	0.69
Medium follicles (no.)	0.24	0.22	0.28	0.19	0.094	0.91	0.58	0.75
Large follicles (no.)	0.12	0.20	0.25	0.35	0.080	0.06	0.26	0.90
All follicles diameter (mm)	6.72	7.57	7.61	8.12	1.113	0.51	0.53	0.87
Small follicles diameter (mm)	3.51	3.62	3.30	3.34	0.111	0.03	0.48	0.73
Medium follicles diameter (mm)	7.44	8.07	7.28	6.85	0.839	0.36	0.89	0.48
Large follicles diameter (mm)	15.1	14.0	13.6	13.0	1.61	0.24	0.42	0.87
Both ovaries								
All follicles (no.)	3.23	4.16	3.04	3.64	0.448	0.42	0.09	0.70
Diameter of all follicles (mm)	7.89	7.69	7.45	7.31	0.763	0.58	0.82	0.96
No. of corpus luteum	1.04	1.06	0.97	0.79	0.136	0.14	0.46	0.38

[1]HMP = high metabolizable protein = 90 g/kg MP and LMP = low metabolizable protein = 65 g/kg MP diets for AFI = ad libitum feed intake to achieve NE_L intake in excess (~160%) of NRC (2001) recommendation and RFI = restricted feed intake to restrict NE_L intake to 100% of NRC (2001) recommendation during the close-up period.
[2]MP = metabolizable protein; I = ad libitum or restricted feed intake; MP × I = interaction between MP and I.
[3]Ultrasonography measurements were done on weekly basis from week 2 to week 9 of lactation; small, medium, and large categories represent follicles with < 5.00, 5.00-10.0, and > 10.0 mm of diameter, respectively.

DISCUSSION

Design and Production Data: Prepartum energy balance of ~105 and 165% of NRC (2001) requirements were achieved in RFI and AFI groups, respectively in both LMP and HMP cows. Data for DMI, production performance, body condition score (BCS), and body weight were reported previously (Akhtar *et al.,* 2021). Briefly, the AFI cows had higher serum β-hydroxybutyrate, free fatty acids, and BCS loss during the postpartum period compared with the RFI cows, however, this increase was more pronounced in cows fed LMP versus HMP diet indicating the use of body fat reserves. A decrease in serum β-hydroxybutyrate and free fatty acid concentrations supported an increase in postpartum DMI in HMP cows compared with LMP cows (Akhtar *et al.,* 2021).

Colostrum Production and Composition: Effects of prepartum dietary treatments on colostrum production and composition are in agreement with previous studies (Nowak *et al.,* 2012; Mann *et al.,* 2015). Given the fact that IgG concentration of colostrum is highly variable on dairy farms (Conneely *et al.,* 2013), achieving the highest possible concentration is important to optimize the newborn health. The mean IgG concentration of colostrum of all cows included in the study was 58.0 ± 2.84 mg/mL. This was close to the colostrum concentration found in previous studies (Morrill *et al.,* 2012; Dunn *et al.,* 2017). It indicates that energy supply in RFI cows in our study was sufficient to produce enough quantity of colostrum IgG concentration in addition to the quality of colostrum produced similar to AFI cows. Although no effect of prepartum dietary protein on colostrum IgG concentration observed in our study as reported previously (Santos *et al.,* 2001; Hare *et al.,* 2019), it is observed that increased prepartum dietary MP supply could increase total colostrum IgG yield (Fig. 1).

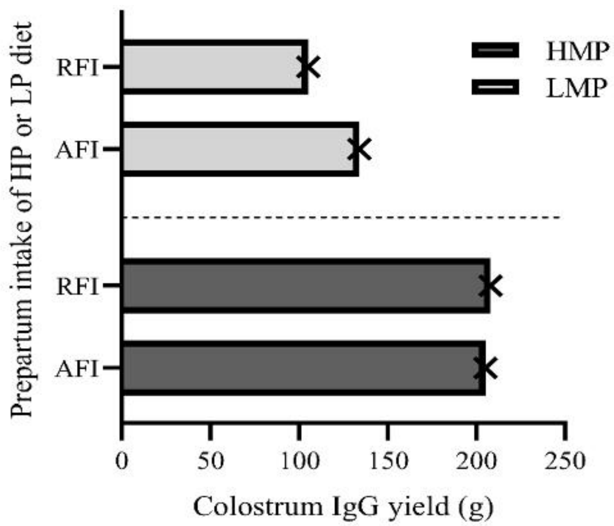

Fig. 1. Colostrum IgG yield (g) of Holstein dairy cows fed different diets prepartum. Close-up period treatment abbreviations: HMP = high metabolizable protein = 90 g/kg MP and LMP = low metabolizable protein = 65 g/kg MP diets for AFI = ad libitum feed intake to achieve NE_L intake in excess (~160%) of NRC (2001) recommendation and RFI = restricted feed intake to restrict NE_L intake to 100% of NRC (2001) recommendation during the close-up period. Prepartum treatment diets were fed from −21 day before expected calving until calving. MP, *P* = 0.04; I, *P* = 0.75; MP × I, *P* = 0.70.

Prepartum dietary MP × I interaction for Calf Birth Weight: An interesting finding of our study was an increasing trend in calf birth weight in RFI cows compared with AFI cows. However, the MP × I interaction indicated that calf birth weight increased in RFI cows compared with AFI cows when received HMP diet compared with LMP diet. Increase in calf birth weight was also observed previously in cows fed 70% of energy requirements than those fed 150% of energy requirements (Perry *et al.,* 1991). Contrarily, a decrease in calf birth weight with low dietary energy was also reported when prepartum energy supply of 70% compared with 130% relative to the requirements (Khan *et al.,* 2004). No difference in calf birth weight of cows fed 100 and 130% energy of requirements during the complete dry period is also observed previously (Dunn *et al.,* 2017). However, the meta-analysis of 48 studies reported lower calf birth weight in cows fed high total digestible nutrients compared with cows fed adequate amount of total digestible nutrients (Zago *et al.,* 2019). It is observed that nutritional restriction during gestation can reduce the fetal growth, increase muscle fiber diameter, and increase adiposity (Gonzalez *et al.,* 2013; Gutiérrez *et al.,* 2014). On the other side, oversupply of nutrients during pregnancy can also be detrimental for the development of fetus (Du *et al.,* 2010). The main effect of overfed cows to reduce fetal growth is metabolic disturbance as indicated by increased free fatty acids in AFI cows around calving in our study (Fig. 2). However, over-expression of genes responsible for adipocytes in fetus and down regulation of myogenesis are also additional reasons (Zago *et al.,* 2019). Conflicting results on the influence of cow nutrition during the prepartum period on calf birth weight could be explained by MP × I interaction observed in this study. However, further research on utilization of prepartum dietary energy and protein from different sources on calf birth weight are required. Excess energy supply than requirements can lead to low nutrient use efficiency in ruminants (Webster *et al.,* 1981), which can explain the increasing tendency in fat and protein corrected colostrum production efficiency and efficiency of N and energy utilization in RFI cows compared with AFI cows in our study.

Calving Ease Tended to Increase in Restricted Cows Prepartum: Ease of calving tended to improve by 31.8% in RFI cows compared with AFI cows. This result is in accordance with previous reports (Zollinger and Hansen, 2003; Beever, 2006). Increased calving difficulty with low dietary protein (≤ 11.7% CP of DM) and repeatedly observed easier calving with low metabolizable energy prepartum (2.15 Mcal/kg of DM) are reported in literature (Park *et al.,* 2002; Beever, 2006). Overfeeding and underfeeding relative to 100% of requirements during prepartum period increases calving difficulty (Proudfoot *et al.,* 2009). Increased calving difficulty in fat cows (BCS ≥ 8) results from fat-filled birth canal and increased abnormal presentations, whereas thin cows (BCS ≤ 3) as a result of restricted diet have no strength to withstand the birth process (Zollinger and Hansen, 2003). Moreover, it is also reported that underfeeding cows relative to (100%) requirements to the point where they are emaciated (BCS = 1) results in calving difficulty similar to the overfed cows relative to (100%) requirements to the point of obesity (BCS ≥ 8; Zollinger and Hansen, 2003). However, in our study, the RFI cows were offered diet to achieve 100% of NRC (2001) energy requirements, which might have resulted in improved calving ease compared to those fed energy in excess of requirements. Richards (2011) also noted 6.09% numerical increase in calving ease with controlled energy diet but they offered diet for ad libitum intake in contrast to our RFI cows.

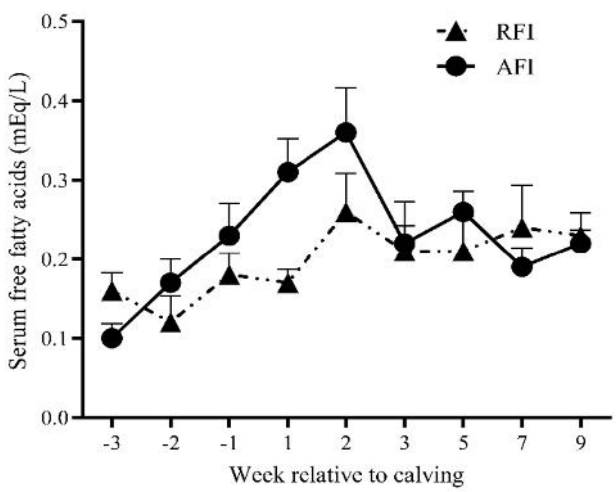

Fig. 2. Least square means for serum free fatty acids (mEq/L) of dairy cows fed different diets prepartum. Prepartum treatment diet abbreviations: RFI = restricted feed intake to restrict NE_L intake to 100% of NRC (2001) recommendation and AFI = ad libitum feed intake to achieve NE_L intake in excess (~160%) of NRC (2001) recommendation during the close-up period. Prepartum treatment diets were fed from −21 day before expected calving until calving. All cows were fed a similar lactation diet after calving.

Dietary MP × I interaction Affected Size of the Largest Follicle: It is interesting that a trend in MP × I interaction was observed on day 35 and 63 for the largest follicle diameter irrespective of the prepartum MP level and intake. A decrease in the largest follicle size on day 35 and an increase on day 63 indicate that sufficient body reserves might have been achieved during first 9 wk of lactation in RFI-LMP cows (Akhtar *et al.,* 2021). Additionally, the HMP diet increased the largest follicle size on day 49 during the postpartum period. These results are in agreement with Santos *et al.* (2001), where they observed an increase of 16.3% in the largest follicle size during first 45 days postpartum by increasing the prepartum dietary crude protein by 2% of DM. Larger, estrogenic follicles may increase uterine immunity, enhance uterine involution, and accelerate resumption of ovarian activity during the postpartum period (Santos *et al.,* 2001).

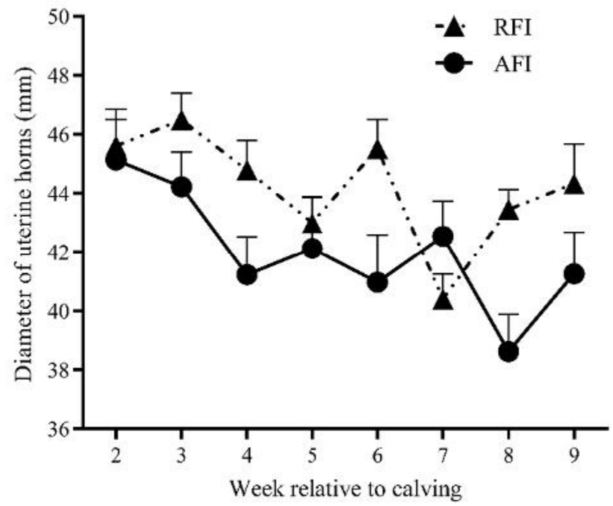

Fig. 3. Least square means for diameter of uterine horns (mm) of dairy cows fed different diets prepartum. Prepartum treatment diet abbreviations: RFI = restricted feed intake to restrict NE_L intake to 100% of NRC (2001) recommendation and AFI = ad libitum feed intake to achieve NE_L intake in excess (~160%) of NRC (2001) recommendation during the close-up period. Prepartum treatment diets were fed from −21 day before expected calving until calving. All cows were fed a similar lactation diet after calving. Ultrasonography measurements were done on weekly basis from week 2 to week 9 of lactation. Week, $P = < 0.01$; I, $P = 0.02$; week × I, $P = 0.02$.

Uterine Horns and Ovarian Follicles Responded to Prepartum Dietary Changes: The AFI cows had lower uterine horn diameter compared with RFI cows without being affected by prepartum dietary MP levels. Although uterine horn diameter decreased by time in all cows but week × intake interaction indicates that change in diameter of uterine horns was relatively lower and smoother in RFI cows (Fig.3). These findings reflect that AFI cows used nutrients less efficiently for reproduction, which might be a result of prepartum adaptation. As previously reported, resumption of ovarian activity in dairy cows is determined by energy status of the cows (Santos *et al.,* 2001) and RFI cows might have used energy more efficiently. Moreover, cows have to make metabolic choices about where to directly use the resources and in early lactation, nutrients will be directed to milk production instead of reproduction for the next pregnancy (Bauman and Currie, 1980). Therefore, intake of MP and energy during both pre- and postpartum periods is important for the continuous supply of these nutrients for reproductive activities. Increasing trend in number of all follicles might be due to improved metabolic status in RFI cows compared with AFI cows (Akhtar *et al.,* 2021). Number and size of follicles on both ovaries separately responded to prepartum dietary MP level. However, an interaction detected between MP × I on medium follicles of right ovary and overall changes in ovarian follicles indicate that both protein and energy prepartum should be considered in balance for different postpartum developmental stages of the ovarian follicles.

Conclusions: In conclusion, restricted intake during the prepartum period tended to improve calving ease, calf birth weight, and total number of ovarian follicles. High metabolizable protein diet increased the largest follicle size on day 49, number of large follicles on right ovary, and size of small follicles on left ovary. The day of first heat, calving to conception interval, and services per conception were not affected by prepartum dietary changes. Interaction between dietary metabolizable protein and feed intake detected for calf birth weight and postpartum ovarian follicles are indicative that dairy cows use prepartum high dietary MP more efficiently when energy supply is controlled to NRC (2001) recommendations.

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