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Identification of diagnostic biomarkers and metabolic pathway shifts of heat-stressed lactating dairy cows

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ABSTRACT

Controlling heat stress (HS) is a global challenge for the dairy industry. However, simple and reliable biomarkers that aid the diagnoses of HS-induced metabolic disorders have not yet been identified. In this work, an integrated metabolomic and lipidomic approach using ^{1}H nuclear magnetic resonance and ultra-fast LC–MS was employed to investigate the discrimination of plasma metabolic profiles between HS-free and HS lactating dairy cows. Targeted detection using LC–MS in multiple reaction monitoring mode was used to verify the reliability of the metabolites as biomarker candidates. Overall, 41 metabolites were identified as candidates for lactating dairy cows exposed to HS, among which 13 metabolites, including trimethylamine, glucose, lactate, betaine, creatine, pyruvate, acetoacetate, acetone, β-hydroxybutyrate, C16 sphinganine, lysophosphatidylcholine (18:0), phosphatidylcholine (16:0/14:0), and arachidonic acid, had high sensitivity and specificity in diagnosing HS status, and are likely to be the potential biomarkers of HS dairy cows. All of these potentially diagnostic biomarkers were involved in carbohydrate, amino acid, lipid, or gut microbiome-derived metabolism, indicating that HS affected the metabolic pathways in lactating dairy cows. Further research is warranted to evaluate these biomarkers in practical applications and to elucidate the physiological mechanisms of HS-induced metabolic disorders.

Biological significance

Heat stress (HS) annually causes huge losses to global dairy industry, including animal performance decrease, metabolic disorder and health problem. So far, physiological mechanisms underlying HS of dairy cows still remain elusive. To our best knowledge, this is the first attempt to elucidate the HS-induced metabolic disorders of dairy cows using integrated ^{1}H NMR and LC–MS-based metabolic study. The results not only provided potential diagnostic biomarkers for HS lactating dairy cows, but also significantly explored the related physiological mechanisms of metabolic pathway shifts induced by HS environment. This work offers comprehensive insights into the global metabolic alterations of dairy cows exposed to HS and provides a new perspective for further study.

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1. Introduction

Environmentally-induced hyperthermia is a world-wide problem for the dairy industry. In southern China, heat stress (HS) causes most dairy farms to be profitless during the summer [1,2]. In the US, HS is responsible for an estimated annual loss of 1 billion dollars to the dairy industry [3]. Furthermore, studies in Germany indicate that HS can reduce the reproductive efficiency of dairy cows from 55% to 23% [4]. All of these losses are linked directly to serious metabolic disorders and health problems induced by HS [5,6]. The HS challenge faced by the dairy industry will be even more severe in the future due to the potential impact of global climate change and the rapid increase in milk productivity pushed by modern molecular genetic technologies [7].

The temperature and humidity index (THI) has traditionally been employed as an index of the HS status of dairy cows. However, THI is based only on air temperature and humidity [5], and is not a direct biomarker of metabolic alterations in response to HS. A gap still exists between the availability of reliable diagnostic biomarkers of HS and the urgent requirements of the dairy industry.

The radical physiological changes that occur in HS-exposed dairy cows are intricate and multifactorial. Wheelock et al. [6] and Baumgard et al. [8] performed a number of studies and concluded that HS-induced decreases in dry matter intake account for only approximately 50% of the reduction in milk yield, and that alteration in post-absorptive metabolism may account for a large portion of the remaining milk loss; however, the mechanisms underlying these changes remain elusive [6]. Metabolomics has been a powerful platform for the identification of low-molecular weight biomarkers in plants, animals, and humans associated with pathophysiological alterations resulting from exposures to specific environmental factors [9–13]. Hence, metabolic profiling may enable the identification of biomarkers and the development of early measures to diagnose or manipulate HS-induced metabolic disorders.

^{1}H nuclear magnetic resonance (NMR) and LC–MS techniques, which are most frequently utilized individually to study metabolic changes, always yield complementary results [10,14–16]. Here, we integrated ^{1}H NMR and LC–MS techniques to detect metabolic differences between HS-free and HS mid-lactation cows. Because lipid metabolism is reportedly disrupted in dairy cows exposed to HS [6], LC–MS-based lipidomic analysis was simultaneously performed to analyze more global metabolic alterations induced by HS [17,18]. The LC–MS targeted analyses were used to verify the reliabilities of the discriminating metabolites by providing high-quality data [19–21]. Receiver operating characteristic (ROC) analysis of the candidates was performed to determine whether they had high sensitivity and specificity in diagnosing HS status. This study demonstrated that integrated ^{1}H NMR and LC–MS, in combination with metabolomic and lipidomic analyses, can provide comprehensive insights into the metabolic alterations in dairy cows exposed to HS. An overview of the study design is shown in Fig. S1 of Supporting information 1.

Table 1 – Characteristics of the Holstein dairy cows used in the study.

Item	HS-free		HS	
Parity	Second		Second	
Lactation days	137	161	137	161
No. of cows	11	11	11	11
Average weight (kg)	645.5 ± 26.7	633.0 ± 20.1	649.3 ± 23.6	638.5 ± 17.6

HS, heat stress.

2. Material and methods

2.1. Reagents and standards

HPLC-grade ACN, isopropanol, methyl tert-butyl ether, water, formic acid, and ammonium formate were purchased from Merck (Darmstadt, Germany); deuterium oxide was purchased from USA Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA); and 3-(trimethylsilyl) propionic-2,2,3,3,d4 propionic acid sodium salt was from Merck Canada Inc. (Kirkland, QC, Canada). All standard compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sample collection

All experiments involving animals were conducted according to the principles of the Chinese Academy of Agricultural Sciences Animal Care and Use Committee (Beijing, China). All 44 Holstein cows were fed the same diet. Information about the numbers, lactation days, and body weight of the dairy cows is shown in Table 1. According to NRC (1971), THI was calculated using the formula: $THI = (1.8 \times T_{db} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26.8)]$, where T_{db} is the dry-bulb temperature (°C) and RH is the relative humidity (%). Fasting blood samples were collected before morning feeding from the caudal veins of Holstein dairy cows raised at Xinghuo Second Dairy Farm (Shanghai, China). The HS-free group consisted of 22 cows, with samples obtained in April (spring season), after natural THI was around 50–55 for 1 month. The HS group consisted of another 22 cows, with samples obtained in July (summer season), after THI gradually increased from 68 to 80 over 1 month and remained stable at 80 for 1 week. Feed composition, temperature, humidity, rectal temperature, respiration rate, and production characteristics of the two groups of dairy cows are shown in Tables S1–S4 of Supporting information 1.

2.3. Sample preparation

Blood samples were collected into K2 EDTA anti-coagulation vacuum tubes and centrifuged at 1600 *g* for 10 min at 4 °C. The supernatants were transferred to tubes, frozen quickly, and stored at −80 °C until use. For LC–MS metabolomic analysis, 150 μL aliquots of plasma were mixed with 600 μL ice-cold acetonitrile, vortexed, and centrifuged for 10 min at 10,000 rpm and 4 °C. The 650 μL supernatant of each sample was transferred to another tube, and concentrated to dryness with a SpeedVac Concentrator (SPD121P, Thermo Savant,