Regulation of Nutritional Metabolism in Different Perinatal Period of Does: Energy Metabolism Efficiency, Lipid Metabolism, Oxidative Stress and Liver Metabolomics Profile in Response to Nicotinamide Supplementation

Xiaoshi Wei

Zhejiang Agriculture and Forestry University: Zhejiang A and F University

Qingyan Yin

Northwest A&F University: Northwest Agriculture and Forestry University

Huihui Zhao

Northwest A&F University: Northwest Agriculture and Forestry University

Jiajun He

Northwest A&F University: Northwest Agriculture and Forestry University

Yangchun Cao

Northwest Agriculture and Forestry University

Junhu Yao (✉ yaojunhu2004@sohu.com)

Northwest Agriculture and Forestry University <https://orcid.org/0000-0001-6318-6576>

Research

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Abstract

Background: Improving energy efficiency is urgent during perinatal period because dairy ruminant is often in negative energy balance, resulting many metabolic disorders thus economic losses. It has been previously highlighted that nicotinamide (NAM) supplementation during perinatal period could benefit the energy balance, while the underlying metabolic alterations and the differences in NAM supplemented during different perinatal period have been poorly studied. This study aimed to elucidate the effects of NAM on energy efficiency, lipid metabolism, oxidative status, and liver metabolites profile of perinatal does during different perinatal period.

Results: Fifteen multiparous does with similar parity, weight, and previous milk yield were paired and allocated to 3 groups (n = 5): control (C), postpartum supplementation (P, NAM supplemented after kidding), and entire-perinatal supplementation (EP, NAM supplemented from -21 to 28 d around kidding). Does were drenched with 5 g/d of NAM and slaughtered on d 28. The ruminal acetic acid tended to decrease, and the propionic acid and valeric acid were increased in P and EP. The does in P and EP had a decreased acetic acid to propionic acid ratio. Does in EP had higher liver mitochondrial respiratory chain complexes. The Complex I was elevated in both P and EP and NAM supplementation tended to increase ATP production. Liver metabolomics analysis and tissues biochemical analysis revealed that lipid was decomposed in abdominal adipose, and in liver, the decomposition of lipid was more than the synthesis, and the amino acid metabolism were enriched by NAM. Moreover, the oxidative status of blood and liver were improved by NAM supplementation, and supplementation of NAM during postpartum only does not appear enough to cope with the metabolites of oxidative phosphorylation.

Conclusions: Overall, these data suggested that NAM supplementation during perinatal period favored the rumen fermentation pattern to propionic acid-type, and improved liver energy efficiency with benefited lipid metabolism. The NAM supplemented from prepartum is needed for better oxidative status and energy metabolism of perinatal does.

Background

The dairy ruminant in perinatal period suffers significant metabolic challenges, with a 4-fold increase in energy requirement. However, the dry matter intake of the animal is limited. As the acetic acid and propionic acid were essential precursors for the synthesis of milk fat and glucose, respectively, the efficient rumen fermentation of the feed is important. Lipolysis occurs as a response to the negative energy balance (NEB) while leaving elevated nonesterified fatty acid (NEFA), which could induce lipid peroxidation and oxidative stress in hepatocytes[1]. Moreover, continuous hypermetabolic reaction results in augmented production of reactive oxygen species (ROS), leading to oxidative stress[2]. Many metabolic disorders occur in perinatal period such as lipidosis, mastitis, also infertility[3]. Thus, nutritional strategies to improve energy efficiency and alleviate oxidative status are urgently required for the health of perinatal dairy ruminant.

Nicotinamide (NAM) is the amide form of vitamin B3 and required for the synthesis of NAD and NADP, participating in many redox reactions. Our previous studies have shown that supplementing both NAM and biotin to transition dairy cows improved the energy balance, mainly through regulating glucose and lipid metabolism thus increasing the ATP production[4, 5]. Mitchell et al.[6] has proven that NAM could improve glucose metabolism by increasing glycogen deposition and flux through the pentose phosphate and glycolytic pathways. Moreover, the NAM was also proven to alleviate oxidative stress through decreasing NEFA, and β -hydroxybutyric acid, ROS, and improving glutathione metabolism in transition dairy cows[4, 5]. The improved energy balance and health status then benefitted the offspring kids on small intestine morphology and function, and the hepatic glucose and lipid metabolism[7, 8]. However, there are still underlying regulatory mechanisms of NAM supplementation during the perinatal period. Since blood lipids are result of lipid absorption, lipid synthesis and lipid utilization rates, additional measures on dynamic lipid metabolism is needed to determine the mechanism of NAM action. In addition, whether the NAM supplemented during different perinatal period could affect the energy metabolism is still unknown.

Based on this, we hypothesized that NAM supplementation during different perinatal period could accelerate energy generation from nutrients oxidation and lipid metabolism accompanied with improved oxidative status. Liver is an important metabolic organ and plays important roles in energy metabolism, in which the energy production accounted for about 20%

energy utilized by dairy ruminant[9]. Therefore, in this study the perinatal does were used as experimental object, and the objectives of this study were not only to evaluate the effects of NAM supplemented during different perinatal period on energy efficiency, lipid metabolism, oxidative status, but to profile the changes in liver metabolites associated with NAM using metabolomics approach.

Materials And Methods

This study was conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Northwest A&F University, Shaanxi, China, and was part of a larger study.

Experimental design

The experimental design was detailed described in Wei et al.[7] Briefly, fifteen Guanzhong dairy does with similar parity, weight, expected kidding date and previous milk yield were paired and randomly assigned within block to 3 groups. The 3 groups were: control (**C**, no supplementation), postpartum supplementation (**P**, NAM supplemented after kidding), and entire-perinatal supplementation (**EP**, NAM supplemented from -21 to 28 d around kidding). The 5 g of NAM was drenched to does daily at 0700. Does were housed in individual tie stalls and fed the same basal diets as total mixed ration (Table S-1). The experiment was conducted from 21 days before expected kidding date to 28 days postpartum. After kidding, does were milked twice daily.

Rumen fluid, tissues and blood collection

On the 28 days after kidding, the does were slaughtered. Ruminal fluid about 100 mL from individual doe was obtained. The pH was immediately measured and then rumen fluid was strained through 2-layer cheesecloth. The 4 mL of filtrate was mixed with 1 mL of 25% (wt/vol) metaphosphoric acid for the analysis of volatile fatty acid (**VFA**). The method we used was referred to former methodology in our lab[10].

Liver and abdominal adipose tissues from each individual were removed within 20 min postmortem, and snap frozen in liquid nitrogen. Blood was collected about 3 h after morning feeding from coccygeal vein once a week (i.e. d -14 (± 1), -7 (± 1) relative to expected kidding day and d 1 (within 24 h), 7, 14, 21, and 28 after kidding). Blood was centrifuged at 3500 g and 4°C for 15 min to obtain serum then stored at -80°C.

Liver mitochondrial respiratory chain status

Quantification of NAD and NADH were carried out using NAD/NADH assay kit from mlbio® (catalog no. ml341820-2 and ml341863-2, Shanghai, China), following the manufacturer's instructions. Livers were extracted at 4°C with extraction buffer provided by the kit. Complex I activity was measured by a modification of the Wallace method, and Complex II/III and complex IV activities were determined by measuring the reduction of cytochrome C or the oxidation of ferricytochrome C at 550-540 nm, respectively, as previously described[11]. The ATP was measured using commercial kit (catalog no. HY-L0049) purchased from Beijing Sino-UK Institute of Biological Technology (Beijing, China).

Lipid metabolism parameters

The TG, NEFA, total cholesterol (**TC**), very low-density lipoprotein (**VLDL**), glycerin, and pyruvate kinase (**PK**) contents were measured using reagent kits purchased from Jiancheng Bioengineering Institute (Nanjing, China) following the instructions from the manufacturer. The activities of glutamyl transaminase (**GPT**) were analyzed using an auto analyzer at Northwest A&F University (Shaanxi, China). The phosphoglycerol transferase 6 (**AGPAT6**), diacylglycerol transferase 2 (**DGAT2**), adipose-triglyceride-lipase (**ATGL**), hormone-sensitive lipase (**HSL**) activities were measured using ELISA method (goat catalog no. ml7089092, ml7098192, ml7091187, and ml7099870, respectively, mlbio®, Shanghai, China). The values of the liver and abdominal adipose tissues were normalized by the protein concentration.

Oxidative status measurements

The blood activities of glutathione peroxidase (**GSH-PX**), superoxide dismutase (**SOD**) and catalase (**CAT**), the hydrogen peroxide (**H₂O₂**) and malondialdehyde (**MDA**) concentrations, and total antioxidant capacity (**T-AOC**) were measured using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's instructions. Moreover, the SOD, T-AOC, CAT and MDA values of liver were normalized by protein concentration.

Liver metabolomics analyses

About 50 ± 1 mg of liver sample was taken into a 2 mL EP tubes, added with 450 μ L extraction liquid ($V_{\text{Methanol}}: V_{\text{Chloroform}} = 3:1$) and 10 μ L of L-2-Chlorophenylalanine (1 mg/mL, stock in dH₂O, serving as internal standard), vortexed, homogenized in ball mill at 45 Hz for 4 min, and then ultrasound treated for 5 min (incubated in ice water). The solution was centrifuged at 12000 rpm, 4°C for 15 min, and a total of 350 μ L of the supernatant were transferred into fresh 1.5 mL EP tubes and dried in the vacuum concentrator. The dried residue was added with 50 μ L methoxy amination hydrochloride (20 mg/mL in pyridine), mixed and incubated at 80°C for 30 min. After that, 70 μ L of the BSTFA reagent (1% TMCS, v/v) was added to the sample aliquots and incubated at 70°C for 1.5h. All samples were analyzed by gas chromatograph system coupled with a Pegasus HT time-of-flight mass spectrometer (**GC-TOF-MS**)[4].

Statistical analysis

Data were analyzed using the MIXED procedure of SAS. The REPEATED procedure was used for variables of blood, and the linear model was used for rumen and tissues parameters. The difference was considered significant at $P \leq 0.05$ and as a trend at $0.05 < P \leq 0.10$. Moreover, the methods used to statistically analyze the liver metabolomics profile were followed the procedures we reported in Wei et al.[4] And in this study, the comparisons were made between every 2 groups, P vs. C, EP vs. C and EP vs. P, and named $C_{P:C}$, $C_{EP:C}$, $C_{EP:P}$, respectively. The significant thresholds were variable importance in projection (**VIP**) > 1 , and $P < 0.05$ (Student's t-test).

Results

Rumen fermentation parameters

In this study, compared to C, the ruminal propionic acid (expressed as percentage of total VFA) was increased in P and EP, and the propionic acid in P was higher than that in EP ($P < 0.001$, Table 1). The ruminal acetic acid tended to decrease ($P = 0.069$). The valeric acid was increased in P and EP ($P = 0.018$). Moreover, does in P and EP had a decreased acetic acid to propionic acid ratio ($P = 0.001$).

Mitochondrial respiratory chain status

The liver NADH concentration was elevated in EP as compared with P and C ($P = 0.001$, Table 2). The NAD⁺ or NAD⁺/NADH ratio were not affected in this study ($P > 0.05$). The mitochondrial complex I and II were increased in EP as compared with P and C ($P = 0.001$), and complex III was higher in P and EP ($P = 0.036$). Compared to C, the complex III was increased in EP ($P = 0.046$). The production of ATP tended to increase ($P = 0.069$).

Parameters related to lipid metabolism in abdominal adipose

The TG content was lower in EP compared to P and C ($P = 0.001$, Table 3), and the NEFA content was decreased in both P and EP ($P = 0.006$). Compared to C, the TC content was decreased in P and EP, and the TC in EP was lower than that in P ($P = 0.002$). The enzyme activities of DGAT2 tended to decrease and the ATGL tended to increase by NAM supplementation ($P = 0.072$ and 0.063 , respectively). The activities of AGPAT6 and HSL were not affected ($P > 0.10$).

Hepatic parameters and enzyme activities

In liver, the TG content tended to decrease and the VLDL tended to increase by NAM supplementation ($P = 0.079$ and 0.083 , respectively, Table 4). The NEFA content was not affected ($P > 0.10$). Compared to C, the glycerin was decreased in P and EP ($P = 0.010$). The GPT was elevated in P as compared with EP and C ($P = 0.009$). The enzyme activity of ATGL was increased in both P and EP ($P = 0.037$), and the PK and AGPAT6 activities tended to increase by NAM supplementation ($P = 0.087$ and 0.091 , respectively). The activities of DGAT2 and HSL were not affected ($P > 0.10$).

Blood parameters and oxidative status of blood and liver

The blood TG and NEFA tended to decrease by NAM supplementation ($P = 0.078$ and 0.099 , respectively, Table 5), without the week $\times$ NAM effect. Serum TC was not affected by NAM, nor the week $\times$ NAM effect ($P > 0.005$).

Does in P had higher serum SOD compared to C ($P = 0.035$, Table 5), and a week $\times$ NAM effect was observed ($P = 0.017$). The CAT and CAT/SOD were increased in EP as compared with P and C ($P < 0.001$), and tendencies existed for week $\times$ NAM effects ($P = 0.062$ and 0.057 , respectively). Tendencies of increased GSH-PX and GSH-PX/SOD were present ($P = 0.067$ and 0.061 , respectively) with no week $\times$ NAM effects. Serum H_2O_2 and MDA concentrations were not affected by NAM, and the week $\times$ NAM effect on H_2O_2 was found ($P = 0.007$). Time effects were noted for all blood parameters ($P < 0.001$). Liver SOD was increased in both P and EP ($P = 0.034$, Figure 1), and T-AOC was increased in EP compared to C ($P = 0.031$).

Liver metabolomics profile

The results of regulated metabolites in $C_{P:C}$, $C_{EP:C}$, and $C_{EP:P}$ were shown in Table 6. The diglycerol and cystine concentrations were decreased in $C_{P:C}$ ($P < 0.05$). The concentrations of sulfuric acid, malonic acid, 3-methylamino-1,2-propanediol, D-glyceric acid, ciliatine, hypoxanthine, palmitoleic acid and guanosine were found decreased in $C_{EP:C}$ ($P < 0.05$). And the uracil concentration was higher in $C_{EP:C}$ ($P < 0.05$). The liver 3-aminoisobutyric acid, cytidine-monophosphate and prostaglandin were decreased in both $C_{P:C}$ and $C_{EP:C}$ ($P < 0.05$). In addition, we found that the malonamide was increased in $C_{P:C}$, while lower in $C_{EP:P}$ ($P < 0.05$). The adenine tended to decrease in $C_{P:C}$, while was higher in $C_{EP:P}$ ($P = 0.073$ and 0.036 , respectively). The octadecanol tended to increase in $C_{P:C}$ and was higher in $C_{EP:C}$ ($P = 0.096$ and 0.030 , respectively).

The enriched pathways of altered metabolites were presented in metabolome view map ($P < 0.05$, Figure 2). There were no enriched pathways in $C_{EP:P}$ ($P > 0.05$). The pyrimidine metabolism was enriched in both $C_{P:C}$ and $C_{EP:C}$. The cysteine and methionine metabolism was enriched in $C_{P:C}$. And the $C_{EP:C}$ had additional enriched pathway of purine metabolism, sulfur metabolism, pantothenate and CoA biosynthesis, glyoxylate and dicarboxylate metabolism, beta-alanine metabolism, glycerolipid metabolism, and glycine, serine and threonine metabolism.

Discussion

During perinatal period, about 70% glucose was provided from hepatic gluconeogenesis[12]. Our previous studies have shown that NAM supplementation would relieve NEB[5] through improving glucose circulation[4]. The propionic acid is the main source of liver gluconeogenic substrate, and the ruminal propionic acid ratio was increased with NAM supplementation, suggesting the improved gluconeogenesis might be due to increased gluconeogenic substrate. This was in accordance with the result in Khan et al.[13] The increased propionic acid could be due to the altered NAD/NADH ratio in microbes, as NAM is a precursor of NAD. Another reason would be that niacin increased rumen protozoa numbers[14], which was shown to contribute to higher ruminal propionic acid level[15]. For ruminant, the acetic acid is the main precursor for *de novo* synthesis of fatty acid in mammary gland, and provides the majority of the carbon. The milk fat yield was increased by 20% when the acetate was ruminal infused to lactating dairy cows, and the *de novo* synthesized fatty acids and palmitic acid were also increased in milk[16]. In this study, however, the milk fat was not affected by the acetic acid that tended to be decreased[8]. The altered rumen fermentation pattern, tended to propionic acid-type, suggested that the NAM supplementation contributed to the effective fermentation of feed in the rumen and produced more energy metabolism substrates, favoring the NAM supplementation in perinatal period.

The mitochondrion plays vital role in energy generation from nutrients oxidation, in which the electrons were transferred originating from metabolites to NAD[17]. As a precursor of NAD, NAM supplementation should increase NAD⁺ level and subsequently increase its redox, i.e., NAD⁺/NADH ratio. However, we did not find increased liver NAD⁺ or NAD⁺/NADH ratio, while elevated NADH concentrations in does supplemented NAM during entire-perinatal period. We presumed that NAM became NAD⁺ when supplemented to does, rapidly being driven force to participate in the increased nutrients oxidation for the urgent energy requirement. Moreover, NAM supplementation provided a larger pool of pyridine nucleotides and the NADH pool may eventually get larger via the supply of NAD⁺[18]. The enriched pyridine metabolism found in both C_{P:C} and C_{EP:C} agreed with this discussion. Liver is an important metabolic organ and the mitochondrion has been referred as a power plant while passing electrons to produce ATP. The mitochondrial complex I catalyzes NADH oxidation to regenerate NAD⁺. It is a key enzyme in cellular energy metabolism and provides approximately 40% of the proton-motive force that is utilized during mitochondrial ATP production[19]. The complex I, II, and III are essential in the following reaction of mitochondrial respiratory chain, which were also increased in this study. The production of ATP would be depressed when the expression of complex I, II, and III decreased[20]. The NAM supplementation caused an elevation in both mitochondrial respiration and ATP production, also supporting the results of improved NEB by NAM in our previous report[5].

However, the generation of ATP is usually accompanied by ROS production, which is the byproducts of oxidative phosphorylation[21]. A good balance between energy level and redox status is required to ensure optimal health during the perinatal period. Measuring enzymatic antioxidants (CAT, GSH-PX and SOD) together with MDA values could give more accurate information on oxidative stress level, rather than using such data separately. As a result of NAM supplementation, the serum antioxidant system (SOD, CAT, and GSH-PX activity) of antioxidant barrier in animals was enhanced, which was in accordance with the report of John et al.[22] The SOD reduces the superoxide radical into H₂O₂, which is the substrate then used by CAT to produce H₂O. When the imbalance between the CAT and SOD, the overexpression of SOD without a proportional compensatory increase in CAT, existed, it would cause a peroxide overload challenge and be deleterious upon the cell[23]. The does supplemented with NAM during entire-perinatal period had an increase in serum CAT/SOD ratio, indicating the ability of blood to cope with oxidative stress. The ROS production was elevated from a week prior kidding, so the NAM supplementation during postpartum was not effective to clear the H₂O₂. Moreover, in order to better understand the antioxidative protection, the ratio of GSH-PX to SOD was considered as its increase indicated an activation of the antioxidant enzyme to defense against ROS, while a decrease may be indicative of a lower scavenging efficiency then the oxidative damage may occur[24]. These alterations manifested as an enhancement in antioxidant capacity by NAM supplementation, further indicating the production and breakdown of ROS remained in homeostasis in does. And the NAM supplementation starting from prepartum was better for the does.

During the perinatal period, the body fat mobilization occurred in response to NEB. The AGPAT6 and DGAT2 are important enzymes in TG synthesis process, and ATGL and HSL are the key enzymes for TG decomposition[25, 26]. The enhanced ATGL was shown to reduce the fat content of the dorsal longest muscle in cattle steers[27]. In this study, with the supplementation of NAM, the synthesis of TG in abdominal adipose was decreased and the decomposition was increased, leaving TG, NEFA and TC in abdominal adipose were significantly reduced in this study. This result indicated that NAM supplementation during entire perinatal period helped the fat metabolization to provide large amount of NEFA into liver for metabolic energy supply. However, the accumulation of BHBA (incomplete oxidation of NEFA) and TG (re-esterification of NEFA) usually caused fatty liver, ketosis and oxidative stress[28, 29]. It is worth noting that the decomposition of TG did not cause an increase, while a decrease in tendency, in the content of TG and NEFA in blood and liver. This would be due to the increased enzyme activity of ATGL and the VLDL concentration in the liver, thus the TG was decomposed to utilize or delivered. The differences in the effects of NAM on abdominal adipose and liver suggested the tissue specific of NAM during perinatal period. In study of Zachut et al.[30], the occurrence of insulin resistance in dairy cows during the perinatal period is tissue-specific, and there was no insulin resistance in liver tissue, while existed in fat tissue. We speculated that the tissue specific of NAM could be related to the tissue-specific of insulin resistance during perinatal period. A summary of the principal findings regarding the lipid metabolism of the abdominal adipose and liver was presented in Fig. 3. The interesting finding in this study was that the VLDL concentration tended to elevate in the liver. Our group previously found that the choline and methionine promoted liver fatty

acid oxidation and VLDL synthesis, and reduced liver TG accumulation in dairy cows during the perinatal period[31]. As we know, the choline and methionine are important methyl donors, participating in the one-carbon unit cycle. When the amount of NAM supplementation was sufficient, it can participate in the methionine-homocysteine cycle and the methylation of substrate[32]. In addition, the enriched amino acid metabolism pathways were found by NAM supplementation. Therefore, the increasing trend of VLDL in the liver might be related to the NAM participation in the methionine cycle, which needs further validation.

Conclusion

The results presented in this study provided the first evidence that NAM supplementation during postpartum and entire perinatal period favored the rumen fermentation pattern to propionic acid-type, and altered the hepatic mitochondrial respiratory chain to generate ATP. Supplementation of NAM during postpartum only does not appear enough to cope with the metabolites of oxidative phosphorylation. The summary of the findings in this study was presented in Fig. 4. What is more, it is one-sided to judge the effect and mechanism of additive only through blood changes as the blood concentration is the comprehensive results of body metabolism. Our data suggested that NAM supplemented from prepartum is needed for better oxidative status and energy metabolism of perinatal does. More research is warranted to look at the effect and mechanism of NAM on urea metabolites.

Abbreviations

AGPAT6

phosphoglycerol transferase 6; ATGL:adipose-triglyceride-lipase; C, control group; P, postpartum group, NAM supplemented after kidding; EP, entire-perinatal group, NAM supplemented from - 21 to 28 d around kidding; C_P:C, compare the P with the C; C_{EP}:C, compare the EP with the C; C_{EP}:P, compare the EP with the P; CAT, catalase; DGAT2:diacylglycerol transferase 2, GPT:glutamyl transaminase; GSH-PX, glutathione peroxidase; H₂O₂, hydrogen peroxide; HSL:hormone-sensitive lipase; MDA, malondialdehyde; NEFA:nonesterified fatty acid; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; NADH, ubiquinone oxidoreductase; NAM, nicotinamide; NEB, negative energy balance; PK:pyruvate kinase; ROS, reactive oxygen species; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TC:total cholesterol; VLDL:very low-density lipoprotein; VIP, variable importance in projection.

Declarations

Ethics approval and consent to participate

The use of animals and all experimental protocols (protocol number 100403) were authorized by the Institutional Animal Care and Use Committee of Northwest A&F University (Yangling, Shaanxi, China).

Consent for publication

Not applicable.

Availability of data and materials

All data were presented in the main manuscript and available to readers.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

The authors' contributions were as follows: J. H. Y. and X. S. W. designed the experiment; X. S. W., Q. Y. Y., H. H. Z. and J. J. H. performed the experiment; X. S. W., Q. Y. Y., and Y. C. C. analyzed the data; X. S. W. wrote the manuscript. All the authors read and approved the final manuscript.

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Tables

Table 1. Effect of nicotinamide supplementation during different period on rumen fermentation parameters of perinatal does.

Items	Treatments			SEM	P-value
	C ¹	P ¹	EP ¹		
pH	7.73	6.50	7.01	0.694	0.307
Total VFA (mmol/L)	62.43	56.72	62.79	4.072	0.816
Individual VFA, % of total VFA					
Acetic acid (A)	67.91	65.72	65.96	0.454	0.069
Propionic acid (P)	15.68 ^c	17.40 ^a	16.79 ^b	0.230	<0.001
Isobutyric acid	1.72	2.01	2.03	0.105	0.476
Butyric acid	10.59	11.06	10.48	0.328	0.787
Isovaleric acid	2.32	2.76	2.76	0.135	0.363
Valeric acid	1.11 ^b	1.25 ^a	1.29 ^a	0.030	0.018
A:P ratio	4.36 ^a	3.78 ^b	3.93 ^b	0.080	0.001

¹C: control; P: postpartum supplementation; EP: entire-perinatal supplementation.

^{a, b}Values in the same row with different small letter superscripts mean significant difference ($P \leq 0.05$), while the same or no letter superscripts mean no significant difference ($P > 0.05$); n=5.

Table 2. Effect of nicotinamide supplementation during different period on liver mitochondrial respiratory chain status of perinatal does.

Items	Treatments			SEM	P-value
	C ¹	P ¹	EP ¹		
NAD ⁺ (ng/mL)	3.29	3.50	3.72	0.156	0.578
NADH (ng/mL)	4.77 ^b	5.14 ^b	6.16 ^a	0.187	0.001
NAD ⁺ /NADH	0.69	0.64	0.60	0.023	0.289
Complex I (ng/mL)	7.05 ^b	7.06 ^b	9.02 ^a	0.291	0.001
Complex II (ng/mL)	5.70 ^b	6.89 ^a	6.35 ^a	0.198	0.036
Complex III (ng/mL)	5.46 ^b	5.55 ^b	6.75 ^a	0.188	0.001
Complex IV (ng/mL)	3.15 ^b	3.74 ^{ab}	4.57 ^a	0.245	0.046
ATP (nmol/mL)	1.69	1.83	2.11	0.762	0.069

¹C: control; P: postpartum supplementation; EP: entire-perinatal supplementation.

^{a, b}Values in the same row with different small letter superscripts mean significant difference ($P \leq 0.05$), while the same or no letter superscripts mean no significant difference ($P > 0.05$); n=5.

Table 3. Effect of nicotinamide supplementation during different period on abdominal adipose tissue parameters of perinatal does.

Items ²	Treatments			SEM	P-value
	C ¹	P ¹	EP ¹		
TG (mmol/gprot)	10.28 ^a	9.57 ^a	6.57 ^b	0.59	0.001
NEFA (mmol/gprot)	1.83 ^a	0.57 ^b	0.40 ^b	0.23	0.006
TC (mmol/gprot)	1.69 ^a	1.14 ^b	0.64 ^c	0.15	0.002
AGPAT6 (U·L)	47.7	47.2	44.7	1.21	0.666
DGAT2 (U·L)	89.9	80.2	67.7	1.42	0.072
ATGL (IU·L)	182.3	188.4	203.4	3.81	0.063
HSL (U·L)	631.3	647.3	680.5	18.3	0.580

¹C: control; P: postpartum supplementation; EP: entire-perinatal supplementation.

²TG = triglyceride; NEFA= nonesterified fatty acid; TC = total cholesterol; AGPAT6 = 1-acylglycerol-3-phosphate acyltransferases 6; DGAT2 = diacylglycerol acyltransferase 2; ATGL = adipose triglyceride lipase; HSL = hormone-sensitive lipase; ATP = adenosine triphosphate.

^{a, b}Values in the same row with different small letter superscripts mean significant difference ($P \leq 0.05$), while the same or no letter superscripts mean no significant difference ($P > 0.05$); n=5.

Table 4. Effect of nicotinamide supplementation during different period on liver parameters of perinatal does.

Items ²	Treatments			SEM	P-value
	C ¹	P ¹	EP ¹		
TG (mmol/gprot)	0.330	0.333	0.319	0.003	0.079
NEFA (mmol/gprot)	0.033	0.035	0.037	0.002	0.694
VLDL (mmol/gprot)	0.058	0.126	0.087	0.012	0.083
glycerin (U·g ⁻¹)	0.043 ^a	0.037 ^b	0.038 ^b	0.001	0.010
GPT (U/g)	3.62 ^b	5.21 ^a	2.22 ^b	0.42	0.009
PK (U/gprot)	8.36	15.74	10.96	1.42	0.087
AGPAT6 (U·L ⁻¹)	70.3	76.4	73.3	1.19	0.091
DGAT2 (U·L ⁻¹)	121.8	114.7	113.5	3.36	0.589
ATGL (IU·L ⁻¹)	231.4 ^b	265.2 ^a	252.1 ^a	6.08	0.037
HSL (U·L ⁻¹)	181.9	190.5	199.9	6.64	0.579

¹C: control; P: postpartum supplementation; EP: entire-perinatal supplementation.

²TG = triglyceride; NEFA= nonesterified fatty acid; VLDL = very low-density lipoprotein; GPT = glutamic-pyruvic transaminase; PK = pyruvate kinase; AGPAT6 = 1-acylglycerol-3-phosphate acyltransferases 6; DGAT2 = diacylglycerol acyltransferase 2; ATGL = adipose triglyceride lipase; HSL = hormone-sensitive lipase;

^{a, b}Values in the same row with different small letter superscripts mean significant difference ($P \leq 0.05$), while the same or no letter superscripts mean no significant difference ($P > 0.05$); n=5.

Table 5. Effect of nicotinamide supplementation during different period on blood fatty acid and oxidative status of perinatal does.

Items ²	Treatments			SEM	P-value		
	C ¹	P ¹	EP ¹		Wk.	NAM	Wk. × NAM
TG (mmol/L)	0.22	0.16	0.15	0.01	<0.001	0.078	0.291
NEFA (mmol/L)	0.17	0.11	0.10	0.01	<0.001	0.099	0.182
TC (mmol/L)	2.12	1.96	1.86	0.08	0.016	0.405	0.341
H ₂ O ₂ (U/mL)	52.69	54.04	51.61	1.505	<0.001	0.537	0.007
MDA (mmol/mL)	1.43	1.37	1.39	0.067	<0.001	0.829	0.677
SOD (U/mL)	57.31 ^b	62.59 ^a	59.31 ^{ab}	1.259	<0.001	0.035	0.017
CAT (U/mL)	2.454 ^b	2.677 ^b	3.636 ^a	0.152	<0.001	<0.001	0.062
GSH-PX (U/mL)	625.7	779.0	748.7	61.11	<0.001	0.067	0.257
CAT/SOD	0.045 ^b	0.045 ^b	0.062 ^a	0.002	<0.001	<0.001	0.057
GSH-PX/SOD	10.62	11.11	12.45	0.502	<0.001	0.061	0.434

¹C: control; P: postpartum supplementation; EP: entire-perinatal supplementation.

² TG = triglyceride; NEFA= nonesterified fatty acid; TC = total cholesterol; H₂O₂: hydrogen peroxide; MDA: malonaldehyde; SOD: superoxide dismutase; CAT: catalase; GSH-PX: glutathione peroxidase.

^{a, b}Values in the same row with different small letter superscripts mean significant difference ($P \leq 0.05$), while the same or no letter superscripts mean no significant difference ($P > 0.05$); n=5.

Table 6. Identification of different hepatic metabolites of does supplemented with nicotinamide.

Metabolites	RT ¹	C _{P:C} ²			C _{EP:C} ²			C _{EP:P} ²		
		VIP ³	P-value	FC ³	VIP ³	P-value	FC ³	VIP ³	P-value	FC ³
sulfuric acid	8.962	0.551	0.438	1.963	2.018	0.048	0.735	1.026	0.338	0.374
malonic acid	9.394	0.817	0.374	1.701	2.525	<0.001	0.763	1.298	0.258	0.448
3-methylamino-1,2-propanediol	9.574	0.688	0.726	1.234	1.652	0.025	0.304	0.574	0.187	0.246
3-aminoisobutyric acid	9.582	1.861	0.041	0.166	2.088	0.027	0.244	0.911	0.425	1.472
D-glyceric acid	11.229	0.900	0.332	1.619	1.998	0.035	0.775	1.315	0.214	0.479
uracil	11.356	0.743	0.278	4.602	2.379	0.006	4.532	0.891	0.981	0.985
malonamide	14.085	1.061	0.023	2.647	0.104	0.778	0.855	1.247	0.012	0.323
ciliatine	16.283	0.854	0.568	0.792	2.320	0.020	0.536	0.698	0.489	0.677
diglycerol	16.292	1.737	0.014	0.169	0.672	0.936	0.963	1.679	0.118	5.694
hypoxanthine	17.054	1.294	0.101	0.424	2.218	0.008	0.149	0.855	0.378	0.351
adenine	17.683	1.619	0.073	<0.001	0.956	0.458	1.430	2.274	0.036	36228.2
palmitoleic acid	19.242	0.422	0.471	1.371	1.282	0.039	0.496	1.092	0.118	0.362
octadecanol	20.462	1.023	0.096	2.523	1.093	0.030	2.453	0.084	0.934	0.972
cystine	21.658	1.694	0.009	0.351	1.185	0.345	0.667	0.939	0.330	1.901
cytidine-monophosphate	23.011	1.744	0.005	0.165	2.109	0.010	0.194	0.315	0.910	1.182
prostaglandin	24.829	2.179	0.030	<0.001	2.673	0.030	<0.001	1.050	0.314	0.485
guanosine	25.368	0.437	0.523	1.361	1.101	0.037	0.506	0.735	0.156	0.372

¹ RT: retention time, minute.

² C_{P:C}: hepatic metabolite in postpartum versus that in control, C_{EP:C}: hepatic metabolite in entire-perinatal versus that in control, and C_{EP:P}: hepatic metabolite in entire-perinatal versus that in postpartum.

³ VIP: variable importance in projection. FC: fold change, calculated as the mean value of peak area obtained from treatment group/mean value of peak area obtained from control group. If the FC value is less than 1, it means that there is less metabolite in treatment group than in control group.

Figures

Figure 1

Effect of nicotinamide supplementation on liver oxidative status of perinatal does. C: control; P: postpartum supplementation; EP: entire-perinatal supplementation. Data were presented as mean $\pm$ SEM. a, b Values with different small letter superscripts mean significant difference ($P \leq 0.05$), while the same or no letter superscripts mean no significant difference ($P > 0.05$).

Figure 2

Metabolome view map of common metabolites identified in liver. CP:C and CEP:C were used to represent the comparison of P and EP with C, respectively. C: control; P: postpartum supplementation; EP: entire-perinatal supplementation. The x axis represents the pathway impact, and y axis represents the pathway enrichment.

Figure 3

Interrelationships among metabolites of lipid metabolism in the abdominal adipose and liver tissues in P and EP. C: control; P: postpartum supplementation; EP: entire-perinatal supplementation. Note: blue numbers denote the changes in P group, and red numbers denote the changes in EP group.

Figure 4

The summary of the findings in this study.

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