Elevated thyroid hormone caused by high concentrate diet participates in hepatic metabolic disorders in dairy cows

Qu Chen

Nanjing Agricultural University - Weigang Campus: Nanjing Agricultural University

Chen Wu

Nanjing Agricultural University - Weigang Campus: Nanjing Agricultural University

Zhihao Yao

Nanjing Agricultural University - Weigang Campus: Nanjing Agricultural University

Liuping Cai

Nanjing Agricultural University - Weigang Campus: Nanjing Agricultural University

Yingdong Ni (✉ niyingdong@njau.edu.cn)

Nanjing Agricultural University

Shengyong Mao

Nanjing Agricultural University - Weigang Campus: Nanjing Agricultural University

Research

Keywords: high concentrate diet, lactating dairy cows, glycometabolism, lipid metabolism, thyroid hormone

Posted Date: April 27th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-443111/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Animal Bioscience on January 5th, 2022.
See the published version at <https://doi.org/10.5713/ab.21.0397>.

Abstract

Background

Feeding a high concentrate diet is widely used to satisfy high milk yields, however, if used over a longer term it can cause metabolic disorders. Abnormal secretion of hormones is one of the important causes of metabolic disorders caused by high concentrate diet, however, the related research is still limited. In total, 12 mid-lactating dairy cows were randomly assigned to HC group (60% concentrate of dry matter, $n = 6$) or LC group (40% concentrate of dry matter, $n = 6$). All cows were slaughtered at the 21st day, and blood and liver samples were collected.

Results

Compared with LC group, HC group showed reduced serum triglyceride (TG), free fatty acid (FFA), total cholesterol (TC), low density lipoprotein (LDL-C), and increased liver glycogen and glucose ($P < 0.05$). Real-time PCR results showed that genes related to gluconeogenesis, namely, glucose-6-phosphatase (G6PC) and phosphoenolpyruvate carboxykinase 1 (PCK1) were significantly upregulated and accompanied greater gluconeogenic protein expression in HC group ($P < 0.05$). The expression of hepatic genes and proteins related to lipogenesis, including sterol regulatory element-binding protein 1 (SREBP1), long-chain acyl-CoA synthetase 1 (ACSL1), and fatty acid synthase (FAS) were downregulated in HC group ($P < 0.05$). However, the expression of genes and proteins related to β -oxidation, including carnitine palmitoyltransferase 1 α (CPT1 α) and peroxisome proliferator activated receptor α (PPAR α) were significantly increased in HC group ($P < 0.05$). The levels of serum cortisol, thyroxine (T4), triiodothyronine (T3), free triiodothyronine (FT3) and liver FT3 elevated significantly in HC group ($P < 0.05$). Moreover, the expression of thyroid hormone receptor (THR) upregulated at gene and protein levels ($P < 0.05$).

Conclusions

Taken together, this study first reports that abnormal secretion of TH induced by high concentrate diet participates in metabolic disorders, and it regulates glycometabolism and lipid metabolism through its actions on the liver in lactating dairy cows.

1. Introduction

With the increased demand for dairy products, dairy industry is gradually developing towards a large-scale and intensive farming model. Excessive amounts of non-structural carbohydrates and highly fermentable forages are fed to dairy cows to satisfy energy requirement for maintenance and high milk yields (Keunen et al., 2002). However, feeding excessive high concentrate diet to ruminants are the common reasons for subacute ruminal acidosis (SARA), and SARA is a popular health and production problem in intensive production systems (Metre et al., 2000, Enemark et al., 2004, Plaizier et al., 2008).

The common consequences of SARA include reduction of food intake, diarrhea, laminitis and inflammation, which are harmful to animal health and milk production performance (Alegre et al., 1988, Kleen et al., 2003, Plaizier et al., 2008, Dong et al., 2011).

In ruminants, liver is an important metabolic organ responsible for gluconeogenesis and lipogenesis (Dong et al., 2017). Hepatic gluconeogenesis is responsible for maintaining an adequate glucose supply to the mammary glands (Zhao and Keating, 2007). In addition, hepatic lipid metabolism is vital to the physical properties and quality of the milk (Dong et al., 2013). Glucose-6-phosphatase (G6PC) and phosphoenolpyruvate carboxykinase 1 (PCK1) are the rate-limiting enzymes involved in hepatic gluconeogenesis (Al-Trad et al., 2010). In the liver, sterol regulatory element- protein 1 (SREBP1), fatty acid synthase (FAS), and long-chain acyl CoA synthet binding ase 1 (ACSL1) are important for de novo fatty acid synthesis (Phillips et al., 2010, Dong et al., 2017). In addition, CPT1 α and PPAR α are responsible for dairy cows to regulate the entry of fatty acids into the mitochondria for oxidation (Dann and Drackley, 2005).

During lactation period, hormones play important roles in glycometabolism and lipid metabolism (Cronjé, 2000). Endocrine disorders can result in metabolic disorders, affecting milk production performance. Our previous studies found that high concentrate diet can induce chronic stress in dairy goats, with the activation of hypothalamic–pituitary–adrenal axis and increased plasma cortisol, and ultimately caused nutrient partitioning and re-distribution of energy in the liver and a decrease in milk quality (Dong et al., 2013, Jia et al., 2014). TH has extensive biological effects on physiological processes, including energy metabolism, glycometabolism, and lipid metabolism (Arrojo et al., 2013, Mullur et al., 2014a). TH contains thyroxin (T4) and triiodothyronine (T3), T3 is the active form of TH. These hormones are secreted by the thyroid gland, circulate in serum mostly bound to proteins, and only free triiodothyronine (FT3) enter cells to bind to the thyroid hormone receptor (TR) (Lakshmanan et al., 1992). TR is a transcription factor that belongs to the nuclear receptor superfamily (Abdalla and Bianco, 2014). It acts as a ligand-dependent transcription factor to regulate genes involved in the glycometabolism and lipid metabolism (Flamant and Gauthier, 2013). Non-pathological elevation of TH promotes liver glycogen synthesis and gluconeogenesis; It also promotes lipolysis, including reduce fat storage and serum TG content (Sinha et al., 2014). Although TH does not belong to stress hormones, some research evidences indicate that TH has close relationship with stress. High altitude is an important stress stimulus, Barnholt et al. found that people who experienced high altitude stress for 3 weeks showed elevated serum T4 content (Barnholt et al., 2006). However, animal experiments show that short-term cold stress significantly increases T3 content, and has little effect on T4 (Fukuhara et al., 1996, Goundasheva et al., 2010). In addition, Sejian et al. found walking stress significantly reduces plasma T3 and T4 contents in sheep (Sejian et al., 2012). Although high concentrate diet can cause increased cortisol and chronic stress in ruminants, however, to date, data about the effects of high concentrate diet on TH are scarce. Accordingly, this research focuses on evaluating the effects of TH on hepatic metabolism in dairy cows fed high concentrate diet.

2. Materials And Methods

2.1. Ethics Statement

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Agricultural University. The “Guidelines on Ethical Treatment of Experimental Animals” (2006) No. 398 set by the Ministry of Science and Technology, China and the Regulation regarding the Management and Treatment of Experimental Animals” (2008) No. 45 set by the Jiangsu Provincial People’s Government, was strictly followed during the slaughter and sampling procedures.

2.2. Experimental Animals

Twelve healthy Chinese Holstein cows (mean body weight, 651 ± 54 kg) during mid-lactation (mean milk yield, 17.43 ± 4.04 kg/d) were raised in dairy farm (Jiangsu Province, China) for the study. All the cows were in their second parity or third parity when the liver tissues were collected. These cows were kept in freestall housing in the same cubicle partition of the barn during the experimental period. After a week of prefeeding, they were randomly assigned to LC and HC group. LC group received low concentrate diet (40% of dry matter) ($n = 6$), and HC group received HC diet (60% of dry matter) ($n = 6$) for 3 weeks. The ingredients in the diets and the nutritional composition are showed in the Table 1. Cows were free access to fresh water throughout the experimental time. At the 21st day, the pH of ruminal fluid in LC and HC groups were 6.31 ± 0.87 and 5.79 ± 0.92 ($P < 0.01$), respectively.

2.3. Samples collection

All cows were killed using electric stunning slaughtering after fasting. Immediately after slaughtering, liver tissue samples were frozen immediately in liquid nitrogen and stored at -80°C for subsequent extraction of RNA and proteins.

2.4. Staining for Periodic Acid-Schiff (PAS)

Liver tissue samples were washed three times with normal saline and fixed with a 10% formalin solution for 48 h at 4°C , dehydrated with ethanol, and embedded with paraffin. The paraffin-embedded slices were deparaffinized, hydrated with distilled water and oxidized for 10 min at 40°C in a 0.5% periodic acid solution. Then washed with distilled water, immersed in Schiff reagent, washed in distilled water, counterstained with Mayer’s hematoxylin, washed in distilled water, dehydrated in a gradient of ethanol and ultimated sealed using a synthetic mounting medium.

2.4. Measurement of serum and liver biochemical parameters

All serum biochemical parameters, including glucose, TG, FFA, TC, LDL-C and HDL-C were measured using an automatic biochemical analyzer (7020, HITACHI, Tokyo, Japan). Liver glycogen was detected by using a glycogen kit (Jiancheng Co. Ltd, Nanjing, China). Liver TG was detected by using a TG kit (Applygen Co. Ltd, Beijing, China). Liver FFA was detected by using a FFA kit (Jiancheng Co. Ltd, Nanjing,

China). Liver glucose was detected by using a glucose kit (Comin Co. Ltd, Suzhou, China). All kits were strictly following the manufacturer's instructions.

2.5. Determinations of hormones in serum and liver

The method for extracting liver TH was described as Beckett et al. ([Beckett et al.](#)). The levels of cortisol, insulin, T3, T4 and FT3 were determined using RIA cortisol, insulin, T3, T4 and FT3 kits, respectively (North institute of biotechnology Co. Ltd, Beijing, China). All kits were strictly following the manufacturer's instructions.

2.6. RNA isolation, cDNA synthesis and real-time PCR

Liver tissues were quickly collected, immediately frozen in liquid nitrogen and stored at -80 °C until RNA isolation. Total RNA was extracted from liver samples with TRIzol reagent (Sangon, Shanghai, China). The concentration and quality of the RNA were measured with a NanoDropND-1000 Spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA). Then 2µg of total RNA was treated with RNase-Free DNase (M6101; Promega, Madison, WI, USA) and reverse transcribed according to manufacturer's instructions. Two micro liters of diluted cDNA (1:40, v/v) was used for real-time PCR, which was performed in an Mx3000P (Stratagene, USA). GAPDH, which is not affected by the experimental factors, was chosen as the reference gene. All the primers were listed in Table 2, were synthesized by Tsingke Company (Nanjing, China). The method of 2-Ct was used to analyze the real-time PCR results, and gene mRNA levels were expressed as the fold change relative to the mean value of the control group.

2.7. Western blotting analysis

One hundred milligrams of frozen liver was minced and homogenized in 1 ml of ice-cold RIPA buffer containing the protease inhibitor cocktail Complete EDTA-free (Roche, Penzberg, Germany). The homogenates were centrifuged at 12235 g for 20 min at 4 °C and then the supernatant fraction was collected. The protein concentration was determined using a BCA Protein Assay kit (Thermo, USA). Fifty micrograms of protein extract from each sample was then loaded into 10% SDS-PAGE gels, and the separated proteins were transferred onto nitrocellulose membranes (BioTrace; Pall Corp., New York, NY, USA). After transfer, membranes were blocked for 2 h at room temperature in blocking buffer and then incubated with the following primary antibodies: rb-anti-PCK1 (1:1000; 12940S; CST), rb-anti-SREBP1 (1:1000; 14088-1-AP; Proteintech), rb-anti-FAS (1:1000; BS6050; Bioworld), rb-anti-G6PC (1:1000; an83690; Abcam), rb-anti-CPT1α (1:1000; 15184-1-AP; Proteintech), rb-anti-PPARα (1:1000; 15540-1-AP; Proteintech), rb-anti-THRB (1:1000; AF8157; Beyotime), rb-anti-DIO2 (1:1000; bs-3673r; Bioss), ACTB-HRP (1:10,000; KC-5A08; Kangchen bio-tech) in dilution buffer overnight at 4°C. After several washes in Tris-buffered-saline with Tween, membranes were incubated with goat anti-rabbit HRP-conjugated secondary antibodies (1:10,000; BS13278, Bioworld) in dilution buffer for 2 hours at room temperature. Finally, the blot was washed and detected by enhanced chemiluminescence using the LumiGlo substrate (Super Signal West Pico Trial Kit; Pierce, Thermo Fisher Scientific, Madison, WI, USA) and the signals were recorded by an imaging System (Bio-Rad, Hercules, CA, USA) and analyzed with Image J software.

3. Result

3.1. High concentrate diet markedly decreased the levels of TG, FFA, TC and LDL-C in serum.

As is shown in Fig. 1, the levels of serum TG, FFA, TC and LDL-C were significantly decreased in HC group ($P < 0.05$), whereas there was no significant difference in serum glucose and HDL-C concentrations ($P > 0.05$).

3.2. High concentrate diet significantly increased the levels of glycogen and glucose in the liver

No obvious lipid deposition, inflammation or damages were observed in the liver in both LC and HC groups demonstrating by HE staining sections (Fig. 2A). Concentrations of liver TG and FFA were not significantly changed by high concentrate diet (Fig. 2E and F) ($P > 0.05$). However, PAS staining showed that high concentrate diet significantly promoted glycogen deposition in the liver (Fig. 2B). Biochemical detection also confirmed an increase in the levels of liver glycogen and glucose in HC group (Fig. 2C and D) ($P < 0.05$).

3.3. Expression of genes and proteins related to glycometabolism in the liver

Real-time PCR result showed that genes expression of PCK1 and G6PC in the liver were significantly increased in HC group (Fig. 3A) ($P < 0.05$). Other genes including phosphogluconate dehydrogenase (PGD), succinate dehydrogenase complex subunit D (PGL), hexokinase 1 (HK1), solute carrier family 2 member 1 (GLUT1), and solute carrier family 2 member 4 (GLUT4) did not show significant difference (Fig. 3A) ($P > 0.05$). In accordance with the genes expression, the proteins expression of G6PC and PCK1 were significantly increased in HC group (Fig. 3B) ($P < 0.05$).

3.4. Expression of genes and proteins related to lipid metabolism in the liver

Real-time PCR showed that genes expression of SREBP1 and ACSL1 in the liver were significantly decreased in cows fed high concentrate diet (Fig. 4A) ($P < 0.05$), while PPAR α and CPT1 α were significantly increased (Fig. 4A) ($P < 0.05$). However, other genes including acetyl-CoA carboxylase alpha (ACC), hormone-sensitive lipase (HSL), and adipose triglyceride lipase (ATGL) mRNA expression were not changed by high concentrate diet (Fig. 4A) ($P > 0.05$). Consistent with the mRNA expression, the protein expression of SREBP1 and FAS were significantly reduced (Fig. 4B) ($P < 0.05$), while CPT1 α and PPAR α were significantly increased in HC group (Fig. 4C) ($P < 0.05$).

3.5. High concentrate diet elevated cortisol and TH concentrations

The levels of serum cortisol significantly upregulated by high concentrate diet (Fig. 5A) ($P < 0.05$), whereas serum insulin did not reach statistical difference (Fig. 5B) ($P > 0.05$). Thyroid hormones including T3 and T4 showed dramatically increased in HC group (Fig. 5C and D) ($P < 0.01$). Furthermore, the levels of serum and liver FT3 were higher in HC group than in LC group (Fig. 5E and F) ($P < 0.05$).

3.6. High concentrate diet increased the expression of THR in the liver

Gene expression analysis showed that the expression of THRA and THRB significantly upregulated by high concentrate diet (Fig. 6A) ($P < 0.05$). Moreover, the protein expression of THRB were increased in HC group, too (Fig. 6B) ($P < 0.05$).

3.7. Expression of iodothyronine deiodinases did not show obvious changes in the liver

Genes associated with the iodothyronine deiodinases, including DIO1, DIO2 and DIO3 were detected by qPCR, however, such genes did not show obvious difference between LC and HC groups (Fig. 7A) ($P > 0.05$). Western blot analysis also confirmed that DIO2 was unaltered by high concentrate diet at the protein level (Fig. 7B) ($P > 0.05$).

4. Discussion

Although high concentrate diet can meet the energy demand of high-yielding cows over the short-term. If used over a longer term it also causes nutritional and metabolic diseases (Jouany, 2006). As SARA affects animal health and reduces productive performance, it is considered to be the most important nutritional disorder for ruminants with excessive high concentrate diet intake (Kleen et al., 2003). In this study, a significant decrease of serum TG, NEFA, TC and LDL-C concentrations were observed in HC group. TG and NEFA are the main precursors of milk fat, and their levels in blood circulation are closely related to milk fat rate. Previous studies found that high concentrate diet significantly induced the reduction in the yield and percentage of milk fat (Hussein et al., 2013, Argov-Argaman et al., 2014). Kadegowda et al. found abomasal infusion of butterfat increased milk fat in lactating dairy cows (Kadegowda et al., 2008). Zebeli et al. found high concentrate diet significantly decreased serum NEFA content, accompanied with significant reduction of milk fat rate (Zebeli et al., 2011). These results are highly consistent with our data, with the decrease of TG and NEFA, the milk fat rate of dairy cows also decreased significantly in HC group (data not shown).

Liver is an important organ for the regulation of glycometabolism and lipid metabolism (Knegsel et al., 2005, Xu et al., 2015). Therefore, we further investigated hepatic morphology and metabolism. Previous studies reported that high concentrate diet can induce TG accumulation, inflammation, and tissue damages in ruminants (Dong et al., 2017, Guo et al., 2017). Although no obvious inflammation, steatosis, and damages were shown in HE staining sections, we observed remarkable glycogen deposition in HC group via PAS staining. Furthermore, biochemical analysis also confirmed that levels of liver glucose and glycogen significantly in HC group. Unlike non-ruminant mammals, ruminants primarily depend on hepatic gluconeogenesis as their source of glucose to meet their energy demand (Overton et al., 1999). Dong et al. found that high concentrate diet significantly increased the enzyme activities involved in glucogenesis, including G6PC and PCK1, whereas no obvious changes in the enzyme activities involved in glycolysis (Dong et al., 2017). It is highly consistent with our data, high concentrate diet significantly up-regulated the expression of G6PC and PCK1 at gene and protein levels, however, the other genes involved in glycolysis and glucose transport did not show statistical significance.

In addition, high concentrate diet can cause lipid metabolism disorders in liver. Jiang et al. employed a comparative proteomic approach to investigate the effect of high concentrate diet on the hepatic metabolism in lactating dairy goats. They found high concentrate diet enhanced hepatic TG synthesis and accumulation, and reduced TG output, eventually reducing milk fat rate (Jiang et al., 2013). However, Xu et al. found that high concentrate diet decreased TG synthesis and increased fatty acids decomposition, accompanied with reduced plasma TG and NEFA contents in hepatic vein, eventually reducing milk fat rate (Xu et al., 2015). In our data, the genes or proteins related to TG synthesis, including SREBP1, ACSL1 and FAS significantly reduced in HC group. Furthermore, high concentrate diet upregulated the genes and proteins related to β -oxidation, including CPT1 α and PPAR α . The result indicated that enhanced hepatic β -oxidation and decreased fatty acids synthesis may ultimately cause the reduction in the serum TG, FFA, and milk fat rate.

Abnormal secretion of hormones is an important cause of metabolic disorders. Previous studies reported that high concentrate diet affected many hormones involved in glycometabolism and lipid metabolism, including cortisol and insulin (Jia et al., 2014, Xu et al., 2015, Maeda et al., 2019, Takemura et al., 2019). Our result showed that although elevated insulin did not reach statistical significance, the levels of serum cortisol was significantly increased in HC group. Increased cortisol concentration in stressful situations is an adaptation response of the organism to the changing environment (A et al., 2006, Jarvis et al., 2006). The studies on dairy goats also showed that high concentrate diet significantly elevated plasma cortisol and glucocorticoid receptor protein expression in the liver, indicating animals under a state of stress (Dong et al., 2013, Jia et al., 2014). Our previous studies on goats confirmed that intramuscular injection dexamethasone, a synthetic glucocorticoid, significantly increased liver glycogen deposition, accompanied with increased expression of proteins related to gluconeogenesis in the liver (Niu et al., 2018).

Long-term treatment with cortisol can lead to the development of fatty liver (Rahimi et al., 2020). Treat primary bovine hepatocytes with dexamethasone resulting lipid deposition via inhibiting the genes related

to β -oxidation (Yin., 2015). However, in animal experiment, high concentrate diet elevates plasma cortisol levels, accompanied with hepatic β -oxidation enhancement (Dong et al., 2013). Similarly, our previous study found dexamethasone injection enhanced β -oxidation and reduced TG content in the liver (Chen et al., 2018). The contradiction between in-vitro and in-vivo outcomes indicated that other hormones may be involved in the metabolic disorders induced by high concentrate diet. We noticed that TH, including serum T3, T4, FT3, and liver FT3 remarkably increased in HC group. TH regulates fatty acid, cholesterol, and glycometabolism through its actions on the liver (Mullur et al., 2014b). THR consists of 2 subtypes, including THRA and THRB. THRA expressed in almost all tissues, mainly in the brain and heart. Whereas THRB highly expressed in the liver (Bookout et al., 2006). Our qPCR result showed that both THRA and THRB were highly increased in HC group. Furthermore, we examined THRB protein expression. And the protein expression of THRB was consistent with the gene expression. Current studies generally considered that TH can promote gluconeogenesis, researchers found that TH can directly elevate PCK1 (Park et al., 1999) and G6PC (Suh et al., 2013) expression which are rate-limiting enzymes for gluconeogenesis. The mechanisms include 2 aspects, one is that THR combines with promoters to directly upregulate genes expression of PCK1 and G6PC, another is that THR increased deacetylation and activation of the master gluconeogenic transcription factor forkhead box O1 (FoxO1) to increase gluconeogenesis (Sinha et al., 2014). For the effects of TH on lipid metabolism, Hashimoto et al. found T3 negatively regulates the mouse SREBP1 gene expression in the liver which is confirmed by ribonuclease protection assays and qPCR. Moreover, promoter analysis on hepatocytes using luciferase assays also showed that T3 negatively regulates the SREBP1 gene promoter (Hashimoto et al., 2006). Instead of inhibiting fatty acid synthesis, TH also induces CPT1 α expression to promote fatty acid shuttling into hepatic mitochondria for oxidation (Sinha et al., 2014). In addition, elevated TH contributes to the clearance of TC and LDL-C (Sinha et al., 2014). And this effect may result in the reduction in serum TC and LDL-C in HC group. T3 can be directly secreted by the thyroid gland and released into the circulation, however, most of blood T3 comes from T4 removing an iodine atom from peripheral tissue. Therefore, we examined the gene and protein expression of iodothyronine deiodinases (DIO) in the liver. However, there was no obvious change in the expression of DIO1, DIO2 and DIO3 between LC and HC groups, demonstrating that elevated T3 was not caused by hepatic iodothyronine deiodinases.

5. Summary And Conclusions

Overall, our data show that high concentrate diet results in abnormal secretion of cortisol and TH. Elevated cortisol and TH induce metabolic disorders, including lower serum TG, NEFA, TC, LDL-C and higher liver glycogen and glucose. Our study preliminarily reveals that TH regulates glycometabolism and lipid metabolism through its actions on the liver in dairy cows, including enhancing gluconeogenesis, β -oxidation and reducing fatty acid synthesis. However, the causes of elevated TH induced by high concentrate diet and the regulatory ways of TH on metabolism in ruminants still need further investigation.

References

- A, K. R., M. J. H. B, C. G. C, and A. B. L. B. 2006. The responses of growing pigs to a chronic-intermittent stress treatment - ScienceDirect. *Physiology & Behavior* 89(5):670-680.
- Abdalla, S. M. and A. C. Bianco. 2014. Defending plasma T3 is a biological priority. *Clinical endocrinology* 81(5):633-641.
- Al-Trad, B., T. Wittek, G. B. Penner, K. Reisberg, and J. R. Aschenbach. 2010. Expression and activity of key hepatic gluconeogenesis enzymes in response to increasing intravenous infusions of glucose in dairy cows. *Journal of animal science* 88(9):2998.
- Alegre, M., C. J. Ciudad, C. Fillat, and J. J. Guinovart. 1988. Determination of glucose-6-phosphatase activity using the glucose dehydrogenase-coupled reaction. *Analytical Biochemistry* 173(1):185-189.
- Argov-Argaman, N., R. Mesilati-Stahy, Y. Magen, and U. Moallem. 2014. Elevated concentrate-to-forage ratio in dairy cow rations is associated with a shift in the diameter of milk fat globules and remodeling of their membranes. *J Dairy Sci* 97(10):6286-6295.
- Arrojo, E. D. R., T. L. Fonseca, J. P. Werneck-de-Castro, and A. C. Bianco. 2013. Role of the type 2 iodothyronine deiodinase (D2) in the control of thyroid hormone signaling. *Biochimica et biophysica acta* 1830(7):3956-3964.
- Barnholt, K. E., A. R. Hoffman, P. B. Rock, S. R. Muza, C. S. Fulco, B. B. raun, L. Holloway, R. S. Mazzeo, A. Cymerman, and A. L. Friedlander. 2006. Endocrine responses to acute and chronic high-altitude exposure (4,300 meters): modulating effects of caloric restriction. *American journal of physiology. Endocrinology and metabolism* 290(6):E1078.
- Beckett, G. J., A. Russell, F. Nicol, P. Sahu, C. R. Wolf, and J. R. Arthur. Effect of selenium deficiency on hepatic type I 5-iodothyronine deiodinase activity and hepatic thyroid hormone levels in the rat. *Biochemical Journal*.
- Bookout, A. L., Y. Jeong, M. Downes, R. T. Yu, R. M. Evans, and D. J. Mangelsdorf. 2006. Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. *Cell* 126(4):789-799.
- Chen, Qu, Niu, Liqiong, Hua, Canfeng, Geng, Yali, Cai, and Liuping. 2018. Chronic dexamethasone exposure markedly decreased the hepatic triglyceride accumulation in growing goats. *General & Comparative Endocrinology*.
- Cronjé, P. 2000. *Digestion, Metabolism, Growth and Reproduction*.
- Dann, H. M. and J. K. Drackley. 2005. Carnitine Palmitoyltransferase I in Liver of Periparturient Dairy Cows: Effects of Prepartum Intake, Postpartum Induction of Ketosis, and Periparturient Disorders - ScienceDirect. *Journal of Dairy Science* 88(11):3851-3859.

Dong, G., S. Liu, Y. Wu, C. Lei, and S. Zhang. 2011. Diet-induced bacterial immunogens in the gastrointestinal tract of dairy cows: Impacts on immunity and metabolism. *Acta Veterinaria Scandinavica* 53(1).

Dong, H., S. Wang, Y. Jia, Y. Ni, Y. Zhang, S. Zhuang, X. Shen, and R. Zhao. 2013. Long-term effects of subacute ruminal acidosis (SARA) on milk quality and hepatic gene expression in lactating goats fed a high-concentrate diet. *PloS one* 8(12):e82850.

Dong, H. B., L. L. Sun, R. H. Cong, S. Y. Tao, Y. Q. DuanMu, J. Tian, Y. D. Ni, and R. Q. Zhao. 2017. Changes in milk performance and hepatic metabolism in mid-lactating dairy goats after being fed a high concentrate diet for 10 weeks. *Animal* 11(3):418-425.

Enemark, J., R. J. Jrgensen, and N. B. Kristensen. 2004. An evaluation of parameters for the detection of subclinical rumen acidosis in dairy herds. *Veterinary research communications* 28(8):687-709.

Flamant, F. and K. Gauthier. 2013. Thyroid hormone receptors: the challenge of elucidating isotype-specific functions and cell-specific response. *Biochimica et biophysica acta* 1830(7):3900-3907.

Fukuhara, K., R. Kvetnansky, G. Cizza, K. Pacak, H. Ohara, D. S. Goldstein, and I. J. Kopin. 1996. Interrelations between Sympathoadrenal System andHypothalamo-Pituitary-Adrenocortical/Thyroid Systemsin Rats Exposed to Cold Stress. *Journal of Neuroendocrinology*.

Goundasheva, D., M. Andonova, and V. Ivanov. 2010. Changes in some parameters of the immune response in rats after cold stress. *Zentralblatt Für Veterinrmedizin.reihe B.journal of Veterinary Medicine* 41(10):670-674.

Guo, J., G. Chang, K. Zhang, L. Xu, D. Jin, M. S. Bilal, and X. Shen. 2017. Rumen-derived lipopolysaccharide provoked inflammatory injury in the liver of dairy cows fed a high-concentrate diet. *Oncotarget* 8(29):46769-46780.

Hashimoto, K., M. Yamada, S. Matsumoto, T. Monden, T. Satoh, and M. Mori. 2006. Mouse sterol response element binding protein-1c gene expression is negatively regulated by thyroid hormone. *Endocrinology* 147(9):4292-4302.

Hussein, M., K. H. Harvatine, W. M. Weerasinghe, L. A. Sinclair, and D. E. Bauman. 2013. Conjugated linoleic acid-induced milk fat depression in lactating ewes is accompanied by reduced expression of mammary genes involved in lipid synthesis. *J Dairy Sci* 96(6):3825-3834.

Jarvis, S., C. Moinard, S. K. Robson, E. Baxter, E. Ormandy, A. J. Douglas, J. R. Seckl, J. A. Russell, and A. B. Lawrence. 2006. Programming the offspring of the pig by prenatal social stress: neuroendocrine activity and behaviour. *Hormones & Behavior* 49(1):68-80.

Jia, Y. Y., S. Q. Wang, Y. D. Ni, Y. S. Zhang, S. Zhuang, and X. Z. Shen. 2014. High concentrate-induced subacute ruminal acidosis (SARA) increases plasma acute phase proteins (APPs) and cortisol in goats.

Animal 8(9):1433-1438.

Jiang, Xueyuan, Zeng, Tao, Zhang, Shukun, and Yuanshu. 2013. Comparative Proteomic and Bioinformatic Analysis of the Effects of a High-Grain Diet on the Hepatic Metabolism in Lactating Dairy Goats. PloS one.

Jouany, J. P. 2006. Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. Animal reproduction science 96(3-4):250-264.

Kadegowda, A. K., L. S. Piperova, P. Delmonte, and R. A. Erdman. 2008. Abomasal infusion of butterfat increases milk fat in lactating dairy cows. J Dairy Sci 91(6):2370-2379.

Keunen, J. E., J. C. Plaizier, L. Kyriazakis, T. F. Duffield, T. M. Widowski, M. I. Lindinger, and B. W. McBride. 2002. Effects of a subacute ruminal acidosis model on the diet selection of dairy cows. J Dairy Sci 85(12):3304-3313.

Kleen, J. L., G. A. Hooijer, J. Rehage, and J. P. Noordhuizen. 2003. Subacute ruminal acidosis (SARA): a review. Journal of Veterinary Medicine A Physiology Pathology Clinical Medicine 50(8):406.

Knegsel, A. T. M. v., H. V. D. Brand, J. Dijkstra, S. Tamminga, and B. Kemp. 2005. Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. Reprod Nutr Dev 45(6):665-688.

Lakshmanan, M., E. Goncalves, A. Pontecorvi, and J. Robbins. 1992. Differential effect of a new thyromimetic on triiodothyronine transport into myoblasts and hepatoma and neuroblastoma cells. Biochimica et biophysica acta 1133(2):213-217.

Maeda, M., K. Kawasumi, S. Sato, and T. Arai. 2019. Evaluation of blood adiponectin levels as an index for subacute ruminal acidosis in cows: a preliminary study. Veterinary research communications 43(4):215-224.

Metre, D., J. W. Tyler, and S. M. Stehman. 2000. Diagnosis of enteric disease in small ruminants. Veterinary Clinics of North America Food Animal Practice 16(1):87-115.

Mullur, R., Y. Y. Liu, and G. A. Brent. 2014a. Thyroid hormone regulation of metabolism. Physiol Rev 94(2):355-382.

Mullur, R., Y. Y. Liu, and G. A. Brent. 2014b. Thyroid Hormone Regulation of Metabolism. Physiological Reviews 94(2):355.

Niu, L., Q. Chen, C. Hua, Y. Geng, L. Cai, S. Tao, Y. Ni, and R. Zhao. 2018. Effects of chronic dexamethasone administration on hyperglycemia and insulin release in goats. J Anim Sci Biotechnol 9:26.

- Overton, Thomas, R., Drackley, James, and K. 1999. Substrate Utilization for Hepatic Gluconeogenesis Is Altered by Increased Glucose Demand in. *Journal of animal science* 77(7):1940-1940.
- Park, E. A., S. Song, C. Vinson, and W. J. Roesler. 1999. Role of CCAAT enhancer-binding protein beta in the thyroid hormone and cAMP induction of phosphoenolpyruvate carboxykinase gene transcription. *The Journal of biological chemistry* 274(1):211-217.
- Phillips, C. M., L. Goumide, S. Bertrais, M. R. Field, L. A. Cupples, J. M. Ordovas, C. Defoort, J. A. Lovegrove, C. A. Drevon, and M. J. Gibney. 2010. Gene-nutrient interactions with dietary fat modulate the association between genetic variation of the ACSL1 gene and metabolic syndrome. *Journal of lipid research* 51(7):1793-1800.
- Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. *Veterinary Journal* 176(1):21-31.
- Rahimi, L., A. Rajpal, and F. Ismail-Beigi. 2020. Glucocorticoid-Induced Fatty Liver Disease. *Diabetes, metabolic syndrome and obesity : targets and therapy* 13:1133-1145.
- Sejian, V., V. P. Maurya, and S. M. Naqvi. 2012. Effect of walking stress on growth, physiological adaptability and endocrine responses in Malpura ewes in a semi-arid tropical environment. *Int J Biometeorol* 56(2):243-252.
- Sinha, R. A., B. K. Singh, and P. M. Yen. 2014. Thyroid hormone regulation of hepatic lipid and carbohydrate metabolism. *Trends in endocrinology and metabolism: TEM* 25(10):538-545.
- Suh, J. H., D. H. Sieglaff, A. Zhang, X. Xia, A. Cvor, G. E. Winnier, and P. Webb. 2013. SIRT1 is a direct coactivator of thyroid hormone receptor beta1 with gene-specific actions. *PloS one* 8(7):e70097.
- Takemura, K., H. Shingu, H. Mizuguchi, Y. H. Kim, S. Sato, and S. Kushibiki. 2019. Effects of forage feeding on rumen fermentation, plasma metabolites, and hormones in Holstein calves during pre- and postweaning periods¹. *Journal of animal science* 97(5):2220-2229.
- Xu, T., H. Tao, G. Chang, K. Zhang, L. Xu, and X. Shen. 2015. Lipopolysaccharide derived from the rumen down-regulates stearyl-CoA desaturase 1 expression and alters fatty acid composition in the liver of dairy cows fed a high-concentrate diet. *BMC Veterinary Research*.
- Yin, L. 2015. The effects of prolactin and glucocorticoid on lipid metabolism in bovine adipocytes and hepatocytes. Ph.D Thesis, Jilin University (in Chinese).
- Zebeli, Q., S. M. Dunn, and B. N. Ametaj. 2011. Perturbations of plasma metabolites correlated with the rise of rumen endotoxin in dairy cows fed diets rich in easily degradable carbohydrates. *J Dairy Sci* 94(5):2374-2382.

Zhao, F. Q. and A. F. Keating. 2007. Expression and regulation of glucose transporters in the bovine mammary gland. J Dairy Sci 90 Suppl 1:E76-86.

Tables

Table 1 The ingredients in the diets and the nutritional composition

Ingredients (%)	Low concentrate diet	HC diet
Corn	19.4	24.923
Soybean meal	13.5	13.476
DDGS	3.8	5.908
Stone meal	0.8	1.477
CaHCO ₃	1.1	0.923
Salt	0.4	0.37
Premix ¹	1	0.923
Barley	/	12
HC ratio	40	60
Silage corn	12	6
Alfalfa	24	17
Oat grass	24	17
Low concentrate ratio	60	40
Nutritional Composition ²		
NE Mcal/kg	1.64	1.66
CP %	16.12	16.2
Ca %	1.18	1.15
P %	0.51	0.53
NDF %	29.92	27.75
NFC %	42.34	44.47
ASH %	4.87	4.56
EE %	3.05	3.02

¹The premix contained VA, 1,900ku/kg; VD, 250ku/kg; VE, 3,000 mg/kg; Niacin, 4,000 mg/kg; Cu, 1,200 mg/kg; Fe, 525 mg/kg; Zn, 13,000 mg/kg; Mn, 5,500 mg/kg; I, 170 mg/kg; Co, 50 mg/kg; Se, 27 mg/kg.

²The calculated nutritional composition values.

Table 2
Primer sequences of the target genes

Gene	Sequence number	Primer sequences (5'-3')	Length (bp)
SREBP1	NM_001113302.1	F: CATCAGCTCCAGCATGGCT R: TGGGTAGGGGTTTCTCGGA	214
G6PC	NM_001076124.2	F: ATGTTGTGGTTGGGATTCTGG R: CACCTTCGCTTGGCTTTCTC	151
PCK1	NM_174737.2	F: GCCGTGAGGAGTTTCGTG R: TGATGATGACCGTCTTGCT	120
ACC	NM_174224	F: AGCTGAATTTTCGCAGCAAT R: GGTTTTCTCCCCAGGAAAAG	117
ACSL1	NM_001076085.1	F: TCGGAACCTGAAGCCATCACC R: GCCTCGTTCCAGCAGATCAC	144
ATGL	NM_001046005.2	F: TCTGCCTGCTGATTGCTATG R: GGCCTGGATAAGCTCCTCTT	98
HSL	NM_001080220.1	F: ATTGCCGACTTCCTACGAGA R: AGTCCGATGGAGATGGTCTG	113
GLUT1	NM_174602.2	F: GGGCATGTGCTTCCAGTATGTG R: TGTCTCGGGAACCTTTGAAGTAGGTG	106
GLUT4	NM_174604.1	F: TCGCTCTCCAGTTCCTAAGACAAG R: AAGGACCAAGGTCCCAGTGA	153
HK1	NM_001012668.2	F: ACCCTGGGTGCCATCTTGAG R: TCTTGTGGAAACGCCGAGAATA	146
PGL	NM_174179.2	F: GGCTGAGGACTACGCCAAGAA R: CACCCAGAATCAGCAGGTCAA	157
CPT1 α	FJ415874.1	F: TCGCGATGGACTTGCTGTATA R: CGGTCCAGTTTGCGTCTGTA	100
PPAR α	FJ415874.1	F: CATAACGCGATTTCGTTTTGGA R: CGCGGTTTCGGAATCTTCT	102
THRA	NM_001046329.1	F:GTCAACCACCGCAAACAC R:ACAACATGCACTCCGAGAA	134
THRB	XM_024986440.1	F:GGAAGCAGAAGCGGAAGT R:CACATGGCAGCTCACAAA	112
DIO1	NM_001122593.2	F: TGGGGTAGACACAATGACGAA R: GGCCAGATTTACCCTTGTAGGA	106
DIO2	NM_001010992.7	F: CCACCTTCTGGACTTTGCCA R: GGAAGTCAGCCACGGATGAG	134
DIO3	NM_001010993.3	F: TCACTCCCTGAGGCTCTG R: CCCAGTAAATGCTTACGGATG	120
GAPDH	NM_001034034.2	F: GGGTCATCATCTCTGCACCT R: GGTCATAAGTCCCTCCACGA	176

Abbreviations

TG: Triglyceride; TC: Total cholesterol; FFA: Free fatty acid; HDL-C: High density lipoprotein-cholesterol; LDL-C: High density lipoprotein-cholesterol; PGD: Phosphogluconate dehydrogenase; PGL: Succinate dehydrogenase complex subunit D; HK1: Hexokinase 1; PCK1: Phosphoenolpyruvate carboxykinase 1; G6PC: Glucose-6-phosphatase catalytic subunit 1; GLUT1: Solute carrier family 2 member 1; GLUT4: Solute carrier family 2 member 4; ACC: Acetyl-CoA carboxylase alpha; SREBP1: Sterol regulatory element-binding protein 1; ACSL1: Long-chain acyl-CoA synthetase 1; FAS: Fatty acid synthase; ATGL: Patatin like phospholipase domain containing 2; HSL: Hormone-sensitive lipase; CPT1 α : Carnitine palmitoyltransferase 1 α ; PPAR α : Peroxisome proliferator activated receptor α ; T4: Thyroxine; T3: Triiodothyronine; FT3: Free triiodothyronine and; TH: Thyroid hormone; THR: Thyroid hormone receptor; DIO: Iodothyronine deiodinase; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; ACTB: Actin beta; HE: Hematoxylin-eosin; PAS: Periodic Acid-Schiff.

Declarations

Authors' contributions

QC coordinated and ran the animal trial with the help of CW, ZY, and LC. SM and YN designed the study and participated in its coordination. QC performed statistical analysis of phenotypic and biochemical data. QC wrote the main draft of the manuscript, with inputs from YN.

Acknowledgements

The authors acknowledge the financial contributions of the Fundamental Research Funds for the Central Universities (JCQY201905) and National Key Research and Development Project (2016YFD0501203).

Funding

This study is supported with Fundamental Research Funds for the Central Universities (JCQY201905) and National Key Research and Development Project (2016YFD0501203).

Availability of data and materials

The data reported in this manuscript is available upon reasonable request from the corresponding author (YN).

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

All authors declare no conflicts of interest.

Figures

Figure 1

Effects of low or high concentrate diet on serum biochemical parameters at the 21st day. (A) The levels of serum TG. (B) The levels of serum FFA. (C) The levels of serum glucose. (D) The levels of serum TC. (E) The levels of serum LDL-C. (F) The levels of serum HDL-C. Values are mean $\pm$ SEM. The data were analyzed by Independent-Samples T test using the Compare Means of SPASS 19.0 for Windows (StaSoft Inc, Tulsa, OK, USA). * $p < 0.05$, ** $p < 0.01$ (LC vs HC).

Figure 2

Effects of low or high concentrate diet on histological changes and biochemical parameters in the liver. Photomicrograph of HE (A) and PAS (B) staining in cow liver, these pictures are from different cows. (C) The levels of liver glycogen. (D) The levels of liver glucose. (E) The levels of liver TG. (F) The levels of liver FFA. Values are mean $\pm$ SEM. The data were analyzed by Independent-Samples T test using the Compare Means of SPASS 19.0 for Windows (StaSoft Inc, Tulsa, OK, USA). * $p < 0.05$ (LC vs HC).

Figure 3

Expression of genes and proteins related to glycometabolism in the liver. (A) Expression of genes related to glycometabolism in the liver. (B) Expression of proteins related to gluconeogenesis in the liver. The data were analyzed by Independent-Samples T test using the Compare Means of SPASS 11.0 for Windows (StaSoft Inc, Tulsa, OK, USA). Values are mean $\pm$ SEM. * $p < 0.05$ (LC vs HC).

Figure 4

Expression of genes and proteins related to lipid metabolism in the liver. (A) Expression of genes related to lipid metabolism in the liver. (B) Expression of proteins related to fatty acid synthesis in the liver. (C) Expression of proteins related to fatty acid β -oxidation in the liver. The data were analyzed by Independent-Samples T test using the Compare Means of SPASS 11.0 for Windows (StaSoft Inc, Tulsa, OK, USA). Values are mean $\pm$ SEM. * $p < 0.05$, ** $P < 0.01$ (LC vs HC).

Figure 5

Effects of low or high concentrate diet on hormones. (A) The levels of serum cortisol. (B) The levels of serum insulin. (C) The levels of serum T4. (D) The levels of serum T3. (E) The levels of serum FT3. (F) The levels of liver FT3. The data were analyzed by Independent-Samples T test using the Compare Means of SPASS 11.0 for Windows (StaSoft Inc, Tulsa, OK, USA). Values are mean $\pm$ SEM. * $p < 0.05$, ** $P < 0.01$ (LC vs HC).

Figure 6

The expression of genes and protein related to thyroid hormone receptor in the liver. (A) Genes expression of THRA and THRB in the liver. (B) Protein expression of THRB in the liver. The data were analyzed by Independent-Samples T test using the Compare Means of SPASS 11.0 for Windows (StaSoft Inc, Tulsa, OK, USA). Values are mean $\pm$ SEM. * $p < 0.05$ (LC vs HC).

Figure 7

The expression of genes and protein related to iodothyronine deiodinases in the liver. (A) Genes expression of DIO1, DIO2 and DIO3 in the liver. (B) Protein expression of DIO2 in the liver. The data were analyzed by Independent-Samples T test using the Compare Means of SPASS 11.0 for Windows (StaSoft Inc, Tulsa, OK, USA). Values are mean $\pm$ SEM. * $p \leq 0.05$ (LC vs HC).