Pre-calving energy density and rumen protected lysine impacted blood metabolites and biomarkers of liver functions in dairy cows during the transition period.

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Abstract

Dairy cow usual faces negative energy balance and disorder of normal organs function due to mismatch between energy intake and energy demands. Negative energy balance directly affects liver function and blood metabolites because of liver used as source of energy supply and center of metabolic activity. The study aimed to determine the effect of pre-calving energy density and rumen-protected lysine on blood metabolites and biomarkers of liver functions in dairy cows during the transition period. Forty 3rd lactation Holstein cows were randomly allocated to one of the four dietary treatments (High energy with rumen-protected lysine (**HERPL)** = 1.53NE_L plus 40 g Lys, High energy without lysine **(HECK) =** 1.53NE_L, Low energy with rumen-protected lysine **(LERPL) =** 1.37NE_L plus 40 g Lys, and Low energy without lysine (**LECK)** = 1.37NE_L arranged in a 2 x 2 factorial design. Blood samples were collected during the transition period and concentrations of blood metabolites and biomarkers of liver functions were measured. Interaction between pre-calving high energy diet and RPL tended to increase plasma albumin, numerically increased glucose, decreased TG, total bilirubin and AST concentrations. The result revealed that pre-calving high energy diet increased insulin, albumin and decreased blood urea nitrogen and total bilirubin concentrations and substantial favor liver functions during the transition period.

Introduction

Preparation for parturition and lactation caused profound physiological, immunological, and endocrine change in dairy cows during transition period. The demand for glucose and metabolizable energy of the cow increases two to three times during transition period. Due to dry matter intake significantly reduced dairy cows unable to meet the required energy and usual face negative energy balance which has great impact on body hormonal balance and organ functions. This mismatch between energy intake and energy demand results in development of negative energy balance and disorder the proper function of the organs (Butler, 2000). Liver function and performance of dairy may impair due to the occurrence of inflammatory responses during the periparturient period (Bertoni et al., 2008).

Liver is one of the most organ which directly affected by negative energy balance because of liver is used as the source of energy supply and center of metabolic activity (Gross et al., 2011). Liver plays important role in controlling nutrient partitioning, glucose, lipid, and protein metabolism, kitogenesis, immune function, ammonia detoxification and steroid hormone catabolism (Donkin, 2012). Moreover, liver oxidize up to 20% free fatty acids during close-up period. However, excess mobilization of free fatty acids beyond the oxidizing capacity of liver and secrete TAG as VLDL resulted in accumulation of TAG. Over accumulation of TAG in the liver tissue cause fatty liver disease eventually leads to liver dysfunction (Herdt, 2000; Bobe et al., 2004). As liver used for center of metabolic activity and source of energy supply, the health of transition cow totally tied with the liver to coordinate these process and cope up with metabolic and hormonal changes (Donkin, 2012).

Consumption of body protein to cope up with NEB leads to depleting important structural and enzyme proteins (Herdt, 2000). On the other hand concentrations of glucose, NFEA, BHBA, insulin, cholesterol, enzymes and protein reflect the nutrients status, and liver status of cows (Kaneko et al., 2008; Sakowski et al., 2012). A variety of enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin concentrations in the blood very useful to assess liver function (Sakowski et al., 2012).

Growth hormone stimulates hepatic synthesis and secretion of IGF-1, which serves as feedback signal to control GH release (Le Roith et al., 2001). In early lactation, growth hormone play an important role in controlling metabolism mainly mediated through IGF-I in dairy cows which is characterized by a hypo insulinemic state (Lucy, 2008).

The negative energy balance status is characterized by alterations in blood metabolite and hormone profile (Piccione et al., 2012). Occurrence of negative energy balance impaired normal liver function and affected biomarkers of liver functions and other blood metabolites in transition cows. However, pre calving feeding of dietary energy density played significant role to ameliorate the negative impact of negative energy balance on proper organ function and transition cow health during the transition period. Lysine is a constituent of carnitine a compound needed for β-oxidation of long fatty acid in mitochondria to maintain cellular energy during excess mobilization free fatty acid from adipose tissue. Therefore, in the present study we hypothesized that feeding transition cows with close-up high energy diet together with rumen-protected lysine would improve glucose supply and significantly impact biomarkers of liver functions, and substantially improve liver function status during the transition period. The objective of the current study was to determine the effect of feeding close up energy density together with rumen-protected lysine to transition cows on blood metabolites and biomarkers of liver functions during the transition period.

Materials And Methods

Experimental design and experimental cows

All procedures were approved by the Animal Care and Use Committee of the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing. In brief, forty the same parity (3^{rd} lactation) Holstein cows entering their 4^{th} lactation were chosen based on body condition scores (BCS) ≥ 3.25 and ≤ 3.5 (1 to 5 scale) from a large herd. During cow selection the expected calving date of each cow was considered to select all cows with similar expected calving dates and previous milk yield. All selected cows were balanced for their BCS, expected calving date, and previous milk yield (13679.65 ± 2370.4 kg 305 d milk yield, *P = 0.80*) before being randomly assigned to dietary treatment groups. All forty cows were randomly allocated to one of the four dietary treatment groups (n = 10 each) arranged in a 2 x 2 factorial design using two dietary energy density and rumen protected lysine. Four dietary treatment rations such as low energy diet with rumen-protected lysine (LERPL) = 1.37 NE_L Mcal/kg DM plus 40 g rumen-protected lysine/cow per day, only low energy diet (LECK) = 1.37 NE_L Mcal/kg DM without rumen-protected lysine (as control), or high energy diet with rumen-protected lysine (HERPL) = 1.53 NE_L Mcal/kg DM plus 40 g rumen-protected lysine/cow per day, only high energy diet (HECK) = 1.53 NE_L Mcal/kg DM without rumen-protected lysine.

Dietary treatment rations and feeding of cows

Prior to feeding the close-up LERPL, LECK, HERPL, or HECk all cows were fed the same dry off energy diet (NE_L=1.34 Mcal/kg DM) during the dry period. Cows were switched to either HE or LE diet at d -21 through d 0 (calving date). After calving up to d 21 all cows received the same lactation diet (NE_L=1.69 Mcal/kg DM). Lysine was top-dressed on the TMR once per day at a rate of 40g Lysine/cow per day by using 50 g of ground corn as a carrier. The same amount 50 g ground corn was also top dressed to control diets. The rumen-

protected lysine (Ascor Chimici Srl, Beijing) supplement contained 55% lysine with 44% bioavailability [39, 45] (Tucker et al., 2015; Reiners, 2017) therefore per 40 g of RPL product the pre and post-partum Lysine-fed cows received 9.68 g of metabolizable lysine. The values for RUP, RDP, Lys, and Met were predicted using the NRC [35] (2001) model and are presented in Table 2.

All experimental cows were housed in a ventilated enclosed barn during the experimental period and were individually fed their respective diet. Cows had access to stand and bedding areas until 3 d before expected parturition, when they were moved to individual maternity pens bedded with straw until parturition. After parturition, cows were individually fed a common lactation diet, with or without Lysine supplementation. Total mixed rations were mixed daily and provided twice per day at 0600 and 1400 h (Table 1 and Table 2). All cows were fed ad libitum with their respective dietary rations.

Table 1. Ingredient composition of diets fed transition cows that were supplemented or were unsupplemented with rumen-protected lysine during the transition period.

Ingredients (%)	Close-up cow diet[1]				Fresh cow diet			
	LECk	LEByls	HECK	HEByls	LECK	LEByls	HECK	HEByls
Corn silage processed[2]	22.6	22.6	-	-	-	-	-	-
Grass hay	24.3	24.3	14.7	14.7	-	-	-	-
Oats hay	22.1	22.1	21.7	21.7	4.9	4.9	4.9	4.9
Alfalfa [3]	-	-	-	-	20.0	20.0	20.0	20.0
Corn silage	-	-	22.1	22.1	22.8	22.8	22.8	22.8
Steam flaked corn	-	-	-	-	8.9	8.9	8.9	8.9
Cornmeal	-	-	9.9	9.9	9.4	9.4	9.4	9.4
Soybean meal [4]	3.7	3.7	3.6	3.6	12.4	12.4	12.4	12.4
Cottonseed meal	3.8	3.8	2.2	2.2	-	-	-	-
DDGS[5]	1.5	1.5	3.6	3.6	-	-	-	-
Extracted soy bean[6]	-	-	-	-	2.5	2.5	2.5	2.5
Canola meal solvent	4.3	4.3	5.0	5.0	2.5	2.5	2.5	2.5
Molasses cane	-	-	-	-	1.8	1.8	1.8	1.8
Corn gluten meal	1.5	1.5	1.1	1.1	1.5	1.5	1.5	1.5
Brewers grains	9.6	9.6	10.6	10.6	6.1	6.1	6.1	6.1
Fresh cow mineral premix[7]	-	-	-	-	3.7	3.7	3.7	3.7
Close up mineral premix	5.3	5.3	4.3	4.3	-	-	-	-
Sta-chol (choline)	0.2	0.2	0.5	0.5	0.1	0.1	0.1	0.1
Bergafat100[8]	-	-	-	-	0.8	0.8	0.8	0.8
DCAD Premix DCAD[9]	0.8	0.8	0.4	0.4	-	-	-	-
KHCO3	-	-	-	-	0.3	0.3	0.3	0.3
MT-BOND	-	-	-	-	0.1	0.1	0.1	0.1
Optigen-slow[10]	-	-	-	-	0.2	0.2	0.2	0.2
Dimond V-XPC Yeast product[11]	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1
Glycoline	-	-	-	-	1.6	1.6	1.6	1.6

Lactation B vitamin	-	-	-	-	0.2	0.2	0.2	0.2
Transition B vitamin	0.4	0.4	0.4	0.4	-	-	-	-

[1]Close-up cow diet: HEByls = high energy (1.53 NE_LMcal/Kg DM) with 40 g/cow per day rumen-protected lysine (Ascor Chimici Srl, Beijing), HE = high energy (1.53 NE_LMcal/kg DM) without rumen-protected lysine, LEByls = low energy (1.37 NE_LMcal/kg DM) with 40g/cow per day rumen-protected lysine, LE = low energy (1.37 NE_LMcal/kg DM) without rumen-protected lysine, lactation diet = 1.69 NE_LMcal/kg DM. After parturition, the same 40 g/cow per day rumen-protected lysine has supplemented for those cows that had been fed on rumen-protected lysine before calving.

[2]Corn silage processed: sieve used 4.75mm, and kernel processing score = 50-70%.

[3]Extracted soy bean: 92.6% DM and 36.4% CP.

[4]Soybean meal: 89.1% DM and 42.6% CP

[5]DDGS = distiller's dried grains with soluble are the nutrient rich co-product of dry-milled ethanol production.

[6]Alflafa: Alfalfa hay was used to formulate total mixed ration.

[7]Close-up and fresh cow mineral premix: Ca, P, Mg, K, Na, Cl and S.

[8]Berga Fat100: by-pass fats for ruminants: extra energy without a carrier.

[9]DCAD = Dietary Cation-Anion Difference used to prevent to hypocalcemia in close-up cow.

[10]Optigen is Alltech's non-protein nitrogen (NPN) source for ruminants.

[11]Diamond V XP yeast culture supplement (FD00365CHN-XP, Diamond V, Cedar Rapids, lowa, USA).

Table 2. Chemical composition of total mixed ration fed in the experiment

Chemical components	Close-up cow diet[1]				Fresh cow diet			
	LECK	LEByls	HECK	HEByls	LECK	LEByls	HECK	HEByls
DM%	54.07	54.07	53.502	53.502	55.6	55.6	55.6	55.6
NE_L Mcal/kg DM	1.37	1.37	1.53	1.53	1.69	1.69	1.69	1.69
CP, % of DM	15.1	15.1	15.1	15.1	18.0	18.0	18.0	18.0
ADF, %	26.10	26.10	23.265	23.262	17.2	17.2	17.2	17.2
NDF, %	44.7	44.7	40.755	40.755	27.7	27.7	27.7	27.7
Ash, %	5.6	5.6	5.6	5.6	5.5	5.5	5.5	5.5
NFC % DM	25.48	25.48	31.85	31.85	41.4	41.4	41.4	41.4
Starch, %	8.79	8.79	15.46	15.46	23.3	23.3	23.3	23.3
Sugar, %	4.99	4.99	4.96	4.96	5.91	5.91	5.91	5.91
RDP % of DM	9.92	9.92	9.91	9.92	11.35	11.34	11.35	11.34
RUP % of DM	5.17	5.21	5.17	5.21	6.71	6.74	6.71	6.74
RDP supplied (g/d)	1194	1198	1191	1192	1928	1931	1928	1931
RUP supplied (g/d)	623	629	621	626	1157	1148	1157	1148
MP supplied (g/d)	1018.47	1024.19	1108.26	1110.32	2126.10	2133.16	2126.10	2133.16
MP balance (g/d)	1126	1127	1075	1074	2126.10	2133.16	2126.10	2133.16
Lys:Met	2.85:1	3.1:1	2.85:1	3.07:1	2.96:1	3.12:1	2.96:1	3.12:1
Lys (% of MP)	6.52	7.06	6.62	7.11	6.5	6.84	6.5	6.84
MP-Lys (g)	66.44	72.29	73.34	78.95	138.27	145.88	138.27	145.88
Met (% of MP)	2.29	2.28	2.32	2.31	2.2	2.19	2.2	2.19
MP-Met (g)	23.33	23.32	25.77	25.68	46.77	46.72	46.77	46.72
NE_L allowable milk (kg/d)	-	-	-	-	27.4	27.80	27.4	27.80

MP allowable milk (kg/d)	-	-	-	-	25.8	25.90	25.8	25.90
EE (% of DM	3.23	3.40	3.4	3.57	4.56	4.67	4.56	4.67
Ca (% of DM)	1.61	1.61	1.56	1.57	0.91	0.92	0.91	0.92
P (% of DM)	0.45	0.45	0.46	0.46	0.40	0.40	0.40	0.40
Mg (% of DM)	0.55	0.55	0.54	0.54	0.43	0.43	0.43	0.43
Cl (% of DM)	0.91	0.93	0.86	0.88	0.57	0.59	0.57	0.59
K (% of DM)	1.04	1.04	0.98	0.97	1.32	1.32	1.32	1.32
Na (% of DM)	0.3	0.3	0.28	0.28	0.55	0.55	0.55	0.55
S (% 0f DM)	0.42	0.42	0.41	0.20	0.20	0.20	0.20	0.20

[1]Close-up cow diet: HEByls = high energy (1.53 NE_LMcal/Kg DM) with 40 g/cow per day rumen-protected lysine (RPL), HE = high energy (1.53 NE_LMcal/kg DM) without rumen- protected lysine, LEByls = low energy (1.37 NE_LMcal/kg DM) with 40 g/cow per day rumen-protected lysine, LE = low energy (1.37 NE_LMcal/kg DM) without rumen-protected lysine, lactation diet = 1.69 NE_LMcal/kg DM.

[2]RPL = rumen-protected lysine.

Feed samples collection and analysis

Samples of feed offered were collected at 0700 h 4 times per week. Samples were frozen at -20°C until further analysis. TMR samples were frozen at -20°C and composited monthly for analysis of DM, CP, NDF, ADF, and Ash. Dry matter intake was determined by measuring feed provided and subtracting the orts remaining. Samples of TMR and orts from each treatment were analyzed for DM content by oven-drying at 60°C until they maintained a constant weight. The dried samples were ground through a 1-mm screen using a Cyclotec 1093 Mill (Tecator 1093, Tecator AB, Höganäs, Sweden) before analysis. Samples were further dried at 105°C for 2 h to determine the absolute DM, and chemical analyses were expressed on the basis of the final absolute DM. The CP (N × 6.25) content of feed samples was determined using the macro-Kjeldahl nitrogen test (AOAC, 2000; method 976.05) with a Kjeltec digester 20 and a Kjeltec System 1026 distilling unit (Tecator AB). The contents of NDF and ADF were determined using the Van Soest procedure [47] (1991) using heat-stable amylase (type XI-A of *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO). The ash content was determined by incineration at 550°C overnight, and the OM content calculated (AOAC, 2000; method 942.05). The ether extract content was determined using a a soxhlet system HT6 apparatus (Tecator AB) according to AOAC (2000; method 920.39). NFC were calculated according to the [35] (NRC, 2001), total sugars were quantified by incubation with invertase followed measurement of reducing sugars [32] (Martel et al. 2011) and starch was determined by alpha-amylase and glucoamylase digestion followed by colorimetric glucose quantification with a commercial kit.

Blood samples collection and analyses of plasma metabolites

Blood samples were collected into evacuated tubes containing heparin from coccygeal vein at 0700 h immediately after provision of morning feeds. Tubes for plasma preparation were put on ice immediately before centrifugation at 3,000 × g for 20 minutes at 4°C. Plasma was decanted and stored at -20 °C for subsequent analysis. Plasma biochemistry analyses of glucose (Glu), triglyceride (TG), albumin (ALB), total bilirubin (TBIL), total cholesterol (TC), blood urea nitrogen (BUN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were performed using automatic biochemical analyzer (Hitachi 7080, Beijing Zhangtong Lanbo Clinical Laboratory Co.Ltd, China) according to the manufacturer's protocols.

The radioimmunoassay technique was used for analyses of insulin-like growth factor -1 and Insulin (DFM-96.r.radioimmunocount, Zhangtong Lanbo Clinical Laboratory Co.Ltd. Beijing, China) based on manufacturer's instructions.

Statistical analysis

The data for glucose (Glu), triglyceride (TG), albumin (ALB), total bilirubin (TBIL), total cholesterol (TC), blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), insulin-like growth factor-1 (IGF-1), and Insulin (INS) were analyzed as a completely randomized design with repeated measures using PROC MIXED of SAS (version 9.2, SAS Institute Inc., Cary, NC). The MIXED statistical model used for analysis was as follow:

$y_{ijkl} = \mu + L_i + E_j + LE_{ij} + A_{ijk} + T_l + TL_{jl} + TE_{kl} + TLE_{jkl} + \varepsilon_{ijkl,}$ where y_{ijkl} was the dependent, continuous variable, μ is the overall mean; L_i was the fixed effect of Lysine (I = with or without supplementary lysine); E_j was the fixed effect of energy (j = 1.37 Mcal/kg DM or 1.53 Mcal/kg DM); A_{ijk} was the random effect of the k'th cow in the ij'th combination of lysine and energy; T_l was the fixed effect of time (day) of the experiment; the two and three way interactions of the time, lysine and energy, all considered fixed, and ε_{ijkl} was the residual error. Plasma Glu, TG, ALB, TBIL, TC, BUN, ALT, AST, IGF-1 and INS were analyzed at various time point that were not equally spaced, hence the covariance structure for the repeated measurements was modeled using the spatial power option. The Kenward-Roger option was used for the computing the denominator degrees of freedom for testing hypotheses. Least squares mean were compared using LSD and statistical differences were declared significant at $P \leq 0.05$ and tendencies were determined at $P \leq 0.10$.

Results

Effect on glucose and Insulin concentrations

Main effect energy, RPL, time and interactions for liver functions biomarkers are summarized in Table 3 and Table 4. The result revealed that dietary treatments ration did not influence pre and postpartum glucose concentrations in dairy cows.

Table 3. Main effect of close up dietary energy density and rumen-protected lysine (RPL) supplementation on blood metabolites and biomarkers of liver functions in dairy cows during the transition period

Variables[1]	RPL[2]		Energy[3]		SEM[4]	P-values[8]				
	0	40	1.37	1.53		RPL	Energy	Time[5]	Time × RPL[6]	Time × Energy[7]
Prepartum										
Glucose	3.8	3.9	3.8	3.9	0.06	0.13	0.07	0.001	0.26	0.001
BUN	5.9	5.5	6.8[a]	4.6[b]	0.25	0.37	0.001	0.03	0.67	0.005
ALT	13.69	13.63	14.1	13.2	0.55	0.94	0.24	0.001	0.09	0.20
AST	37.1	34.1	35.9	35.3	1.9	0.26	0.63	0.57	0.83	0.83
ALB	61.8	61.4	59.8[b]	66.3[a]	1.08	0.10	0.02	0.001	0.60	0.001
TC	2.3	2.2	2.2	2.2	0.08	0.35	0.6	0.001	0.60	0.34
TG	0.14	0.12	0.14	0.12	0.01	0.14	0.19	0.35	0.86	0.02
INS	15.1	14.8	13.4[b]	16.5[a]	0.54	0.74	0.003	0.001	0.78	0.007
TBIL	2.9	2.7	3.7[a]	2.0[b]	0.2	0.46	0.001	0.001	0.84	0.006
IGF-1	149.6	140.3	159.6[a]	130.4[b]	7.0	0.35	0.005	0.20	0.26	0.19
Postpartum										
Glucose	3.8	3.7	3.6	3.9	0.12	0.43	0.19	0.001	0.20	0.20
BUN	4.7	4.6	4.7	4.5	0.13	0.84	0.47	0.001	0.60	0.02
ALT	12.9	13.1	13.4	12.7	0.83	0.91	0.59	0.001	0.15	0.39
AST	75.5	67.1	70.7	71.9	5.3	0.26	0.86	0.001	0.14	0.76
ALB	61.3	62.08	61.2	62.1	1.29	0.66	0.62	0.001	0.95	0.47
TC	2.4	2.5	2.5	2.3	0.09	0.63	0.34	0.001	0.37	0.03
TG	0.02	0.03	0.03	0.02	0.00	0.34	0.33	0.46	0.34	0.39
INS	9.4	9.2	9.6	8.9	0.33	0.67	0.12	0.001	0.92	0.21
TBIL	4.9	4.4	4.8	4.6	0.37	0.33	0.74	0.001	0.31	0.34
IGF-1	282.9	294.9	312.4[a]	265.4[b]	9.7	0.39	0.001	0.001	0.63	0.006

a, b Means from main effect in the same row with different superscripts are significantly different, $P < 0.05$, using the Least Significant difference method.

[1]Variables: glucose (Glu), triglyceride (TG), albumin (ALB), total bilirubin (TBIL), total cholesterol (TC), blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), insulin-like growth

factor-1 (IGF-1), and Insulin (INS) [2]RPL effect = rumen-protected lysine was top dressed on total mixed rations at a rate of 40 g/cow per day from d -21 to d 21 after calving.

[3]Energy effect: low energy (LE) = 1.37 NE_L Mcal/kg DM); high energy, (HE = 1.53 NE_L Mcal/kg DM) fed from d -21 to d 0 (calving date).

[4]SEM = Standard error of mean

[5]Time effect = d -21, -14, -7, 0, 7, 14, and 21 relative to parturition.

[6]Time × RPL = Interaction effect of time and rumen-protected lysine (RPL was top dressed into total mixed rations at a rate of 40 g/cow per day from d -21 to d 21 after calving).

[7]Time × Energy: interaction effect of time and energy; low energy (LE) = 1.37 NE_L Mcal/kg DM; high energy (HE) = 1.53 NE_L Mcal/kg DM fed from d -21 to d 0 (calving date).

[8]P-Value = statistical differences were declared significant at $P \leq 0.05$ and tendencies were determined at $P \leq 0.10$.

Table 4. Interaction effect of close up dietary energy density and rumen-protected lysine (RPL) supplementation on blood metabolites and biomarkers of liver functions in dairy cows during the transition period

Variables[1]	RPL × Energy [2]				*P – values [3]*			
	LECK[4]	LEByIs[5]	HECK[6]	HEByIs[7]	SEM[8]	RPL × Energy	Time[9]	Time ×RPL × Energy[10]
Prepartum								
Glucose	3.6	3.9	3.9	3.9	0.09	0.12	0.01	0.46
BUN	7.1	6.5	4.7	4.6	0.36	0.48	0.03	0.54
ALT	14.2	14.0	13.1	13.3	0.77	0.80	0.01	0.03
AST	36.5	35.2	37.7	32.9	2.7	0.52	0.57	0.62
ALB	57.21	62.5	66.4[a]	66.3[b]	1.53	0.08	0.01	0.44
TC	2.2	2.3	2.3	2.0	0.11	0.15	0.01	0.45
TG	0.15	0.13	0.13	0.11	0.01	0.91	0.35	0.27
INS	12.9	13.8	17.2	15.7	0.77	0.13	0.01	0.12
TBIL	3.8	3.7	2.2	1.9	0.28	0.64	0.01	0.80
IGF-1	163.8	155.3	135.4	125.3	9.9	0.93	0.20	0.01
Postpartum								
Glucose	3.7	3.6	3.9	3.8	0.17	0.91	0.01	0.86
BUN	4.7	4.6	4.5	4.5	0.19	0.95	0.01	0.48
ALT	13.0	13.7	12.9	12.5	1.18	0.62	0.01	0.31
AST	67.2	74.2	83.9	60.1	7.5	0.04	0.01	0.63
ALB	60.1	62.4	62.5	61.8	1.82	0.41	0.01	0.97
TC	2.5	2.6	2.4	2.4	0.13	0.60	0.01	0.65
TG	0.02	0.04	0.02	0.02	0.01	0.40	0.46	0.37
INS	9.5	9.8	9.2	8.6	0.47	0.30	0.01	0.49
TBIL	4.6	4.9	5.3	3.9	0.53	0.10	0.01	0.30
IGF-1	318.6[a]	306.1[b]	247.2	283.7	13.8	0.08	0.01	0.18

a, b Means from interactions effect in the same row with different superscripts are significantly different, *P < 0.05*, using the Least Significant difference method.

[1]Variables: glucose (Glu), triglyceride (TG), albumin (ALB), total bilirubin (TBIL), total cholesterol (TC), blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), insulin-like growth factor-1 (IGF-1), and Insulin (INS).

[2]RPL × Energy: interaction effect of rumen-protected lysine and energy diet.

[3]P- Values are from a repeated measures analysis of variance. Statistical differences were declared significant at $P \leq 0.05$ and tendencies were determined at $P \leq 0.10$.

[4]LECK : close-up low energy diet (LE = 1.37 NE_L Mcal/kg DM) with 0 g rumen-protected lysine.

[5]LEByls : close-up low energy diet (HE = 1.37 NE_L Mcal/kg DM) with 40 g/ day per cow rumen-protected lysine.

[6]HECK : close-up high energy diet (LE = 1.53 NE_L Mcal/kg DM) with 0 g rumen-protected lysine.

[7]HEByls : close-up high energy diet (HE = 1.53 NE_L Mcal/kg DM) with 40 g/ day per cow rumen-protected lysine.

[8]SEM: standard error of mean

[9]Time = effect of time (d -21, -14, -7, 0, -3, 7, 14 and 21 relative to calving day).

[10]Time × RPL × Energy: Interaction effect of time × rumen-protected lysine × energy.

However, close-up high energy diet tended to improve glucose concentration during the prepartum period (*P = 0.07;* Figure 1A). Cows in close-up high energy diet numerically higher in glucose concentrations than cows in low energy diet during the pre and postpartum period Table 3 (Figure 2A). Cows fed low energy diet and supplemented with RPL numerically higher in prepartum glucose concentration compared to control group (P = 0.12).

Interaction between time and pre-calving energy diet significantly impact glucose concentrations in prepartum cows (*P = 0.01*) and insulin concentrations during the pre and post partum periods (*P = 0.001;* Figure 2B). Interactive between time and energy significantly affected prepartum plasma insulin concentrations (*p = 0.007*) but not postpartum insulin concentrations (*P = 0.21*). Cows fed high energy diet had higher glucose concentrations at d -14 before calving. The highest glucose concentration (6.3 mM Vs.5.44 mM) was found in cow fed HE diet at d 0 (calving) as compared to cows fed low energy diet. The lowest glucose concentration (2.67 mM) was found in cows fed high energy diet in the first two weeks after calving. The highest plasma insulin concentration 17.4 mM was found at d -21 before calving in cows fed high energy diet. Lowest plasma insulin concentration10.2 mM was found at d -7 before calving in cows fed close-up low energy diet.

Close-up energy diet significantly affected plasma insulin concentration in prepartum cows (*P = 0.003;* Figure 1B*)* but did not impact postpartum insulin concentrations (*P = 0.12).* Close-up high energy diet significantly increased prepartum plasma insulin concentration by 23.1% (P = 0.003) compared to cows fed low energy diet.

Effect on plasma Albumin (ALB) and Blood urea nitrogen (BUN) concentrations

Pre-calving energy diets significantly affected plasma albumin (*P = 0.02*) and BUN concentrations (*P =0.001*) in prepartum cows but not in postpartum cows. Compared with cow in low energy diet cow in high energy diet

significantly increased albumin concentration by 10.8% (P = 0.002; Figure 1C), significantly decreased BUN concentration by 32.4% (*P = 0.001;* Figure 1D) during the prepartum period. Cows fed with low energy diet decreased plasma albumin concentration by 9.8%. Interaction between time and energy significantly affected albumin concentrations (*P = 0.001;* Figure 1C) and on BUN concentrations in prepartum cows (*P = 0.005*) but not in post partum cows (*P = 0.84)*. The highest plasma albumin concentration (73.30 g/L) was recorded at d -14 before calving in cows fed high diet. The lowest plasma albumin concentration 49.04 g/L was found in cows' fed low energy diet at d - 7 before calving.

The highest BUN concentration (7.87 mM) was found in cow fed prepartum low energy diet and the lowest BUN concentration (4.29 mM) was found in cows fed pre-calving high energy diet at -21 before calving. Higher plasma urea concentration 5.1mM was found in cows fed low energy diet at calving but the lowest BUN concentration 4.1mM was found in cow fed high energy diet in the first 2 weeks after calving. Cows fed high energy diet numerically had higher plasma albumin concentrations during the postpartum period compared to cows fed low energy diet. Interaction between pre-calving energy diet and RPL tended to influence prepartum plasma albumin concentrations (*P = 0.08*). Cow fed high energy diet and supplemented with RPL had higher albumin concentrations during the prepartum period as compared to cows fed low energy diet with RPL. Rumen-protected lysine supplementation numerically increased plasma albumin concentration as compared to non supplemented cows (Figure 2F). Plasma BUN concentrations did not affect by rumen-protected lysine supplementation both during the prepartum and postpartum periods. However, RPL supplementation numerically decreased plasma BUN concentration by 6% and 2.1 % in pre and postpartum cows respectively.

Effect on Plasma IGF-1and total bilirubin (TBIL) concentrations

Pre-calving energy density significantly affected prepartum plasma TBIL concentrations and IGF-1 concentrations in pre and postpartum cows. Close-up high energy diet significantly decreased plasma IGF-1 concentration by 18.3% (*P = 0.005;* Figure 1E), significantly decreased TBIL concentration by 45.9% (*P = 0.001,* Figure 1F) in preparum cows and significantly lowered IGF-1 concentrations by 15.2% (*P =0.001;* Figure 2E) in postpartum cows but not affected postpartum TBIL concentrations (Figure 2D).

Cows in low energy diet numerically increased post partum TBIL concentration by 4.5%. Supplementation of RPL did not affect plasma IGF-1 concentrations both in pre and post partum cows. However, feeding RPL to transition cows numerically decreased prepartum plasma IGF-1concentrations by 6.2% and increased plasma IGF-1concentration by 4% in post partum cows. Interaction between rumen-protected lysine and energy tended to impact IGF-1 concentration in postpartum cows. Cows fed low energy and supplemented with RPL increased postpartum IGF-1 concentration as compared to cow fed high diet with RPL. Time, RPL and energy interaction had significant effect on IGF-1 concentration (*P = 0.001*). Time significantly affected TBIL concentrations during the pre and postpartum period (*P = 0.001*). Time and energy interaction affected TBIL concentration in prepartum cows but not in postpartum cows (Figure 1F). The lowest TBIL concentration of 1.65µmol/L and 3.6µmol/L were found at d -7 and d 14 relative to calving in cows fed HE diet respectively. The highest pre and post partum TBIL concentrations of 4.94 µmol/L and 5.99µmol/L were found in cows fed LE diet at d -14 and d 7 relative to calving respectively. Dietary treatment rations did not impact TBIL concentrations in post partum cows. However, cows fed HE diet numerically reduced post partum TBIL concentration compared to cows fed LE diet. Cows fed high energy diet together with RPL numerically

decreased TBIL concentration (1.85μmol/L vs. 2.2μmol/L) compared to control group. Cows fed low energy diet with RPL had higher TBIL concentration (3.6μmol/L vs. 3.7μmol/L) compared to cow fed low energy diet alone.

Effect on ALT, AST, TC and TG concentrations

Dietary treatments ration did not significantly influence plasma ananine tansaminase (ALT), aspartate tansaminase (AST), total cholesterol (TC) and triglyceride (TG) concentrations during the pre and postpartum periods (Table 3 and Table 4). However, time, RPL and energy interaction significantly affected ALT concentrations during the prepartum period (*P = 0.03*). Time significantly affected ALT, AST, and TC concentrations (*P= 0.001*) but not TG concentration (*P = 0.46*) during the pre and postpartum periods.

We found the highest plasma ALT concentration (16.03 U/L) at -14 before calving in cow fed LE and lowest ALT concentrations (12.45 U/L) at d -7 relative to calving in cows fed high energy diet. In postpartum cows the lowest ALT concentration (9.7 U/L) was found in the first week after calving in cows fed high energy diet alone.

The highest AST concentration was observed in cows fed pre-calving HE diet alone in the first week after calving. Compared to cows fed low energy diet together with RPL, cows fed high energy diet with RPL significantly decreased ALT concentration by 6.4% and AST concentration by 19% during the pre and postpartum periods respectively. Cow fed high energy diet with RPL significantly increased AST concentrations by 28.4% in postpartum cows compared to cows fed high energy diet with RPL. Concentration of plasma ALT concentration tended to affect by the interaction between time and RPL (*P = 0.09*) in prepartum cows not in postpartum cows.

Cows fed HE diet and supplemented with rumen-protected lysine numerically decreased prepartum plasma AST concentration by 0.13%. Cow supplemented by RPL numerically lower in AST concentrations than cows did not supplemented with RPL during the pre and postpartum periods. Pre and postpartum plasma TG concentration exhibited no difference among dietary treatment groups. Plasma TG concentrations numerically decreased during the pre and post partum periods in cow fed close-up high energy diet. RPL supplementation numerical decreased plasma TG concentrations during the pre and postpartum periods. Time and energy interaction significantly affected TC concentration in pre and postpartum cows (*P = 0.001*). Cows fed high energy diet had lowest TC concentration at d -21 before calving.

Discussion

Plasma glucose and insulin concentrations

Dietary ration that leads to low blood glucose and insulin concentrations contribute to fatty liver because of insulin suppresses fat mobilization from adipose tissue. Insulin is the major homeostatic hormone and its functions primarily to stimulate lipogenesis and glucose utilization to inhibit lipoysis in dairy cattle. In the present study plasma glucose concentrations did not affect by pre-partum energy density but it tended to improve glucose concentrations in pre-partum cows. In contrast to this study, close-up energy density affected blood glucose concentration during early lactation (Zhang et al., 2015). In our results, glucose concentration

slightly increased at d -14 relative to calving before reached peak level at calving. Similarly, plasma glucose level rapidly increased in the first 2 weeks before calving and reached peak level at d 0 (calving) and then gradually decreased (Wang et al., 2017).

We found that cows fed high energy diet together with rumen-protected lysine did not differ in pre and postpartum plasma glucose concentrations. In previous study, dry period energy density did not affect glucose concentrations in pre and postpartum cows (Nowak et al., 2013). But our data revealed that interaction between week and energy significantly influenced plasma glucose levels during the transition period. Cows in high energy (**HE**) diet had higher plasma glucose concentration at d -14 before calving whereas cows in low energy (**LE**) diet had the lower plasma glucose concentration at d -7 before calving. This result indicated that close-up energy diet had a carryover effect to influence plasma glucose concentrations during the transition period. Cows fed HE diet had highest plasma glucose concentration at calving than late pregnancy cows. This implies that feeding pre-calving high energy diet lower mobilization of fat from adipose tissue at calving compared to cows fed with pre-partum low energy diet.

Similar to our results, previous research indicated that cows in high energy diet (1.55Mcal/kg) had higher blood insulin and glucose concentration at parturition compared to cows in low energy (1.25Mcal/kg) diet (Zhang et al., 2015). Cows fed high energy diet may be produce more gluconegenic precursors particularly propionate for hepatic glucose production and increased glucose concentration in the blood stream. High energy intake increased glucose and insulin concentrations (Holtenius et al., 2003; Douglas et al., 2006).

Plasma insulin concentration peaks at parturition, but is maintained at lower concentrations postpartum than prepartum (Grum et al., 1996). Low insulin concentration causes lipolysis (fat breakdown) in adipose tissue through stimulating the sympathetic nervous system (Drackley, 2010). Decreased insulin concentration and increased growth hormone levels during the transition period leads to increase adipose tissue lipolysis and free fatty acids concentrations in blood (Drackley, 1999). In the present study, cows fed close-up high energy diet had higher insulin concentration in blood plasma during the transition period. In contrast to this, cows fed high energy and medium energy rations had low insulin concentration at d 1 after calving compared to cows fed low energy diet (Zhang et al., 2015).

Insulin concentrations steadily increased in cow fed low energy diet in the first 3 weeks after calving in our study. Lipid metabolism and breaking down of triglycerides depend on insulin regulation. Insulin stimulates lipogenesis and inhibits lipolysis in adipose tissue (Herdt, 2000). Both lipolysis and lipogenesis create a cycle to move fatty acids from triglycerides to NEFA or from NEFA to triglycerides. Insulin secrete under ketone bodies and NEFA stimulation to create feedback loop to suppress additional mobilization of NEFA (Herdt, 2000).

Plasma total cholesterols and triglyceride concentrations

In transition cows glucose, lipid and cholesterol metabolism have to adapt to the lactating state (Kessler et al., 2014). Excessive intracellular triglyceride accumulation in liver cells results in disturbed normal liver function and leads to cell damage. Plasma cholesterylesters and triglyceride concentrations increased in cows with fatty liver syndrome (Ametaj et al., 2005). In our results, plasma cholesterols levels were varied across the transition period and TG concentration was more pronounced during the prepartum period than postpartum

period. This indicate that transition cow fed low pre-partum energy diet put dairy cows in more negative energy balance during preparum period than postpartum period.

A higher cholesterol level was observed at d -14 before calving in cows fed high energy diet. But it gradually decreased towards parturition and then steadily increased in early lactation and reached peak level at d 21 after calving. Low amount of cholesterol and phospholipid indicate the cows had fatty liver (BAŞOĞLU et al., 1998; SEVİNÇ et al, 1998). Despite of cholesterol synthesis machinery increased at the onset of lactation total cholesterol concentration and individual lipoprotein- associated cholesterol fractions in the plasma were dramatically decreased (Kessler et al., 2014). Plasma triglyceride (TG) and cholesterol concentrations decreased from week 3 before calving to 1 week after calving and then gradually increased until week 14 postpartum (Kessler et al., 2014). During negative energy balance excess NEFA in the liver reesterified to TG and very low density lipoproteins are the most important carriers of TG in blood (Bauchart, 1993). In the current study, there was a decreased trend in TG concentration during the post partum period in cows prepartum fed high energy diet which was in agreement with Van den Top et al. (2005) who reported TG concentration in plasma was distinctly decreased after calving. In our results, cow in low energy diet showed a trend of increased TG concentrations during the postpartum period. Similarly, TG concentration increased steadily from 1 week to week 14 after calving (Kessler et al., 2014). In the case of cows fed low energy diet the secretion of very low density lipoprotein (VLDL) from the liver may be limited in early lactation and the excess TG accumulate in the liver leading to fatty liver as reported and confirmed by Gross et al. (2013). Loor et al. (2007) Hypothesized that cholesterol might be the limiting parameters of VLDL synthesis, similar to this hypothesis the low cholesterol levels in cows fed low energy diet in our results may be decreased in VLDL synthesis and increased TG concentration in postpartum cows. Moreover the limited ability of ruminant to export TG as VLDL may contributes and leads the accumulation of TG in the liver (Kleppe et al., 1988; Pullen et al, 1990). Since phospholipids involved for secretion of liver triglycerides as VLDL decreased level of phospholipids may resulted in excessive accumulation of triglycerides in the liver (Artegoitia et al, 2014). This excessive accumulation of TG in the liver impaired proper liver function (Murondoti et al., 2004). The steadily increased TG concentration from 1 week p.p. to week 3 post partum periods in our results may be impaired normal liver function.

Albumin, blood urea nitrogen (BUN) and total bilirubin (TBIL) concentrations

Albumin is totally synthesized in liver and classified as negative acute phase protein implies that hepatic production commonly decreased during the onset of inflammation (Bertoni et al., 2008)

Albumin contains about 60% of measured serum protein and the most abundant plasma protein. In sever hepatic change the plasma albumin concentration significantly reduces (West, 1990). In our results, cows in high energy diet significantly increased preparum plasma albumin concentration. But there was a substantial decrease in albumin concentration in cow fed prepartum low energy at d 14 after calving which is consistent with those animals experienced more pronounced inflammatory conditions (Osorio et al., 2014). The concentration of plasma negative acute phase protein such as albumin and total cholesterol in plasma have been used to classify liver function into upper, upper-intermediate, lower-intermediate and lower quartiles (Bionaz et al., 2007; Osorio et al., 2014). Cows in the upper postpartum liver function quartile have albumin concentrations ranging between 33 and 35 g/L (Bionaz et al., 2007; Osorio et al., 2014). In our study albumin

concentrations was above 35 g/L throughout the experiment period in all cows fed different dietary treatment rations. In moderate and sever fatty liver condition, the serum bile concentration was significantly higher and albumin concentration was significantly reduced than normal cows (Sevinc et al, 2001). Our data indicated that plasma albumin concentrations increased at d -21 and d -14 and decreased at d -7 in cow fed high energy diet as dairy cows approach to parturition. Albumin concentration lower in prepartal cows compared to postpartum cows which was similar (Osorio et al., 2014) who reported lower albumin concentration in cow did not Met-supplemented. Urea is an end product of protein metabolism, turns into ammonia primarily in the liver. Feeding dietary protein beyond the level needed by the microbes it resulted in conversion of ammonia into urea in the liver and excreted in the urine because of excess ammonia is toxic to the animal while urea is not. Feeding more energy will increase the nitrogen requirement by the microbes and promote the use of excess ammonia (Van Soest, 1994. In the present study, cows both in low and high energy diet significantly increased blood urea nitrogen (BUN) concentration at d -21 and -14 before calving. BUN concentration of cows in high energy diet significantly lowered as compared to cow in low energy diet during the prepartum period. During postpartum period cows in high energy diet steadily decreased in blood urea concentration in the first two weeks after calving and elevated at d 21 after calving.

A cow with higher inflammation indices is indirectly confirmed by bilirubin concentrations (Bertoni et al., 2008). In fact the greater value of bilirubin is a consequence of the reduction of bilirubin clearance which is a usual metabolic function of liver. In this trial, cows fed low energy diet during the prepartum period significantly increased bilirubin concentrations after calving. The plasma total bilirubin concentration is an important indicator of liver function that is increased during severe lipidosis (Bossaert et al., 2012; Sejersen, Sørensen et al, 2012). Plasma bilirubin and bile acids concentrations increased in cows with fatty liver syndrome (Ametaj et al., 2005). In the present study, cows in high energy diet significantly decreased in total bilirubin (TBIL) concentration around calving. A decreased in the plasma TBIL concentration in this experiment may be indicate a healthy liver status and improvement of hepatic function in transition cows receiving high energy diet. The reduction in the TBIL level could be a result of accelerated NEFA β-oxidation and TG transport. Cow in low energy diet had higher TBIL concentration at d -21 and d -14 and low TBIL levels at d -7 before calving. Not as cow in high energy diet the TBIL concentrations in cows fed low energy diet steadily increased from calving to the first two weeks after calving then drop at d 21 after calving. We found the highest TBIL concentrations at calving in cows fed low energy diet during the prepartum period. The increment in total and indirect bilirubin may be related to fat infiltration of the liver (Sevinc et al, 2002). Previous study indicated that cows with abnormal displacement had mostly an increase in total bilirubin concentration (Gul and Grunder, 1990). The BUN level is typically used to estimate nitrogen excretion and nitrogen utilization efficiency in animals (Kohn et al., 2005).

The type and the degree of liver cells damage may be investigate by measuring the concentration of AST and ALT in blood plasma of dairy cows (Kew, 2000). ALT is the most sensitive target in the diagnosis of acute liver damage but AST is more sensitive in reflecting the degree of damage (Kew, 2000). In our study, the concentration of ALT in cow fed RPL significantly varied during the prepartum period. In healthy cattle the normal activity of AST in the blood is 78 -132U/L (Ingvartsen, 2006). In the present study the AST concentration was within the normal range (32 -102U/L) in all dietary treatment groups. However, the highest AST concentration (102 U/L) was found at d 7 p. p. in cows did not supplement with RPL. The normal range

of ALT concentration was 11-40 U/L (Ingvartsen, 2006). But in our result the ALT concentration ranged from 9 to 16 U/L. Higher ALT concentrations was found in late pregancy cows than postpartum cows. Plasma ALT concentration steadily increased in postpartum cows from d 0 to d 14 then it declined at d 21 after calving. The lowest ALT concentration was observed at d -21ante partum in cows supplemented with RPL. But in postpartum cows ALT concentration was gradually decreased from d 0 to d 14 then it increased at d 21 P.P. Increased ALT activity can be associated with liver damage, mostly caused by negative energy balance during early lactation. But increasing AST concentration in blood serum could indicate a growing intensity of metabolic changes, mainly of proteins, in fourth (<250 days *postpartum*) lactation phases (Sakowski et al., 2012). Cows with severe fatty liver had higher levels of AST (Sevinc et al., 2001).

Insulin and IGF-1 and concentrations

Lower concentration of IGF-1 during late pregnancy may increase growth hormone secretions and lipolysis, thereby increasing the risk of ketosis (Sevinc et al., 2001). Insulin and IGF-1 concentrations were highest in the far-off period and decreased during early lactation.

In present study we found that cow fed low energy diet significantly increased post partum IGF-1concentration comapred to cows fed high energy diet.The liver is the tissue where IGF-I is primarily synthesized under the control of growth hormone produced by the adenohypophysis (Wathes et al., 2006). IGF-I production will decrease, influencing glucose uptake and normal insulin sensitivity (Wathes et al., 2006). Insulin and IGF-I are metabolic hormones having a multi functional role on the tissue response (Wathes et al., 2006). In our results we observed the highest IGF-1concentration in cows fed low energy diet in the first two week after calving and the lowest IGF-1concentration at calving in cows fed high energy diet. Feeding prepartum high energy diet favouring dairy cows by lower postpartum IGF-1concentration which had postive effect to reduce growth hormone secrtions and lipolysis. IGF-1 is known to be the main mediator of growth hormone (GH) on milk production, regulating milk synthesis by the mammary gland (Alberts et al., 2002).

Conclusion

Feeding close-up high energy diet tended to improve glucose levels, significantly increased insulin and albumin concentrations, significantly decreased blood urea nitrogen and total bilirubin concentrations during the prepartum period and significantly decreased IGF-1 and TG concentration during the pre and post partum periods. Interaction between time and pre-calving energy diet significantly impacted glucose, insulin, albumin, blood urea nitrogen concentrations in prepartum cows and significantly affected TBIL, ALT and AST concentrations in pre and postpartum cows. Interaction between pre-calving high energy diet and RPL tended to increase prepartum plasma albumin concentrations, numerically increased glucose, decreased TG, total bilirubin and AST concentrations during the pre and postpartum period. RPL supplementation numerically decreased plasma prepartum IGF-1concentrations but increased plasma IGF-1concentration in post partum cows. Collectively, the result indicated that feeding close-up high energy diet with rumen protected lysine substantial favoring liver function status during the transition period in dairy cows.

Declarations

Author Contributions: Conceptualization, G.D.D., D.P.B. and L.M.; methodology, D.P.B., G.D.D. and L.M; software, D.P.B.; validation, D.P.B., G.D.D. and F.W.; formal analysis, G.D.D., L.M., and F.W., D.P.B., G.D.D., and L.M. resources, D.P.B. and L.M.; data curation, G.D.D. and D.P.B.; writing—original draft preparation, G.D.D.; writing—review and editing, D.P.B., T.C. and L.M; visualization, G.D.D., D.P.B and L.M.; supervision, D.P.B.; project administration, D.P.B.; funding acquisition, D.P.B.

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Statement of Animal Rights

This study was approved by the Animal Care and Use Committee of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (No. IAS20180115, Beijing). Use of animals in the present study was in strict accordance with the Directions for Caring for Experimental Animals from the Institute of Animal Science, Chinese Academy of Agricultural Sciences.

Conflict of Interest Statement: The authors declare no conflict of interest.

Data availability

The data and materials of this study will be available on request.

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Figures

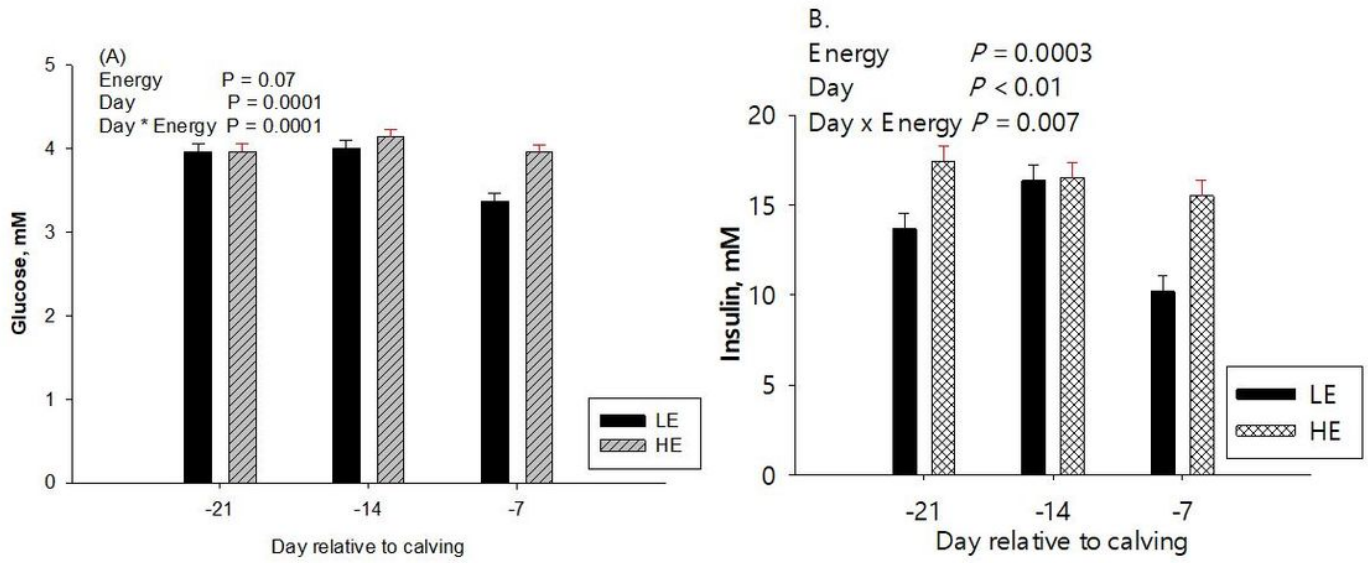

Figure 1

Effect of prepartum energy density and supplementing rumen-protected lysine during the transition period glucose, and insulin in dairy cows. HE = high energy, and LE = low energy

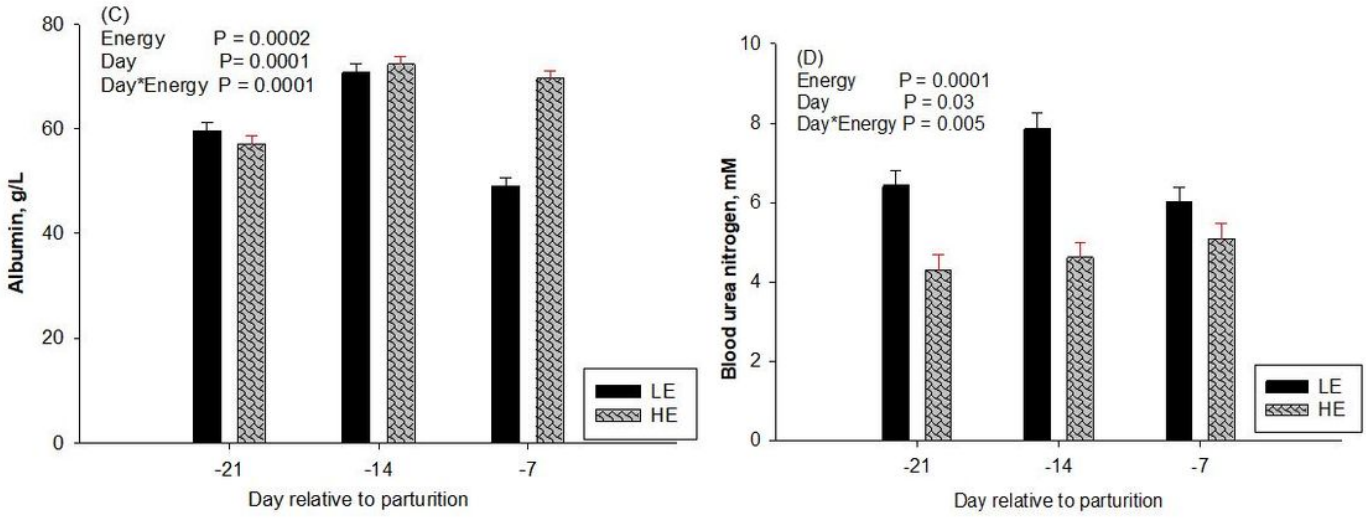

Figure 2

Effect of prepartum energy density and supplementing rumen-protected lysine during the transition period Albumin, and blood urea nitrogen in dairy cows. HE = high energy, and LE = low energy

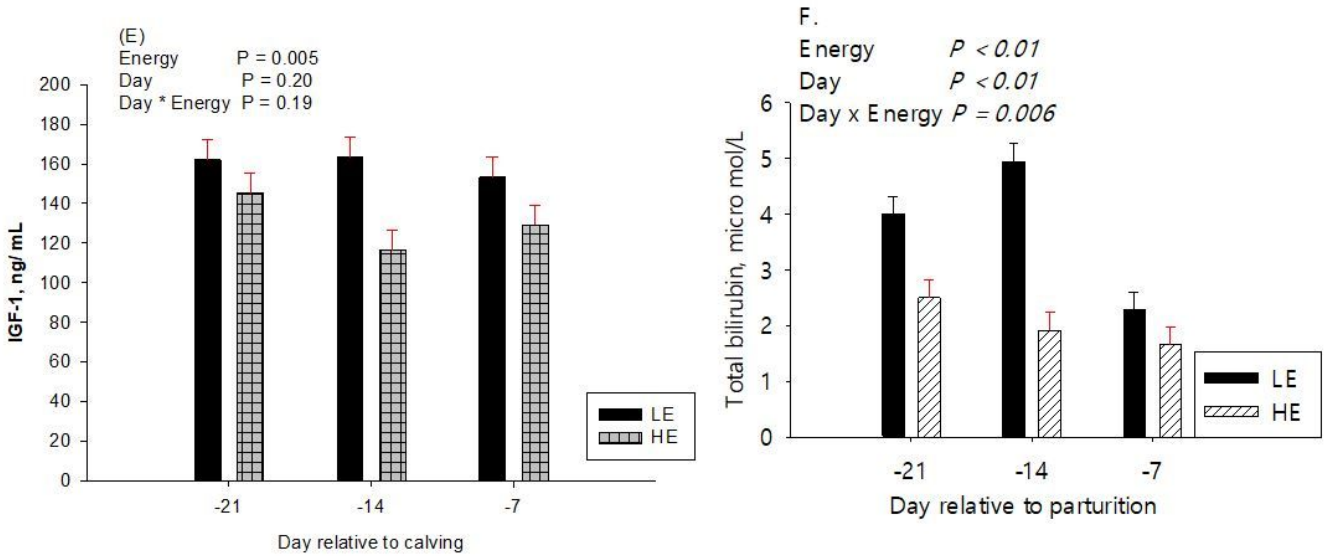

Figure 3

Effect of prepartum energy density and supplementing rumen-protected lysine during the transition period IGF-1 and total bilirubin in dairy cows. HE = high energy, and LE = low energy