Effects of Prepartum Dietary Crude Protein Level on Postpartum Production Performance and Blood Parameters in Holstein Multiparous Cows: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary crude protein levels during the prepartum period on production performance and blood biochemical parameters in postpartum dairy cows. Twenty-one multiparous Holstein dairy cows with similar expected calving dates, parity, body weight, previous lactation duration, and milk yield were randomly divided into 3 groups with 7 cows per group. During the prepartum period (within 21 d before calving), three different diets with crude protein levels of 12.12% (Group A), 13.07% (Group B), and 14.02% (Group C) were fed, all with a net energy for lactation of 5.50 MJ/kg, to study their effects on production performance and blood parameters of dairy cows during 1–30 d postpartum. The results showed that: 1) During 1–30 d postpartum, dry matter intake in Group B was 5.41% and 5.85% higher than that in Groups A and C, respectively ($P<0.05$). At 30 d postpartum, body weight loss in Groups A, B, and C was 6.30%, 5.03%, and 8.22%, respectively, with the highest loss in Group C. Compared with the other two groups, Group B showed a trend toward increased postpartum milk yield, but the difference among groups was not significant ($P>0.05$). During 2–10 d postpartum, lactose percentage in Group B was 4.31% higher than that in Group C ($P<0.05$). The apparent digestibility of crude protein in Group B during the prepartum period was 12.56% higher than that in Group A ($P<0.05$). 2) At 10, 20, and 30 d postpartum, serum glucose (GLU) content in Group B was 31.41%, 29.47%, and 21.38% higher than that in Group C, respectively ($P<0.05$). On the day of calving, serum total protein (TP) content in Group B was 12.98% higher than that in Group C ($P<0.05$). At 30 d postpartum, serum albumin (ALB) and leptin (LP) contents in Group B were 14.69% and 5.97% higher than those in Group C, respectively ($P<0.01$). At 20 d postpartum, serum triglyceride (TG) content in Group B was 3.57% lower than that in both Groups A and C

(P<0.05), and serum insulin (Ins) content was 7.26% and 12.47% lower than that in Groups A and C, respectively (P<0.01). At 30 d postpartum, serum insulin-like growth factor I (IGF-I) content in Group B was 4.94% lower than that in Group C (P<0.01). In conclusion, a dietary crude protein level of 13.07% is the appropriate dietary crude protein level for the prepartum period.

Full Text

Impact of Prepartal Dietary Crude Protein Level on Postpartal Production Performance and Blood Biochemical Indexes of Multiparous Holstein Dairy Cows

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Abstract

This study investigated the effects of prepartal dietary crude protein level on postpartal production performance and blood biochemical indexes in dairy cows. Twenty-one multiparous Holstein cows with similar expected calving dates, parity, body weight, previous lactation duration, and milk yield were randomly assigned to three groups (n=7). During the prepartal period (21 days before calving), cows were fed three diets with crude protein levels of 12.12% (Group A), 13.07% (Group B), and 14.02% (Group C), each with a net energy for lactation of 5.50 MJ/kg. Postpartal performance and blood parameters were measured during days 1–30 of lactation. The results showed: (1) Dry matter intake (DMI) during days 1–30 postpartum was 5.41% and 5.85% higher in Group B compared to Groups A and C, respectively (P<0.05). By day 30 postpartum, body weight losses were 6.30%, 5.03%, and 8.22% for Groups A, B, and C, respectively, with Group C experiencing the greatest loss. Group B exhibited a trend toward higher milk yield compared to the other groups, though differences were not statistically significant (P>0.05). During days 2–10 postpartum, lactose percentage was 4.31% higher in Group B than in Group C (P<0.05). The apparent digestibility of crude protein during the prepartal period was 12.56% higher in Group B than in Group A (P<0.05). (2) Serum glucose (GLU) concentrations on postpartal days 10, 20, and 30 were 31.41%, 29.47%, and 21.38% higher in Group B than in Group C (P<0.05). On the day of calving, serum total protein (TP) content in Group B was 12.98% higher than in Group C (P<0.05). On day 30 postpartum, serum albumin (ALB) and leptin (LP) concentrations in Group B were 14.69% and 5.97% higher than in Group C (P<0.01). On day 20 postpartum, serum triglyceride (TG) concentration in Group B was 3.57% lower than in Groups A and C (P<0.05), while serum insulin (Ins) concentration was 7.26% and 12.47% lower than in Groups A and C, respectively (P<0.01). On day 30 postpartum, serum insulin-like growth factor I (IGF-I) concentration in Group B was 4.94% lower than in Group C (P<0.01). These findings indicate that a dietary crude protein level of 13.07% is optimal for multiparous cows during the prepartal period.

Keywords: perinatal period; Holstein cows; crude protein level; blood indexes; production performance

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The perinatal period in dairy cows extends from 2–3 weeks prepartum to 2–3 weeks postpartum and is characterized by dramatic endocrine changes. Increasing estrogen levels and fetal compression of the digestive tract directly reduce feed intake during this critical period, resulting in insufficient energy intake. However, nutrient requirements for the fetus and placenta peak during the final week of gestation, creating a supply-demand imbalance that leads to negative energy balance [1]. Postpartum dry matter intake (DMI) is a primary factor affecting milk production and body weight changes, and increasing DMI during early lactation can shorten the duration of negative energy balance. The NRC (1989, 2001) recommended a dietary crude protein level of 12% for multiparous cows during the peripartal period, noting that increasing protein by 2–4% beyond 12–13% could reduce postpartum DMI and milk yield [2], whereas primiparous cows required 14.2% crude protein to meet their needs. Greenfield et al. [3] suggested that maintaining prepartal dietary crude protein at 12% positively affected postpartum DMI and milk yield. Conversely, Santos et al. [4] demonstrated that increasing prepartal crude protein from 12.7% to 14.7% through animal-source protein supplementation improved lactation performance. Domestic research [5] found no effect on postpartum DMI or milk yield when multiparous cows were fed diets containing 12.1%, 14.0%, or 9.6% crude protein. These conflicting results indicate that the optimal dietary crude protein level for peripartal dairy cows remains controversial and warrants further investigation. This study examined the effects of prepartal dietary crude protein level on postpartal production performance and blood indexes to determine the optimal protein concentration.

1.1 Experimental Animals

The experiment was conducted at Hongda Dairy Farm in Baoding, Hebei Province. Twenty-one multiparous Holstein cows were selected and randomly divided into three groups (n=7 each). Groups were balanced for expected calving date, parity, body weight, previous lactation duration (~305 days), and milk yield (P>0.05). All cows were healthy and disease-free.

1.2 Experimental Design

Based on NRC (2001) nutrient requirements, three experimental diets were formulated with identical net energy for lactation (5.50 MJ/kg) but different crude protein levels: 12.12% (Group A), 13.07% (Group B), and 14.02% (Group C). Diet composition and nutrient levels are presented in . Postpartum, all groups received the same total mixed ration.

1.3 Feeding Management

All groups were maintained under identical management conditions. Diets were fed at 06:30, 12:00, and 17:00 daily by group. Residual feed was weighed the following morning to determine DMI.

1.4 Sample Collection

1.4.1 Blood Sampling Blood samples (15 mL) were collected from the jugular vein before morning feeding on days 21, 14, and 7 prepartum, on the day of calving, and on postpartal days 5, 10, 20, and 30. Samples were placed in coagulation-promoting tubes, immediately centrifuged at 1,795×g for 15 minutes, and the separated serum was aliquoted into 0.5 mL tubes and stored at -20°C.

1.4.2 Fecal Sampling During the final three days of the experiment, fecal samples were collected from three cows per group. Samples were obtained rectally at 08:00 and 18:00 daily, carefully avoiding urine contamination. Collected feces were divided into two portions: one portion received 20 mL of 10% sulfuric acid (H_2SO_4) per 100 g fresh weight to fix ammonia nitrogen, while the other was stored fresh.

1.4.3 Milk Sampling Daily milk yield was recorded to calculate 10-day average production. Milk samples (10 mL) were collected on postpartal days 1, 10, 20, and 30, with morning and evening samples combined in a 1:1 ratio to create daily composite samples.

1.5 Measurements

1.5.1 Dry Matter Intake and Body Weight Individual cow DMI was determined from daily feed offered and refused. Body weight was measured at the start of the experiment, on the day of calving, and on postpartal days 15 and 30 after milking.

1.5.2 Milk Composition Milk fat, protein, lactose, and solids-not-fat percentages were analyzed at room temperature on the day of collection using a full-component milk analyzer (MILKYWAY-CP4).

1.5.3 Blood Indexes Serum glucose (GLU), urea nitrogen (UN), triglycerides (TG), albumin (ALB), and total protein (TP) were measured using kits from Sino-Biochemical Laboratory and a Vitalab Selectra E semi-automatic biochemical analyzer (Netherlands). Serum insulin (Ins), insulin-like growth factor I (IGF-I), and leptin (LP) concentrations were determined using ELISA kits from R&D Systems (USA) and a PowerWave XS microplate reader.

1.5.4 Apparent Digestibility Apparent digestibility of crude protein, ether extract, neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium, and phosphorus was determined using the internal indicator method (acid-insoluble ash). Crude protein content was measured using a FOSS 8200 semi-automatic Kjeldahl apparatus. Methods for ether extract, NDF, ADF, calcium, and phosphorus analyses followed procedures described in *Feed Analysis and Feed Quality Detection Technology* (2nd edition) edited by Zhang Liying.

1.6 Statistical Analysis

Data were initially processed using Excel and analyzed using SPSS 13.0 software. One-way ANOVA was performed, and Duncan' s multiple range test was used for pairwise comparisons when significant differences were detected. Results are expressed as mean ± standard deviation.

2.1 Dry Matter Intake

As shown in , Group B exhibited higher DMI than Groups A and C across all periods. During days 1-10 postpartum, Group B DMI was 8.90% higher than Group C ($P<0.05$). During days 21-30, Group B DMI was 5.12% and 6.49% higher than Groups A and C, respectively ($P<0.01$). Overall DMI during days 1-30 was 5.41% and 5.85% higher in Group B compared to Groups A and C ($P<0.05$).

2.2 Body Weight

shows that compared to the day of calving, body weight losses on day 15 postpartum were 5.36%, 5.61%, and 6.27% for Groups A, B, and C, respectively, with Group C showing the greatest loss. By day 30 postpartum, weight losses were 6.30%, 5.03%, and 8.22% for Groups A, B, and C, respectively, again highest in Group C.

2.3 Calf Birth Weight

As shown in , no significant differences were observed in calf birth weight among treatment groups ($P>0.05$).

2.4 Milk Yield

indicates that dietary crude protein level tended to increase milk production. Group B produced 2.27 kg/d more milk than Group C (P>0.05) and 1.54 kg/d more than Group A (P>0.05) during days 1–30 postpartum.

2.5 Milk Composition

reveals that during days 2–10 postpartum, lactose percentage in Group B was 4.31% higher than in Group C (P<0.05). During days 11-20, milk protein percentage in Group C was 4.23% higher than in Group A (P<0.05), while solids-not-fat percentage in Group A was 4.32% higher than in Group C (P<0.05). During days 21-30, milk fat percentage in Groups A and C was 3.33% and 3.94% higher than in Group B (P<0.01). Milk protein percentage in Group C was 4.32% higher than in Group A (P<0.05), and solids-not-fat percentages in Groups A and B were 4.04% and 3.79% higher than in Group C (P<0.05).

2.6 Nutrient Apparent Digestibility

shows that during the prepartal period, apparent digestibility of crude protein in Group B was 12.56% and 4.41% higher than in Groups A and C, respectively (P<0.05). No significant differences were observed for other nutrients (P>0.05).

2.7 Blood Biochemical Indexes

Serum GLU concentrations declined from 21 days prepartum to 7 days prepartum, then increased on the day of calving. Postpartum GLU levels continued to rise on days 10, 20, and 30, with Group B significantly higher than Group C (P<0.05). Serum TP concentrations decreased prepartum in all groups; on the day of calving, Group B TP was significantly higher than Group C (P<0.05). Postpartum TP levels recovered but remained similar among groups (P>0.05). Prepartum serum ALB concentrations did not differ among groups (P>0.05), but on the day of calving, Group C was significantly lower than Groups A and B (P<0.05). On postpartal days 20 and 30, Group B ALB was significantly higher than Group C (P<0.01). Serum TG concentrations in Group B were significantly lower than in Groups A and C on the day of calving and on postpartal days 5, 10, and 20 (P<0.05).

Prepartum serum IGF-I concentrations were similar among groups (P>0.05) but declined as parturition approached, reaching the lowest values on the day of calving (Group A lowest, Group C intermediate, Group B highest; P<0.01). On postpartal days 5, 10, and 20, Group B IGF-I remained significantly higher than Groups A and C (P<0.01). Serum Ins concentrations also declined prepartum, reaching nadir on the day of calving, then gradually increased postpartum. On postpartal days 5 and 10, Group B Ins was significantly or extremely significantly lower than Groups A and C (P<0.05 or P<0.01). On day 20, Group B Ins was extremely significantly lower than Group C (P<0.01), while Group

A was significantly lower than Group C (P<0.05). On day 30, Ins concentrations decreased again, with Group B significantly lower than Groups A and C (P<0.05). Prepartum serum LP concentrations declined, with Group B extremely significantly higher than Group C at 14 days prepartum (P<0.01) and extremely significantly higher than both Groups A and C at 7 days prepartum, on the day of calving, and on postpartal days 5 and 10 (P<0.01). No significant differences were observed in serum UN concentrations among groups (P>0.05).

3.1 Effects of Prepartal Dietary Crude Protein Level on DMI and Body Weight

Postpartum DMI is a major determinant of milk production and body weight changes in dairy cows, and increasing DMI can shorten the duration of negative energy balance. Research indicates that low dietary protein levels impair DMI, leading to protein deficiency that insufficiently supports digestive enzyme synthesis, inhibits rumen bacterial fermentation, and causes body protein catabolism, ultimately reducing milk yield and protein content [6]. Conversely, excessively high protein levels provide minimal production benefits while increasing nitrogen excretion and reducing nitrogen utilization efficiency [7]. Greenfield et al. [3] demonstrated that feeding peripartal cows diets containing 12% versus 16% crude protein favored postpartum recovery and higher DMI, possibly because excessive protein prolonged the postpartum dietary adaptation period and increased metabolic demands. Our results align with these findings: among diets containing 12.12%, 13.07%, and 14.02% crude protein, DMI was highest in cows fed 13.07% crude protein (Group B).

DMI is closely associated with body weight changes. Prepartum weight gain reflects positive energy balance, where nutrient intake supports both fetal development and maternal tissue accretion. Postpartum weight loss occurs due to parturition and negative energy balance. In this study, Group C experienced greater weight loss than Groups A and B, likely because high dietary protein reduced DMI. The combination of reduced DMI, high milk production, and altered endocrine status during the peripartal period promoted adipose tissue mobilization [8]. Although differences between Groups A and B were not statistically significant, Group B showed a trend toward reduced weight loss. Since increasing DMI during early lactation shortens negative energy balance [9], this explains why Group B, with higher DMI, experienced less weight loss.

3.2 Effects of Prepartal Dietary Crude Protein Level on Calf Birth Weight

Although differences in calf birth weight among groups did not reach statistical significance, Group B calves tended to be heavier than those in Groups A and C. This trend may be attributed to differences in DMI and subsequent negative energy balance duration among groups, which altered circulating GLU, non-esterified fatty acids, and β-hydroxybutyrate concentrations. These metabolic changes likely triggered adaptive mechanisms that regulated nutrient partitioning and redistribution, ultimately affecting fetal growth [10].

3.3 Effects of Prepartal Dietary Crude Protein Level on Milk Yield

Dietary protein level critically affects lactation performance. Inadequate protein limits milk production, while excessive protein wastes feed resources and increases environmental nitrogen pollution. Optimal dietary protein levels maximize milk production, improve feed efficiency, and minimize environmental impact. Previous studies have reported varying effects of dietary protein on ruminant milk yield. Chew et al. [11] found that feeding 11% versus 9% crude protein during the entire dry period increased milk yield. Santos et al. [4] reported that increasing prepartal crude protein from 12.7% to 14.7% did not improve multiparous cow performance. These findings suggest that while dietary protein can enhance milk production, an optimal level exists beyond which no further benefits occur and protein resources are wasted [12]. Our results support this conclusion. Although milk yield differences among groups were not statistically significant, Group B consistently outperformed the others. This demonstrates that dietary crude protein level can be reduced from high to moderate levels without compromising production performance, thereby lowering feed costs and nitrogen emissions while protecting the environment.

3.4 Effects of Prepartal Dietary Crude Protein Level on Milk Composition

During days 21–30 postpartum, Group B milk fat percentage was extremely significantly lower than in Groups A and C, likely because moderate dietary protein increased milk yield. With consistent dietary concentrate-to-forage ratios, milk fat percentage typically correlates negatively with milk yield [13]. Milk protein percentage increased with dietary crude protein level, consistent with Lu [14], who reported that each 1% increase in dietary crude protein increased milk protein by 0.02%. During days 2–10 postpartum, Group B lactose percentage was 4.31% higher than Group C, possibly because higher serum GLU concentrations in Group B cows met the substantial glucose demand for lactose synthesis in high-producing cows [15]. Solids-not-fat percentage has gained recent attention [16]. In this study, Group A solids-not-fat percentage was significantly higher than Group C during days 11–20 and 21–30, contradicting reports by Zhao et al. [17] and Yin et al. [5], who found no effect of dietary crude protein level on this parameter. These discrepancies may be due to differences in breed, parity, or environmental conditions. Further research is needed to clarify the effects of prepartal dietary crude protein on postpartal milk composition.

3.5 Effects of Prepartal Dietary Crude Protein Level on Nutrient Apparent Digestibility

Yin et al. [5] reported that increasing dietary crude protein tended to reduce apparent digestibility of crude protein and neutral detergent fiber, though differences were not significant. Our results demonstrate that prepartal dietary crude protein level affected nutrient digestibility, with Group B showing improved digestion compared to Groups A and C. This may be because optimal protein levels shortened negative energy balance duration, maintained cow health, and improved digestibility. Alternatively, our use of spot fecal sampling rather than total collection may have contributed to differences from other studies. The underlying mechanisms require further investigation.

3.6 Effects of Prepartal Dietary Crude Protein Level on Blood Biochemical Indexes

Serum TG concentration directly reflects lipid digestion and transport status. Under normal conditions, blood lipid levels are low; impaired lipid transport significantly elevates circulating lipids [18]. Postpartum TG concentrations in Groups A and C were significantly higher than in Group B because low and high dietary protein levels reduced DMI and prolonged negative energy balance. Increased energy demands for lactation mobilized adipose tissue or reduced fatty acid uptake by mammary tissue, leading to incomplete TG oxidation, ketone body accumulation, and risk of ketosis or hepatic lipidosis.

Postpartum GLU demand increases dramatically, challenging the liver to synthesize glucose from precursors like propionate and amino acids. On day 30 postpartum, Group B serum GLU was significantly higher than Group C, indicating that moderate prepartal protein feeding facilitated postpartal GLU recovery through physiological regulation. Serum TP and ALB concentrations reflect liver function and energy metabolism [19]. Group B serum TP was higher than Group C postpartum, and ALB followed similar trends. This may be because adequate prepartal nutrition in Group C increased protein utilization by fetal and maternal tissues, slowing postpartum recovery. Additionally, reduced DMI and prolonged negative energy balance in Group C may have impaired hepatic protein synthesis and secretion.

Serum UN concentration is an important indicator of protein metabolism, reflecting protein deposition efficiency. Lower UN under stable dietary protein conditions indicates higher protein deposition efficiency elsewhere, whereas elevated UN increases urinary nitrogen loss and reduces nitrogen utilization [20]. For ruminants, optimal dietary protein promotes ammonia incorporation into microbial protein, reducing serum UN. Elevated UN may reflect enhanced protein catabolism or increased hepatic urea recycling [21]. In this study, Group B had lower postpartum serum UN than Groups A and C, demonstrating that moderate protein level improved nitrogen utilization and deposition.

IGF-I plays crucial roles in mammary cell development, apoptosis resistance, and functional maintenance [22]. Due to structural similarity to insulin, IGF-I exerts insulin-like effects, reducing serum GLU, promoting lipolysis and protein synthesis, and stimulating mammary epithelial cell proliferation [23]. Prepartum serum IGF-I was extremely significantly higher in Group B than in Groups

A and C, suggesting that moderate protein feeding stimulated protein synthesis to support subsequent lactation. Postpartum IGF-I was extremely significantly lower in Group B, indicating that dietary protein met lactation demands without requiring hormonal mobilization. Insulin is the primary hormone regulating GLU homeostasis, reducing serum GLU by promoting glycogen synthesis, hepatic conversion to fatty acids, or GLU oxidation for energy. Thus, insulin regulates energy metabolism disorders. Postpartum serum Ins was significantly or extremely significantly higher in Groups A and C than in Group B because reduced DMI in these groups necessitated adipose tissue mobilization (increased lipase activity) to compensate for energy deficits, elevating GLU and triggering increased insulin secretion to maintain stability. Zuo [24] reported that insulin inhibits lactation, consistent with our results and explaining why Group B had higher milk yield than Groups A and C.

Leptin, secreted by adipocytes, regulates feeding behavior, reduces energy expenditure, and decreases feed intake [25]. At normal concentrations, leptin inhibits feeding via hypothalamic pathways. Elevated leptin reduces DMI and enhances lipid metabolism, promoting fat utilization. Serum leptin during early lactation correlates with negative energy balance severity and duration; energy deficit-induced weight loss reduces serum leptin, stimulating appetite and increasing DMI [26]. This study found that feeding lower protein diets prepartum resulted in higher postpartum serum leptin, higher DMI, and less weight loss, whereas Groups A and C experienced greater weight loss, lower DMI, and reduced leptin. These findings align with Niu et al. [27] and Pei et al. [28]. The relationship between leptin and dietary nutrients requires further investigation.

Compared to 12.12% and 14.02% crude protein levels, feeding a diet containing 13.07% crude protein during the prepartal period increased postpartum DMI and milk yield, reduced body weight loss, accelerated postpartum serum GLU recovery, maintained lower TG concentrations to reduce hepatic stress, and increased serum leptin to alleviate negative energy balance.

References

[1] Xu P. Study on negative energy balance and metabolic regulation mechanisms in peripartal dairy cows [D]. Master' s thesis. Daqing: Heilongjiang Bayi Agricultural University, 2009.
[2] Crawley DD, Kilmer LH. Effect of level and source of rumen degradable protein fed prepartum on postpartum performance of dairy cows [J]. Journal of Dairy Science, 1995, 78(Suppl.1): 266.
[3] Greenfield RB, Cecava MJ, Johnson TR, et al. Impact of dietary protein amount and rumen undegradability on intake, peripartum liver triglyceride, plasma metabolites, and milk production in transition dairy cattle [J]. Journal of Dairy Science, 2000, 83(4): 703-710.
[4] Santos JEP, DePeters EJ, Jaron PW, et al. Effect of prepartum dietary protein level on performance of primigravid and multiparous Holstein dairy cows [J]. Journal of Dairy Science, 2001, 84(1): 213-224.

[5] Yin Fuquan, Ga' erdi. Effects of different protein levels in prepartal diets on dairy cow performance [J]. Heilongjiang Animal Science and Veterinary Medicine, 2008(11): 22–24.
[6] Broderick GA. Effects of varying dietary protein and energy levels on the production of lactating dairy cows [J]. Journal of Dairy Science, 2003, 86(4): 1370–1381.
[7] Li Depeng. Study on optimal dietary protein level for dairy cows in mid-lactation [D]. Master' s thesis. Tai' an: Shandong Agricultural University, 2014.
[8] Zhang Xingkai. Physiological characteristics and nutritional requirements of peripartal dairy cows [J]. China Dairy Cattle, 2012(4): 62–65.
[9] Liang Yingxiang, Liang Lanxiang, Yan Huiqun, et al. Effects of dietary crude protein level on digestibility and performance of peripartal dairy cows [J]. Zhejiang Journal of Animal Science and Veterinary Medicine, 2007(5): 1–2.
[10] Zhang Zhihong. Effects of different prepartal energy levels on growth performance, immunity, and endocrine function of newborn calves [D]. Master' s thesis. Hohhot: Inner Mongolia Agricultural University, 2013.
[11] Chew BP, Murdock FR, Riley RE, et al. Influence of prepartum dietary crude protein on growth hormone, insulin, reproduction and lactation of dairy cows [J]. Journal of Dairy Science, 1984, 67(2): 270–275.
[12] Xu Hongrui, Shi Jianqing, Zhao Guoqi. Research progress on urea nitrogen metabolism in ruminants [J]. Journal of Animal Science and Veterinary Medicine, 2006, 25(1): 23–24, 27.
[13] Wang Jianhua, Ge Xin, Zhang Baoxun, et al. Effects of different dietary energy and protein levels on digestion and metabolism in Laoshan dairy goats [J]. China Feed, 2011(1): 5–7.
[14] Lu Dexun. Nutritional engineering technology for dairy cows during the peripartal period [J]. Journal of Dairy Science and Technology, 2002, 25(3): 31–34.
[15] Zhong Lelun, Yao Junhu, Xu Ming. Research progress on glucose nutrition in ruminants [J]. Feed Review, 2006(5): 13–15.
[16] Zhang Jici, Jin Shande. On improving the solids-not-fat content of fresh milk [J]. Foreign Animal Science and Technology, 1983(4): 19–22.
[17] Zhao Xiaowei, Zhou Lingyun, Bu Dengpan, et al. Effects of dietary nutritional regulation on milk composition in dairy cows [J]. China Animal Husbandry and Veterinary Medicine, 2011, 38(3): 16–20.
[18] Yang Peilin. Effects of different energy intake levels on production performance and blood parameters in dairy cows [D]. Master' s thesis. Beijing: Chinese Academy of Agricultural Sciences, 2007.
[19] Zuo Caizhi, Deng Junliang, Wang Zhe, et al. Effects of different energy intake levels on serum total bilirubin, protein, and transaminases in healthy peripartal dairy cows [J]. Chinese Journal of Veterinary Science, 2007, 27(6): 865–869.
[20] Stanley CC, Williams CC, Jenny BF, et al. Effects of feeding milk replacer once versus twice daily on glucose metabolism in Holstein and Jersey calves [J]. Journal of Dairy Science, 2002, 85(9): 2335–2343.
[21] Chikhou FH, Moloney AP, Allen P, et al. Long-term effects of cimaterol in

Friesian steers: I. Growth, feed efficiency, and selected carcass traits [J]. Journal of Animal Science, 1993, 71(4): 906-913.
[22] Sun Zhenhua. Study on the application effect of double-low rapeseed meal replacing cottonseed and soybean meal in high-yielding dairy cows [D]. Master' s thesis. Hohhot: Inner Mongolia Agricultural University, 2012.
[23] Wang Qun, Yin Junli, Tang Bo, et al. Effects of dietary 2-hydroxy-4-(methylthio)butanoic acid isopropyl ester supplementation at different protein levels on production performance and blood biochemical indexes in dairy cows [J]. Journal of Nanjing Agricultural University, 2014, 37(3): 126-132.
[24] Zuo Caizhi. Study on effects of different energy levels on production performance, blood biochemistry, and endocrine factors in peripartal dairy cows [D]. Master' s thesis. Ya' an: Sichuan Agricultural University, 2004.
[25] Liu Jianzhong, Li Ning, Xiong Yuanzhu, et al. Research progress on obesity gene and its expression product leptin in humans and mice [J]. Journal of Agricultural Biotechnology, 2004, 8(4): 396-402.
[26] Reist M, Erdin D, von Euw D, et al. Concentrate feeding strategy in lactating dairy cows: metabolic and endocrine changes with emphasis on leptin [J]. Journal of Dairy Science, 2003, 86(5): 1690-1700.
[27] Niu Shuling, Zhang Cai, Zhang Guocai, et al. Effects of dietary treatment on milk yield and leptin gene expression in adipose tissue of dairy cows [J]. Scientia Agricultura Sinica, 2007, 40(10): 2382-2386.
[28] Pei Yan, Li Xiuju, Zhang Cai, et al. Effects of different energy intake levels on milk performance and serum leptin and NPY concentrations in dairy cows [J]. Feed Industry, 2006, 27(21): 31-34.

Note: Figure translations are in progress. See original paper for figures.