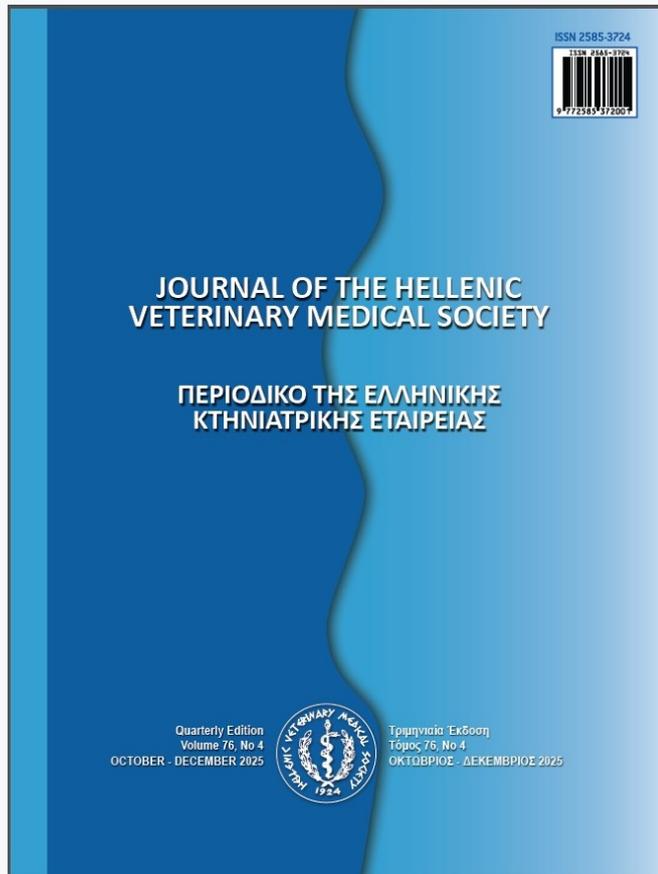


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Nutritional strategies and seasonal adaptations: Enhancing dairy cow productivity and calf development

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ABSTRACT: This study assessed the impact of rumen undegradable protein levels in close-up diets on Holstein Friesian cows, examining milk yield, composition, body condition score, colostrum, and calf weight across seasons. Using a design with two replicates per treatment, 173 multiparous Holstein cows were randomly assigned to COND (Control Diet = 4.2% RUP, 14.3% crude protein) or RUPD (Rumen Undegradable Protein Diet = 6.0% RUP, 17.1% crude protein) diets administered 30 days prepartum. Results showed that dry matter intake was influenced by both season and diet, with RUPD-fed cows exhibiting higher dry matter intake, especially in winter. While milk yield showed no significant seasonal difference, increased dietary rumen undegradable protein correlated with higher milk yield. An inverse relationship existed between milk fat percentage and rumen endegradable protein levels. Milk protein content increased with higher dietary protein, particularly in winter. Calf weight was higher in winter, and colostrum fat and urea nitrogen levels varied with the season. Body condition score remained unaffected by calving season or diet crude protein level. In conclusion, adjusting the rumen undegradable protein content in close-up diets has been shown to enhance dry matter intake, milk production, and calf weight in Holstein cows during the close-up period, thereby positively impacting their nutritional status.

Keyword: Close-up feeding; Calf; Milk production; Dairy cow; Rumen undegradable protein

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INTRODUCTION

The physiological changes occurring in cows during the dry period necessitate adopting appropriate feeding strategies (Delaby et al., 2009). The close-up period is a critical period of fetal development and is important for maternal maintenance and support for the growing fetus (Pascottini et al., 2020; VandeHaar and Donkin, 1999). Meeting nutritional requirements during this period is essential not only for proper fetal development and high milk yield in the postpartum period but also for preventing metabolic disorders, such as retained placenta, metritis, hypocalcemia, and ketosis, that can be influenced by inadequate feeding practices (Bionaz et al., 2020; Cardoso et al., 2020; Curtis et al., 1985; Lopreiato et al., 2020; Rodríguez et al., 2017; Tucho and Ahmed, 2017). Recent studies have highlighted the importance of close-up feeding and the impact of varying protein levels on key outcomes such as prenatal calf development, udder health, colostrum quality, and overall health (Hiltz and Laarman, 2019; Shi et al., 2020; Singh et al., 2020; Wang et al., 2021; Borchardt et al., 2022; Westhoff et al., 2023). However, despite these advances, the seasonal effects on protein requirements during the dry period remain poorly understood, with seasonal fluctuations potentially influencing nutrient metabolism and reproductive performance in ways that require further exploration.

During the close-up period, bypass protein intake is particularly important to support digestive system function, accommodate increased fetal growth, and prepare for lactation (VandeHaar and Donkin, 1999). Insufficient protein intake during this period can negatively impact prenatal calf development, maternal health, and lactation performance. Conversely, excessive protein intake can reduce nitrogen and feed efficiency while increasing nitrogen excretion through feces, urine, and gases (Agle et al., 2010; Scherpenzeel et al., 2018; Toledo et al., 2021).

Seasonal factors, including heat and cold stresses, must be considered when determining nutrient requirements (Kaufman et al., 2018). Exposure to heat stress can alter dry matter intake and nutrient utilization in tissues (West, 1999; Gorniak et al., 2014), potentially leading to problems during the close-up period due to changes in body condition score (BCS) (Friggens et al., 2004). Maintaining an optimal body condition is critical to avoid health issues related to non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) released by adi-

pose tissue postpartum (LeBlanc, 2010; Bünemann et al., 2019; Gärtner et al., 2019).

While recent studies suggest that RUP supplementation during the close-up period may increase postpartum milk yield (Farahani et al., 2019; Girma et al., 2019), conflicting results have been observed regarding the impact of high rumen-degradable protein (RDP) feeding on postpartum amino acid requirements (Abdelmegeid et al., 2018). Although previous research on close-up feeding's effects on calf birth weights has yielded inconsistent results (Dewhurst et al., 2000; Chebel et al., 2018; Daros et al., 2021), few studies have comparatively explored the influence of heat and cold stress during this period (Mulliniks et al., 2015). In light of these gaps, we hypothesize that feeding an RUP-supplemented diet during the close-up period will enhance milk yield, milk composition, BCS, colostrum quality, and calf birth weight. Our objective is to evaluate the combined impact of seasonal factors, such as heat and cold stress, alongside an RUP-supplemented diet on these critical parameters.

MATERIALS AND METHODS

This study was conducted at the Dasht Radin dairy farm in Qazvin, Iran, in August 2018 and February 2019.

Animals, diets, and experimental design

A total of 173 multiparous Holstein cows were divided into two pens per treatment group, with two replicates in both summer and winter. The cows were grouped based on parity, BCS, and previous milk yield, measured 30 days before the expected parturition date.

Two experimental diets were formulated: the control diet (COND) and the rumen undegradable protein supplemented diet (RUPD). The COND diet contained 4.2% RUP and 14.3% crude protein (CP), while the RUPD diet contained 6.0% RUP and 17.1% CP. Both diets were formulated to provide net energy lactation (NEL) levels of 1.60 Mcal/kg DM. To achieve the desired CP levels, RUP sources such as corn gluten meal and fish meal were incorporated. The experimental diets were fed from 30 days before the expected calving date until parturition. Post-calving, all cows were provided the same postpartum diet (PPD) from calving until 21 days in milk (DIM).

The cows were housed in pens of identical dimensions, with uniform bedding, floor covering, and water availability. The experimental diets were ad-

ministered twice daily, at 0900 and 1700 h, ensuring a targeted leftover of 5–10%.

Upon showing primary signs of calving, cows were moved to maternity pens. Calf weights were recorded immediately after birth, before colostrum administration, and the initial milking of colostrum was documented. Colostrum samples were collected for later compositional analysis. After calving, cows were transferred to sand-bedded free stalls until reaching 21 DIM, receiving the same PPD diet ad libitum three times daily (0900, 1700, and 0100 h). Milking occurred three times daily (0800, 1600, and 0000 h).

Table 1 presents the distribution of BCS into categories (< 3.25, 3.25–3.75, and > 3.75) and the Mean±SEM values for previous lactation milk yield and parity at -30 days relative to the expected calving date. Table 2 provides the ingredients and compositions of the postpartum and treatment diets, while Table 3 details their chemical composition.

Meteorological data, sampling and data collection

Daily air temperature (T) and relative humidity (RH) were recorded in the pens throughout the experimental period, with separate records kept for both winter and summer. These data were used to calculate the Temperature-Humidity Index (THI) using the following equation (Dikmen and Hansen, 2009):

$$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$$

In addition, wind velocity (V) data obtained from the meteorological center were used to calculate the Wind Chill Temperature (WCT) using the formula (Tucker et al., 2007):

$$WCT = 13.12 + 0.6215 T - 13.17 V^{0.16} + 0.3965 T \times V^{0.16}$$

where T is temperature in degrees Celsius (°C), RH is relative humidity in percentage (%), and V is wind velocity in kilometers per hour (km/h).

Daily records of feed intake and leftovers were documented throughout the experimental period. Total Mixed Ration (TMR) and leftover samples were collected twice a week and frozen at -20°C for later analysis. After the experimental period, the samples were thawed, dried at 60°C for 48 hours, ground through a 1-mm screen, and analyzed for nutritional components, including Ether Extract (EE) (method 920.39) and Crude Protein (CP) (method 984.13) (Helrich, 1990). Energy intake was calculated using the equations provided by the National Research Council (NRC, 2001), with Net Energy Intake (Mcal) determined by multiplying DMI by Net Energy Lactation (NEL) per kilogram of DM.

Milk yield was recorded daily from 1 to 21 DIM. Milk samples were collected twice a week, covering three consecutive milkings, and analyzed for fat, true protein, lactose, and Milk Urea Nitrogen (MUN) using a MilkoScan (CombiFoss 78110; Foss Analytical A/S, Hillerød, Denmark). BCS was assessed at -30, -3, 0, and 21 days relative to calving by two individuals using a 5-point scale (1 = thin, 5 = obese) (Edmonson et al., 1989), and the average scores were used for analysis.

Post-birth, female calves were separated from their mothers, and their weights were recorded on a digital scale before colostrum feeding. Colostrum was administered within the first 30 minutes after birth, and the calves were housed in metal boxes with wheat straw bedding until 3 days post-birth. Calf weights were recorded twice: at 30 days old and at weaning (70 days of age).

Statistical analysis

Data were analyzed using PROC MIXED in SAS version 9.4 (SAS Institute Inc., NC), employing a split-plot design. The analytical model was represented by the following formula:

$$Y_{ijkl} = \mu + S_i + D_j + E_{aij} + SD_{ij} + C(SD)_{ijk} + E_{bijkl}$$

Table 1. Proportions of BCS (< 3.25, 3.25 - 3.75, and > 3.75), Mean±SEM of previous lactation milk yield, and parity for each treatment group at -30 days relative to expected calving date

Items	Season		Diet		P-value		
	Summer	Winter	COND	RUPD	Season	Diet	Season×Diet
≤ BCS 3.25, [% (no/no)]	63.32(52/85)	71.61(63/88)	70.24(63/90)	63.87(53/83)	0.20	0.37	0.59
3.25<BCS<3.7, [% (no/no)]	26.89(23/85)	18.12(16/88)	21.53(20/90)	22.88(19/83)	0.17	0.83	0.36
≥ BCS 3.75, [% (no/no)]	10.07(9/85)	10.19(9/88)	7.7(7/90)	13.21(11/83)	0.98	0.24	0.62
Milk yield ¹ , kg	11573±140.39	11804±137.84	11757±135.45	11620±140.73	0.24	0.48	0.94
Parity	3.19±0.14	3.23±0.14	3.19±0.13	3.23±0.14	0.81	0.86	0.68

¹ Previous lactation

Table 2. Ingredients and compositions of postpartum and treatment diets¹

Ingredients, % of DM	Treatment		
	PPD	COND	RUPD
Legume forage hay	14.50	23.50	23.50
Corn silage	21.30	33.57	33.60
Wheat straw	1.58	-	-
Beet sugar pulp, dried	3.70	-	-
Barley grain, rolled	13.70	8.60	5.25
Corn grain, ground, dry	19.70	15.03	12.50
Canola meal, mechanical extraction	-	3.00	3.00
Soybean meal, solvent	15.40	8.45	8.45
Meat and bone meal	2.10	2.67	2.67
Fish meal	-	-	3.05
Corn gluten meal	-	-	2.88
Cottonseed, whole with lint	1.80	-	-
Soybean seed, whole heated	1.50	-	-
Calcium soaps of fatty acids	0.32	-	-
Calcium carbonate	0.98	1.42	1.42
Magnesium oxide	0.34	0.16	0.16
Magnesium sulfate	-	0.84	0.84
Calcium chloride	-	0.54	0.54
Ammonium chloride	-	0.32	0.24
Sodium bicarbonate	0.80	-	-
Salt	0.45	-	-
Bentonite	0.49	-	-
Vitamin premix ²	0.68	0.95	0.95
Mineral premix ³	0.68	0.95	0.95

¹ PPD: %16.00 CP (RDP: %10.80, RUP: %5.20), COND: %14.30 CP (RDP: %10.10, RUP: %4.20), RUPD: %17.10 CP (RDP: %11.10, RUP: %6.00)

² (DM basis): 1.200.000 IU of vitA/kg, 250.000 IU of vitD/kg, 10.000 IU of vitE/kg, 200 mg of biotin/kg, and 3.000 mg of monensin/kg.

³ (DM basis): 2.105 mg of Co/kg, 4.200 mg of Cu/kg, 190 mg of I/kg, 14.500 mg of Mn/kg, 80 mg of Se/kg, and 15.000 mg of Zn/kg.

where Y_{ijkl} represents the dependent variable, μ is the overall mean, S_i accounts for the fixed effect of season, D_j is the fixed effect of diet, E_{aij} is the main error term, SD_{ij} captures the interaction between season and diet, $C(SD)_{ijk}$ is the random effect of cows nested within season by diet, and E_{bijkl} is the secondary error term.

Season was considered the whole-plot factor, while CP prepartum served as the subplot factor. Covariance structures for each variable were assessed, and the optimal structure was determined based on the smallest Bayesian Information Criterion (BIC), Akaike Information Criterion (AIC), and correct-

ed Akaike Information Criterion (AICc) (Littell et al., 1998). Least squares means were used to report the data, and statistical significance was assessed at $P < 0.05$. A tendency to significance was considered at $P < 0.10$, and means were compared using ANOVA with Tukey's test to ensure precision and reliability in the statistical analysis.

RESULTS

The research findings have been examined under three subheadings: (i) Environmental conditions, milk yield and compositions, (ii) Feed intake, BCS and colostrum compositions, and (iii) Calf weights and average daily gain.

Table 3. Chemical composition of postpartum and treatment diets

Composition	Treatment		
	PPD	CON	RUPD
NE _L ² , Mcal/Kg DM	1.61	1.63	1.66
CP ¹	14.30	17.10	16.00
RDP ²	10.10	11.10	10.80
RUP ²	4.20	6.00	5.20
RDP balance ² , gr/d	+8.00	+131	+91
RUP balance ² , gr/d	+419	+649	-518
MP balance ³ , gr/d	+196	+400	-421
NDF ¹	32.20	31.60	28.00
ADF ¹	22.20	22.10	18.00
NFC ²	43.10	40.60	45.90
EE ¹	2.80	2.90	3.50
Ca ²	1.60	1.70	1.20
P ²	0.50	0.50	0.40
Mg ²	0.40	0.40	0.41
Na ²	0.04	0.07	0.44
K ²	1.31	1.32	1.22
Cl ²	0.73	0.70	0.49
S ²	0.30	0.35	0.20
DCAD ² , mEq/kg DM	-43	-45	+242

¹ Calculated by chemical analysis.

² Estimated via NRC (2001) based on actual DMI, BW, BCS, and calf weight of the cows in prepartum diets; and based on actual DMI, BW, BCS, and calf weight, milk yield, and milk composition of the cows in postpartum diets.

³ MP requirements were NRC model plus 120 g of MP to account for mammary growth (Bell et al., 2000).

Environmental conditions, milk yield and compositions

The average Temperature-Humidity Index (THI) during the study period was 75.8, indicating moderate heat stress in dairy cows. In line with these conditions, the average WCT during the close-up period was recorded at -1.07°C. The effect of varying RUP levels on milk yield and composition across seasons is shown in Table 4. While seasonal variation did not significantly affect milk yield, cows fed the RUP-supplemented diet produced significantly more milk compared to the control group (37.74 ± 0.61 kg/d for COND vs. 40.39 ± 0.64 kg/d for RUPD; P < 0.01), suggesting that the observed increase in milk yield was primarily due to the RUP supplementation rather than an increase in total dietary crude protein. However, fat-corrected milk (FCM)

Table 4. Effect of feeding different RUP levels during close-up period on milk yields and compositions of Holstein cows in different seasons

Milk yield and composition	P-Value											
	Season				Treatment				Interaction			
	Summer	Winter	SEM	COND	RUPD	SEM	Season	Diet	Time ²	SeasonXDiet	SeasonXTime	SeasonXDietXTime
Milk yield, kg/d	38.76	39.37	0.64	37.74	40.39	0.61	0.49	0.01	0.01	0.95	0.59	0.01
4% FCM ¹ , kg/d	36.88	37.62	0.62	36.74	37.76	0.64	0.41	0.25	0.01	0.73	0.01	0.06
Fat, %	3.70	3.74	0.03	3.85	3.59	0.03	0.42	0.01	0.01	0.28	0.01	0.25
True protein, %	3.13	3.38	0.02	3.18	3.33	0.02	0.01	0.01	0.01	0.04	0.01	0.01
Lactose, %	4.72	4.59	0.01	4.63	4.68	0.01	0.01	0.04	0.01	0.13	0.28	0.77
MUN, mg/dL	13.70	13.91	0.10	13.77	13.84	0.10	0.14	0.63	0.01	0.87	0.01	0.01

¹ 4%FCM [kg/d]: (Milkfat [%] · 0.5 + 0.4) · Milk yield [kg/d] (Gains, 1928)

² From -30 d relative to parturition to calving

remained unaffected by diet or season. Notably, the lower milk fat percentage observed in cows fed the RUP-supplemented diet (COND = $3.85 \pm 0.03\%$ vs. RUPD = $3.59 \pm 0.04\%$; $P < 0.01$) may be attributed to an inverse relationship between dietary CP levels and milk fat content. Higher CP intake leads to increased propionate production in the rumen, which subsequently reduces acetate availability for milk fat synthesis. Conversely, true protein levels were significantly higher in the RUPD group. Additionally, lactose levels were higher in summer compared to winter, with RUP supplementation further increasing lactose content (COND = $4.63 \pm 0.01\%$ vs. RUPD = $4.68 \pm 0.01\%$; $P < 0.05$). No significant effect of season or RUP supplementation was observed on MUN levels.

Feed intake, BCS and colostrum compositions

Table 5 presents a comprehensive analysis of the effects of varying levels of RUP on DMI, BCS, and colostrum composition across different seasons. During the close-up period, the RUP-supplemented diet resulted in significantly higher DMI compared to the control group (RUPD = 14.69 ± 0.14 kg/d vs. COND = 13.48 ± 0.14 kg/d; $P = 0.01$). Both diets were formulated to be isoenergetic and isonitrogenous, ensuring that observed differences in DMI were attributable to RUP supplementation rather than energy or protein content disparities. This pattern was consistently observed across replicates for each group, reinforcing the reliability of the findings. As anticipated, cows exhibited increased feed consumption during the winter season relative to summer (summer = 13.69 ± 0.14 kg/d, winter = 14.56 ± 0.14 kg/d) ($P = 0.01$), emphasizing the seasonal varia-

tion in intake. Furthermore, the interaction between season and diet did not significantly affect DMI or Net Energy (NE) intake, suggesting the stability of these parameters across different dietary conditions. While the RUP-supplemented diet did not significantly impact BCS or colostrum composition, an interaction between season and diet was observed for lactose content. Specifically, lactose concentration in colostrum was higher in the RUPD-fed group during winter (COND = $4.56 \pm 0.02\%$, RUPD = $4.64 \pm 0.02\%$) ($P < 0.01$). Additionally, the MUN concentration was significantly higher in summer (19.45 mg/dL) compared to winter ($P < 0.01$).

Calf weights and average daily gain

The effects of varying levels of RUP supplementation on calf weights are summarized in Table 6. Although the introduction of RUP supplements did not significantly affect calf birth weight, calves born in the winter had notably higher weights compared to those born in the summer (summer COND = 39.41 ± 0.45 kg, RUPD = 40.90 ± 0.82 kg; winter COND = 41.89 ± 0.66 kg, RUPD = 42.45 ± 1.27 kg) ($P < 0.05$). At 30 days of age, calves born in the winter also exhibited higher weights (summer COND = 55.85 ± 1.34 kg, RUPD = 58.28 ± 1.95 kg; winter COND = 58.11 ± 1.22 kg, RUPD = 63.00 ± 2.09 kg), with a significant increase observed ($P < 0.05$). Similar trends were observed at weaning, where winter-born calves had significantly higher live weights (summer COND = 89.65 ± 1.51 kg, RUPD = 96.15 ± 0.95 kg; winter COND = 95.06 ± 1.23 kg, RUPD = 100.30 ± 2.08 kg) ($P < 0.01$). In addition, weaning weights ($P < 0.01$) and Average Daily Gain (ADG) until weaning (summer COND = 0.72 ± 0.02 kg, RUPD = 0.79

Table 5. Effect of feeding different RUP levels during close-up period on feed intake, BCS and colostrum compositions in different seasons

Items		Season			Treatment			P-value		
		Summer	Winter	SEM	COND	RUPD	SEM	Season	Diet	Season×diet
Intake	DMI (kg/d)	13.69	14.56	0.14	13.48	14.69	0.14	0.01	0.01	0.27
	NE (Mcal/d)	8.45	8.98	0.08	8.37	9.02	0.08	0.01	0.01	0.26
BCS	Prepartum	0.19	0.18	0.02	0.17	0.19	0.02	0.70	0.37	0.17
	Postpartum	-0.24	0.021	0.02	-0.22	0.02	0.02	0.30	0.58	0.13
	1 th colostrum,kg	5.73	5.83	0.32	5.73	5.83	0.22	0.76	0.75	0.39
Colostrum	Fat,%	8.13	7.48	0.38	7.75	7.86	0.27	0.09	0.76	0.61
	True protein,%	13.35	13.73	0.37	13.31	13.77	0.26	0.30	0.22	0.14
	Lactose,%	3.08	3.05	0.11	3.0	3.14	0.08	0.78	0.24	0.01
	MUN,mg/dL	19.45	18.35	0.17	18.96	18.84	0.28	0.006	0.76	0.56

Table 6. Effect of feeding different RUP levels on calf weights in different seasons

Calf (kg)	Season		SEM	Diet			P-value		
	Summer	Winter		COND	RUPD	SEM	Season	Diet	Season×diet
Birth wt	40.24	42.17	0.82	40.73	41.68	0.57	0.02	0.25	0.15
30 d wt	57.16	60.31	1.21	57.24	60.75	1.19	0.04	0.04	0.47
30 d ADG	0.56	0.61	0.03	0.54	0.63	0.03	0.33	0.09	0.26
Weaning wt	93.16	97.41	1.10	92.97	98.32	1.12	0.01	0.01	0.68
Weaning ADG	0.76	0.79	0.01	0.74	0.81	0.01	0.01	0.01	0.87

± 0.08 kg; winter COND = 0.76 ± 0.01 kg, RUPD = 0.83 ± 0.02 kg) were significantly higher ($P < 0.01$) in calves fed an RUP-supplemented diet, particularly in the winter season.

DISCUSSION

The findings of the study emphasize the crucial role of RUP-supplemented diet feeding during the close-up dry period in influencing the health and productivity of Holstein Friesian cows. The observed significant increase in milk yield provides a promising strategy for dairy producers aiming to enhance production efficiency. By demonstrating a clear correlation between dietary RUP levels and enhanced lactation performance, this study contributes valuable insights to dairy herd management. The results affirm the effectiveness of RUP supplementation in supporting metabolic conditions post-calving while highlighting cows' nuanced responses to seasonal variations. These findings emphasize the importance of integrating seasonal factors into nutritional strategies to optimize milk composition, calf development, and overall herd health.

The significance of close-up dry period feeding in preventing metabolic disorders and ensuring high milk yield during lactation is well-established (Bionaz et al., 2020; Cardoso et al., 2020; Lopreato et al., 2020). RUP supplementation during this period supports the gravid uterus, mammary gland metabolism, and subsequent lactation performance (VandeHaar and Donkin, 1999; Lapierre et al., 2012; Allen et al., 2015). While prior studies have examined RUP effects on milk yield, composition, BCS, colostrum quality, calf birth weight, reproductive performance, and health status (Hartwell et al., 2020; Contreras et al., 2004; Nowak et al., 2012), few have investigated the impact under heat and cold stress conditions (Mulliniks et al., 2015). This study employed a split-plot design to evaluate RUP effects on milk yields, BCS, and calf weights across seasonal conditions (summer and winter) and dietary treatments (CON diet and RUP diet).

Heat stress typically impairs milk production and quality by disrupting metabolism, reducing insulin and non-esterified fatty acid (NEFA) levels, and altering hormone release, including growth hormone and insulin-like growth factor-I (IGF-I) (Shwartz et al., 2009; Wheelock et al., 2010; Baumgard and Rhoads Jr., 2013). Cows in the RUPD group produced significantly more milk during summer than those on COND, aligning with previous findings (Farahani et al., 2019). This increase may stem from the higher protein content (12-15%), supporting the hypothesis that metabolizable protein, rather than net energy, limits milk production during early lactation (Schwab and Foster, 2009). Amino acids mobilized from muscle, skin, uterine regression, and myometrial protein breakdown drive milk production (Kokkonen, 2014). The seasonal decline in milk protein and lactose levels during heat stress suggests altered amino acid metabolism, including glycine, glutamate, glutamine, threonine, proline, valine, methionine, isoleucine, and leucine, which may be downregulated (Shan et al., 2018; Fan et al., 2019). Our study provides valuable data for exploring the complex relationship between heat stress, amino acid metabolism, and milk protein synthesis.

DMI reductions during heat stress in dry cows, though less severe than in lactating cows, are supported by literature (Adin et al., 2009; Do Amaral et al., 2009). Conflicting reports on increased dietary protein's effect on DMI during the close-up period suggest that diet composition, net energy for lactation (NEL) density, and lactation stage must be considered (Bell et al., 2000; Stockdale and Roche, 2002; Roche et al., 2009; Husnain and Santos, 2019; Cardoso et al., 2020).

BCS at calving did not differ significantly between RUPD and COND groups, but colostrum composition showed seasonal and diet-related interactions, particularly in lactose levels. Further investigation

is warranted to elucidate these interactions. RUP supplementation during the close-up period did not affect calf birth weight; however, winter-born calves were significantly heavier, possibly due to the environmental factors associated with the season, which aligns with previous research (Hozáková et al., 2019; Cho et al., 2021). Though 30-day ADG showed no significant differences by season or diet, weaning weight and ADG until weaning were significantly influenced by both factors. Winter-born calves and those from RUP-supplemented cows exhibited greater live weight gains, underscoring the multifaceted effects of season and dietary interventions on calf development.

The substantial increase in milk yield among RUP-supplemented cows during the close-up period suggests that adjusting dietary RUP levels is an effective strategy for enhancing milk production in Holstein Friesian cows. The study highlights the influence of seasonal factors on dairy cattle performance. While milk yield remained consistent across seasons, variations in DMI and calf weights emphasize the importance of accounting for seasonal effects in nutritional planning. Changes in milk composition, notably increased protein and lactose alongside reduced milk fat percentage, reflect cows' nuanced responses to RUP supplementation, warranting further exploration for implications on dairy product quality and market alignment.

The study offers insights into maternal diet's influence on calf birth weight and growth, with winter-born calves showing increased weights. This suggests that RUP supplementation can positively affect both milk production and offspring development. Fine-tuning RUP levels in the close-up diet emerges as a vital component of effective nutritional strategies, potentially enhancing herd productivity and health. Dairy farmers and nutritionists should consider seasonal variations when formulating diets to mitigate the challenges of heat and cold stress, ultimately promoting animal performance and well-being.

The study's scope, conducted in August 2018 and February 2019, may not capture long-term effects of RUP supplementation, underscoring the need for extended research to assess sustained impacts on milk production and cow health. The study's restriction to a specific dairy farm in Iran limits generalizability. Replication across diverse environments and management systems could enhance external validity. Although seasonal variations were considered, other environmental factors such as humidity and forage quality remain unexplored and warrant future

research. Longitudinal studies assessing maternal RUP supplementation's impact on offspring growth beyond weaning are also recommended. Economic analyses incorporating feed costs, milk prices, and overall farm profitability would further inform the practical viability of RUP-supplemented diets.

In summary, this study illuminates the multifaceted interplay between RUP supplementation, seasonal stressors, milk production, milk composition, and calf development. The results advocate for seasonally adjusted, protein-enriched nutritional strategies to enhance dairy herd productivity, resilience, and economic viability.

CONCLUSIONS

This study demonstrates the critical influence of rumen RUP supplementation and seasonal variation during the close-up period on dairy cow performance. Increased dietary protein levels led to significant improvements in milk yield and composition, characterized by higher protein and lactose concentrations alongside a reduction in milk fat percentage. These effects were more pronounced during colder seasons, highlighting the complex interaction between nutritional strategies and environmental factors. The results emphasize the importance of seasonally adaptive nutritional management to enhance herd productivity and health. However, the findings should be interpreted with caution due to study limitations. Further research is essential to address potential confounding factors and improve the generalizability of these outcomes across diverse dairy production systems, contributing to a more refined understanding of how RUP supplementation and seasonal dynamics influence performance.

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CONFLICT OF INTEREST

The author/s declared that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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