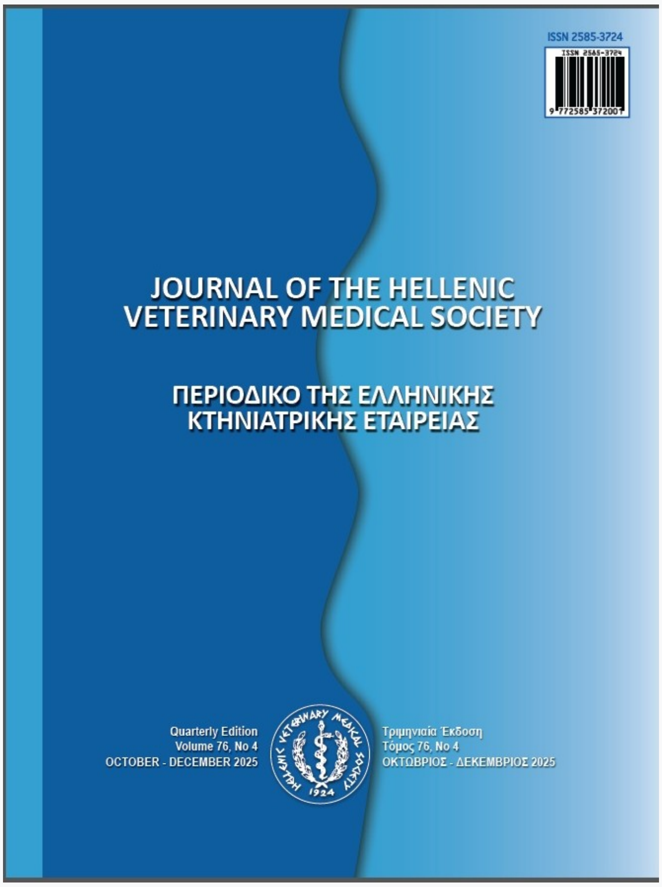
ePublishing
ΕΘΝΙΚΟ ΚΕΝΤΡΟ ΤΕΚΜΗΡΙΩΣΗΣ NATIONAL DOCUMENTATION CENTRE

**Journal of the Hellenic Veterinary Medical Society**

Vol 76, No 4 (2025)




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

*Hİ Tosun, F Ahmadi*

doi: 10.12681/jhvms.40882

Copyright © 2025, Hİ Tosun, F Ahmadi

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0.

**To cite this article:**

Tosun, H., & Ahmadi, F. (2025). Nutritional strategies and seasonal adaptations: Enhancing dairy cow productivity and calf development. *Journal of the Hellenic Veterinary Medical Society*, *76*(4), 10003–10012. https://doi.org/10.12681/jhvms.40882

https://epublishing.ekt.gr | **e-Publisher:** EKT | **Downloaded at:** 25/01/2026 12:00:13

J HELLENIC VET MED SOC 2025, 76 (4): 10003-10012
ΠΕΚΕ 2025, 76 (4): 10003-10012

Research article
Ερευνητικό άρθρο

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

**H. İ. Tosun,*[1] F. Ahmadi[2]**

*[1]Rumico Livestock Management and Nutrition, Aydın, Turkiye*

*[2]Department of Animal Science, University of Zanjan, Zanjan, Iran*

**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 undegredable protein correlated with higher milk yield. An inverse relationship existed between milk fat percentage and rumen endegredable 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 undegredable 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

*Correspondence author:*
Halil İbrahim Tosun,
Rumico Livestock Management and Nutrition, Efeler, 09020, Aydın, Türkiye
E-mail address: halilibrahimtosun@gmail.com

*Date of submission:* **20-3-2025**
*Date of acceptance:* **13-5-2025**

## 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 adipose 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 administered 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):

$$WCT = 13.12 + 0.6215\,T - 13.17\,V0.16 + 0.3965\,T \times V0.16$$

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:

$$Yijkl = \mu + Si + Dj + Eaij + SDij + C(SD)ijk + Ebijkl$$

**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 diets[1]

| 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, $\mu$ is the overall mean, $S_i$ accounts for the fixed effect of season, $D_j$ is the fixed effect of diet, $Ea_{ij}$ 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 $Eb_{ijkl}$ 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 corrected 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).

**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 | Season | | | Treatment | | | P-Value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 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

**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) remained unaffected by diet or season. Notably, the lower milk fat percentage observed in cows fed the RUP-supplemented diet (COND = 3.85 ± 0.03% vs. RUPD = 3.59 ± 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 ± 0.01% vs. RUPD = 4.68 ± 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 variation 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 ± 0.02%, RUPD = 4.64 ± 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 |
| Colostrum | 1$^{th}$colostrum,kg | 5.73 | 5.83 | 0.32 | 5.73 | 5.83 | 0.22 | 0.76 | 0.75 | 0.39 |
| | 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 | | | Diet | | | P-value | | |
|---|---|---|---|---|---|---|---|---|---|
| | Summer | Winter | SEM | 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; Lopreiato 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.

## ACKNOWLEDGEMENTS

The authors would like to thank Dr. Buzz Burhans, Dairy-Tech Group, Twin Falls, Idaho, United States, for his contribution. Special thanks go to Dr. Hamid Amanlou, Professor, Zanjan University, Iran, and Dr. Tahereh Amirabadi, Assistant Professor, Shahrekord University, Iran.

## 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.

## REFERENCES


Abdelmegeid MK, Elolimy AA, Zhou Z, Lopreiato V, McCann JC, Loor JJ (2018) Rumen-protected methionine during the peripartal period in dairy cows and its effects on abundance of major species of ruminal bacteria. Journal of animal science and biotechnology, 9(1), 17. https://doi:10.1186/s40104-018-0230-8

Adin G, Gelman A, Solomon R, Flamenbaum I, Nikbachat M, Yosef E, Zenou A, Shamay A, Feuermann Y, Mabjeesh S J, Miron J (2009) Effects of cooling dry cows under heat load conditions on mammary gland enzymatic activity, intake of food and water, and performance during the dry period and after parturition. Livestock science, 124(1-3), 189-195. https://doi:10.1016/j.livsci.2009.01.014

Agle M, Hristov AN, Zaman S, Schneider C, Ndegwa P, Vaddella VK (2010) The effects of ruminally degraded protein on rumen fermentation and ammonia losses from manure in dairy cows. Journal of dairy science, 93(4), 1625-1637. https://doi:10.3168/jds.2009-2579

Allen JD, Hall LW, Collier RJ, Smith JF (2015) Effect of core body temperature, time of day, and climate conditions on behavioral patterns of lactating dairy cows experiencing mild to moderate heat stress. Journal of dairy science, 98(1), 118-127. https://doi:10.3168/jds.2013-7704

Baumgard LH, Rhoads RP (2013) Effects of heat stress on postabsorptive metabolism and energetics. Annual review of animal biosciences, 1(1), 311-337. https://doi:10.1146/annurev-animal-031412-103644

Bell AW, Burhans WS, Overton TR (2000) Protein nutrition in late pregnancy, maternal protein reserves and lactation performance in dairy cows. Proceedings of the nutrition society, 59(1), 119-126. https://doi:10.1017/s0029665100000148

Bionaz M, Vargas-Bello-Perez E, Busato S (2020) Advances in fatty acids nutrition in dairy cows: from gut to cells and effects on performance. Journal of animal science and biotechnology, 11(1), 110. https://doi:10.1186/s40104-020-00512-8

Borchardt S, Sutter F, Heuwieser W, Venjakob P (2022) Management-related factors in dry cows and their associations with colostrum quantity and quality on a large commercial dairy farm. Journal of dairy science, 105(2), 1589-1602. https://doi:10.3168/jds.2021-20671

Bünemann K, von Soosten D, Frahm J, Kersten S, Meyer U, Hummel J, Zeyner A, Danicke S (2019) Efects of body condition and concentrate proportion of the ration on mobilization of fat depots and energetic condition in dairy cows during early lactation based on ultrasonic measurements. Animals, 9(4), 131. https://doi:10.3390/ani9040131

Cardoso FC, Kalscheur KF, Drackley JK (2020) Symposium review: Nutrition strategies for improved health, production, and fertility during the transition period. Journal of dairy science, 103(6), 5684-5693. https://doi:10.3168/jds.2019-17271

Chebel RC, Mendonça LG, Baruselli PS (2018) Association between body condition score change during the dry period and postpartum health and performance. Journal of Dairy Science, 101(5), 4595-4614. https://doi:10.3168/jds.2017-13732

Cho K, Song Y, Yeo JM, Park JK, Kim DW, Roh SH, Seong P, Lee WY (2021) Analysis of seasonal effect on Korean native cattle (Hanwoo) birth weight. Journal of animal science and technology, 63(4), 759-765. https://doi:10.5187/jast.2021.e72

Contreras LL, Ryan CM, Overton TR (2004) Effects of dry cow grouping strategy and prepartum body condition score on performance and health of transition dairy cows. Journal of dairy science, 87(2), 517-523. https://doi:10.3168/jds.S0022-0302(04)73191-4

Curtis CR, Erb HN, Sniffen CJ, Smith RD, Kronfeld DS (1985) Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. Journal of dairy science, 68(9), 2347-2360. https://doi:10.3168/jds.S0022-0302(85)81109-7

Daros RR, Havekes CD, DeVries TJ (2021) Body condition loss during the dry period: Insights from feeding behavior studies. Journal of dairy science, 104(4), 4682-4691. https://doi:10.3168/jds.2020-19481

Delaby L, Faverdin P, Michel G, Disenhaus C, Peyraud JL (2009) Effect of different feeding strategies on lactation performance of Holstein and Normande dairy cows. Animal, 3(6), 891-905. https://doi:10.1017/S1751731109004212

Dewhurst RJ, Moorby JM, Dhanoa MS, Evans RT, Fisher WJ (2000) Effects of altering energy and protein supply to dairy cows during the dry period. 1. Intake, body condition, and milk production. Journal of Dairy Science, 2000; 83(8), 1782-1794. https://doi:10.3168/jds.S0022-0302(00)75049-1

Dikmen S, Hansen P (2009) Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? Journal of dairy science, 92(1), 109-116. https://doi:10.3168/jds.2008-1370

Do Amaral BC, Connor EE, Tao S, Hayen J, Bubolz J, Dahl GE (2009) Heat-stress abatement during the dry period: Does cooling improve transition into lactation? Journal of dairy science, 92(12), 5988-5999. https://doi:10.3168/jds.2009-2343

Edmonson A, Lean I, Weaver L, Farver T, Webster G (1989) A body condition scoring chart for Holstein dairy cows. Journal of dairy science, 72(1), 68-78. https://doi:10.3168/jds.S0022-0302(89)79081-0

Fan C, Su D, Tian H, Hu R, Ran L, Yang Y, Su Y, Cheng J (2019) Milk production and composition and metabolic alterations in the mammary gland of heat-stressed lactating dairy cows. Journal of integrative agriculture, 18(12), 2844-2853. https://doi:10.1016/S2095-3119(19)62834-0

Farahani TA, Amanlou H, Farsuni N, Kazemi-Bonchenari M (2019) Interactions of protein levels fed to Holstein cows pre-and postpartum on productive and metabolic responses. Journal of dairy science, 102(1), 246-259. https://doi:10.3168/jds.2018-14575

Friggens NC, Andersen JB, Larsen T, Aaes O, Dewhurst RJ (2004) Priming the dairy cow for lactation: a review of dry cow feeding strategies. Animal research, 53(6), 453-473. https://doi:10.1051/animres:2004037

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

Gärtner T, Gernand E, Gottschalk J, Donat K (2019) Relationships between body condition, body condition loss, and serum metabolites during the transition period in primiparous and multiparous cows. Journal of dairy science, 102(10), 9187-9199. https://doi:10.3168/jds.2018-15762

Girma DD, Ma L, Wang F, Jiang QR, Callaway TR, Dracley JK, Bu DP (2019) Effects of close-up dietary energy level and supplementing rumen-protected lysine on energy metabolites and milk production in transition cows. Journal of dairy Science, 102(8), 7059-7072. https://doi:10.3168/jds.2018-15962

Gorniak T, Meyer U, Sudekum KH, Danicke S (2014) Impact of mild heat stress on dry matter intake, milk yield and milk composition in mid-lactation Holstein dairy cows in a temperate climate. Archives of animal nutrition, 68(5), 358-369. https://doi:10.1080/1745039X.2014.950451

Hartwell JR, Cecava MJ, Donkin SS (2020) Impact of dietary rumen undegradable protein and rumen-protected choline on intake, peripartum liver triacylglyceride, plasma metabolites and milk production in transition dairy cows. Journal of dairy science, 83(12), 2907-2917. https://doi:10.3168/jds.S0022-0302(00)75191-5

Helrich, K (1990) Official methods of analysis of the association of official analytical chemists. Association of official analytical chemists.

Hiltz RL, Laarman AH (2019) Effect of butyrate on passive transfer of immunity in dairy calves. Journal of dairy science, 102(5), 4190-4197. https://doi:10.3168/jds.2018-15555

Hozáková K, Bujko J, Vavrisínova K, Zitny J (2019) Evaluation of the factors affecting the growth intensity of Charolais calves. Poljoprivreda i sumarstvo, 65(1), 223-232. https://doi:10.17707/AgricultForest.65.1.22

Husnain A, Santos JEP (2019) Meta-analysis of the effects of prepartum dietary protein on performance of dairy cows. Journal of dairy science, 102(11), 9791-9813. https://doi:10.3168/jds.2018-16043

Kaufman JD, Pohler KG, Mulliniks JT, Rius AG (2018) Lowering rumen-degradable and rumen-undegradable protein improved amino acid metabolism and energy utilization in lactating dairy cows exposed to heat stress. Jıurnal of dairy science, 101(1), 386-395. https://doi:10.3168/jds.2017-13341

Kokkonen T (2014) Investigation of sources of variation in the effect of prepartum protein supplementation on early lactation performance of dairy cows. Livestock science, 163, 41-50. https://doi:10.1016/j.livsci.2014.02.008

Lapierre H, Lobley GE, Doepel L, Raggio G, Rulquin H, Lemosquet

S (2012) Triennial Lactation Symposium: Mammary metabolism of amino acids in dairy cows. Journal of animal science, 90(5), 1708-1721. https://doi:10.2527/jas.2011-4645

LeBlanc S (2010) Monitoring metabolic health of dairy cattle in the transition period. The journal of reproduction and development, 56 Suppl(S), S29-35. https://doi:10.1262/jrd.1056s29

Littell RC, Henry PR, Ammerman CB (1998) Statistical analysis of repeated measures data using SAS procedures. Journal of animal science, 76(4), 1216-1231. https://doi:10.2527/1998.7641216x

Lopreiato V, Mezzetti M, Cattaneo L, Ferronato G, Minuti A, Trevisi E (2020) Role of nutraceuticals during the transition period of dairy cows: a review. Journal of Animal Science and Biotechnology, 11(1), 96. https://doi:10.1186/s40104-020-00501-x

Mulliniks JT, Rius AG, Edwards MA, Edwards SR, Hobbs JD, Nave RLG (2015) Forages and pastures symposium: Improving efficiency of production in pasture- and range-based beef and dairy systems. Journal of animal science, 93(6), 2609-2615. https://doi:10.2527/jas.2014-8595

National Research Council (2001) Committee on Animal Nutrition, and Subcommittee on Dairy Cattle Nutrition. Nutrient requirements of dairy cattle: National Academies Press, 2001.

Nowak W, Mikuła R, Kasprowicz-Potocka M, Ignatowicz M, Zachwieja A, Paczynska K, Pecka E (2012) Effect of cow nutrition in the far-off period on colostrum quality and immune response of calves. Journal of veterinary research, 56(2), 241-246. https://doi:10.2478/v10213-012-0043-z

Pascottini OB, Leroy J, Opsomer G (2020) Metabolic Stress in the Transition Period of 48. Dairy Cows: Focusing on the Prepartum Period. Animals, 10(8), 1419. https://doi:10.3390/ani10081419

Roche JR, Friggens NC, Kay JK, Fisher MW, Stafford KJ, Berry DP (2009) Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. Journal of dairy science, 92(12), 5769-5801. https://doi:10.3168/jds.2009-2431

Rodríguez E, Arís A, Bach A (2017) Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. Journal of dairy science, 100(9), 7427-7434. https://doi:10.3168/jds.2016-12210

Scherpenzeel CGM, Hogeveen H, Maas L, Lam T (2018) Economic optimization of selective dry cow treatment. Journal of dairy science, 101(2), 1530-1539. https://doi:10.3168/jds.2017-13076

Schwab C, Foster G (2009) Maximizing milk components and metabolizable protein utilization through amino acid formulation. in Proceedings of Cornell Nutrition Conference for Feed Manufacturers, Syracuse, NY. New York State College of Agriculture & Life Sciences, Cornell University.

Shan CH, Guo J, Sun X, Li N, Yang X, Gao Y, Qiu D, Li X, Wang Y, Feng M, Wang C, Zhao JJ (2018) Effects of fermented Chinese herbal medicines on milk performance and immune function in late-lactation cows under heat stress conditions. Journal of Animal Science, 96(10), 4444-4457. https://doi:10.1093/jas/sky270

Shi W, Haisan J, Inabu Y, Sugino T, Oba M (2020) Effects of starch concentration of close-up diets on rumen pH and plasma metabolite responses of dairy cows to grain challenges after calving. Journal of dairy science, 103(12), 11461-11471. https://doi:10.3168/jds.2020-18768

Shwartz G, Rhoads ML, VanBaale MJ, Rhoads RP, Baumgard LH (2009) Effects of a supplemental yeast culture on heat-stressed lactating Holstein cows. Journal of dairy science, 92(3), 935-942. https://doi:10.3168/jds.2008-1496

Singh AK, Bhakat C, Mandal DK, Mandal A, Rai S, Chatterjee A, Ghosh MK (2020) Effect of reducing energy intake during the dry period on milk production, udder health, and body condition score of Jersey crossbred cows in the tropical lower Gangetic region. Tropical animal health and production, 52(4), 1759-1767. https://doi:10.1007/s11250-019-02191-8

Stockdale C, Roche J (2002) A review of the energy and protein nutrition of dairy cows through their dry period and its impact on early lactation performance. Australian journal of agricultural research, 53(7), 737-753. https://doi:10.1071/AR01019

Toledo MZ, Stangaferro ML, Gennari RS, Barletta RV, Perez MM, Wijma R, Sitko EM, Granados G, Masello M, Van Amburgh ME, Luchini D, Giordano JO, Shaver RD, Wiltbank MC (2021) Effects of feeding rumen-protected methionine pre- and postpartum in multiparous Holstein cows: Lactation performance and plasma amino acid concentrations. Journal of dairy science, 104(7), 7583-7603. https://doi:10.3168/jds.2020-19021

Tucho TT, Ahmed WM (2017) Economic and reproductive impacts of retained placenta in dairy cows. Journal of reproduction and infertility, 8(1), 18-27. https://doi:10.5829/idosi.jri.2017.18.27

Tucker CB, Rogers AR, Verkerk GA, Kendall PE, Webster JR, Matthews LR (2007) Effects of shelter and body condition on the behaviour and physiology of dairy cattle in winter. Applied animal behaviour science, 105(1-3), 1-13. https://doi:10.1016/j.applanim.2006.06.009

VandeHaar MJ, Donkin SS (1999) Protein nutrition of dry cows. Paper presented at Tri-State Dairy Management Conference Fort Wayne, IN, The Ohio State University.

Wang H, Elsaadawy SA, Wu Z, Bu DP (2021) Maternal supply of ruminally-protected lysine and methionine during close-up period enhances immunity and growth rate of neonatal calves. Frontiers in veterinary science, 8, 780731. https://doi:10.3389/fvets.2021.780731

West JW (1999) Nutritional strategies for managing the heat-stressed dairy cow. Journal of animal science, 77 Suppl 2(suppl_2), 21-35. https://doi:10.2527/1997.77suppl_221x

Westhoff TA, Womack SJ, Overton TR, Ryan CM, Mann S (2023) Epidemiology of bovine colostrum production in New York Holstein herds: Cow, management, and environmental factors. Journal of dairy science, 106(7), 4874-4895. https://doi:10.3168/jds.2022-22447

Wheelock JB, Roads RP, VanBaale MJ, Sanders SR, Baumgard LH (2010) Effects of heat stress on energetic metabolism in lactating Holstein cows. Journal of dairy science, 93(2): p. 644-655. https://doi.org/10.3168/jds.2009-2295