Investigating the impact of heat stress and subsequent recovery on fatty acid profiles in bovine milk

P.A. Tu[1], M.K. Yang[1], Y.H. Yeh[1], J.W. Shiau[1], J.F. Huang[2] and Y.H. Chen[1]

[1]Northern Region Branch, Taiwan Livestock Research Institute, Ministry of Agriculture, No.207-5, Pitoumian, Xihu Township, Miaoli 368003, Taiwan
[2]Taiwan Livestock Research Institute, No.112, Farm Road, Hsinhua, Tainan 71246, Taiwan
Corresponding Author: hsuan@mail.tlri.gov.tw

Abstract

Heat stress (HS), particularly prevalent in tropical regions such as Taiwan, poses significant threats to animal health, dairy production, and the composition of milk. This stress affects not only the welfare and productivity of cows but also increases the costs of herd management, thereby impacting the profitability of dairy farming. Under the ongoing climate change, Taiwan is expected to experience more frequent high-temperature days, emphasizing the need to evaluate and mitigate the adverse effects of HS on dairy production. HS induces various physiological changes in dairy cattle, including increased respiration and heart rates, along with a rise in core body temperature. The most profound impacts of HS are observed in the form of reduced dry matter intake and a decline in milk yield. These changes are attributed to energy-intensive metabolic adaptations that cattle undergo for heat dissipation, which in turn contribute to the decrease in milk production. An experimental study of HS was conducted over 4 consecutive days, with a daily average temperature-humidity index (THI) exceeding 74, followed by a recovery period with an average daily THI below 68. Milk samples were collected bi-daily during this period, which included a baseline phase (days 1-3), the HS phase (days 5 and 7), and the recovery phase (days 9 and 11). These samples were analyzed for their fatty acid (FA) profiles, including saturated FA (SFA), unsaturated FA (UFA), mono-unsaturated FA (MUFA), poly-unsaturated FA (PUFA), short-chain FA (SCFA), medium-chain FA (MCFA), long-chain FA (LCFA), total de novo FA, mixed FA, and preformed FA, using MilkoScan FT$^+$ 300 equipped with Fourier-transform infrared spectra. The results from this experiment showed that HS caused a significant reduction in the relative percentage of SFA, de novo FA, mixed FA, MCFA, C14:0, and C16:0 FAs, accompanied by an increase of that in UFA, preformed FA, LCFA, C18:0, and C18:1 FAs. These changes in the FA profile are expected to alter the physical properties and nutritional value of milk fat. While some FA levels partially returned to normal during the recovery phase, they did not fully revert after short periods of recovery. This study highlights the metabolic adaptations of lactating cattle in response to acute HS. There was a noticeable shift in the milk FA profile, characterized by a decrease in FAs predominantly containing SCFA to MCFA, and an increase in those primarily consisting of LCFA. These alterations in FAs could potentially serve as biomarkers for HS in dairy cattle, providing a valuable tool for daily monitoring and management. In conclusion, HS profoundly influences the FA profile of bovine milk, signifying a metabolic shift towards increased LCFA. This alteration, not completely reversible even in a short-term recovery phase, strengthens the critical need for effective HS management and abatement strategies in dairy farming. This is particularly urgent given the rising global temperatures, which could exacerbate the HS challenges to the dairy industry in Taiwan.

Keywords: milk, fatty acid, heat stress, recovery.
Presented at: Session 2: SC Milk Analysis – New tools to extend the horizon of milk mid-infrared spectrometry

Introduction

HS is particularly prevalent in tropical regions such as Taiwan and poses significant threats to animal health, dairy production, and the composition of milk (Liu *et al*, 2019). This stress not only affects the welfare and productivity of cows but also increases the costs of herd management, thereby impacting the profitability of dairy farming (Kadzere *et al*, 2002). Under ongoing climate change, it is expected that Taiwan will experience more frequent high-temperature days (Ho *et al*, 2016) which emphasizes the need to evaluate and mitigate the adverse effects of HS on dairy production (Summer *et al*, 2019). Physiological changes induced by HS in dairy cattle include increased respiration and heart rates along with a rise in core body temperature (Polsky and Keyserlingk, 2017). The most profound impacts are observed in reduced dry matter intake and decline in milk yield - attributed to energy-intensive metabolic adaptations for heat dissipation contributing to decreased milk production.

The influence of HS on milk composition, beyond milk yield, has been the subject of extensive research. Several studies have reported a decrease in total protein and total fat content in milk under HS conditions (Bernabucci *et al*, 2015; Hill and Wall, 2015). However, conflicting findings exist, with some studies indicating no significant decrease in fat percentage in heat-stressed cows (Hammami *et al*, 2015; Lacetera *et al*, 2003). Additionally, there is evidence suggesting that an increase in the THI is associated with a decrease in the content of short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA), and an increase in long-chain fatty acids (LCFA) (Lacetera *et al*, 2003). Despite these findings, there is a lack of detailed information regarding the impact of HS and subsequent recovery on milk FA profiles. Therefore, our study aims to investigate the effects of HS and subsequent recovery on milk FA profiles.

Material and methods

Cows and experimental design

- The study was conducted using lactating Holstein cows in a commercial dairy farm in Taiwan. A total of 77 Holstein dairy cows in early lactation were subjected to a heat challenge for over 3 consecutive stages. First with a baseline phase with a temperature-humidity index (THI) below 68. Then, an HS phase with a daily average THI exceeding 74, followed by a recovery period with an average daily THI below 68. Milk samples were collected bi-daily during this period, which includedBaseline phase (day 1-3).
- HS phase (day 5 and day 7), and the
- Recovery phase (day 9 and day 11).

Milk FA profile analysis

Milk samples were thoroughly analyzed for a variety of fatty acid profiles, which included saturated FA (SFA), unsaturated FA (UFA), mono-unsaturated FA (MUFA), poly-unsaturated FA (PUFA), short-chain FA (SCFA), medium-chain FA (MCFA), long-chain FA (LCFA), *de novo* FA/newly synthesized FA, mixed FA, C14:0 fatty acid (FA), C16:0 FA, C18:0 FA, and C18:1 FA . The analysis was performed using the MilkoScan FT^{+} 300 equipped with Fourier-transform infrared spectra.

Statistical analysis

Statistical analysis included the use of ANOVA and Tukey's post hoc test to assess differences in FA profiles among the different days in baseline, HS, and recovery phases.

Results

Effects of HS on FA profiles in milk

The results from the study showed that HS caused a significant reduction in the relative percentage of SFA, de novo FA, mixed FA, MCFA, C14:0, and C16:0 FAs, accompanied by an increase in USFA, preformed FA, LCFA, C18:0, and C18:1 FAs (Figure 1 and 2). These changes in FA profiles indicate that HS leads to alterations in milk composition, with a shift towards higher levels of unsaturated and long-chain fatty acids. The metabolic shifts observed during HS, particularly the decrease in FAs predominantly containing SC FA to MCFA, and an increase in those primarily consisting of LCFA, demonstrate the profound influence of HS on the metabolic adaptations of lactating cattle (Table 1).

Recovery of FA Profiles following HS

The recovery phase showed some restoration in the FA profiles, with a partial reversal of the changes observed during HS. While some FA levels partially returned to normal during the recovery phase, they did not fully revert after short periods of recovery (Figure 1 and 2). The *de novo* FA, mixed FA, SCFA, C14:0 FA, C16:0 and MCFAs' levels showed a partial recovery but remained lower than baseline levels during the recovery phase. For UFA, PUFA, LCFAs', C18:0 and C18:1 FAs didn't show significant reduction during the recovery phase. During this period MUFA and preformed FAs significantly decreased ($P < 0.05$), suggesting that HS impact on bovine milk's FA profiles is not completely reversible in the short term (Table 1).

Table 1. Effect of heat stress on fatty acid (FA) composition of milk fat.

Milk FA	mg/g of total FA					
	Baseline	D5 heat stress	D7 heat stress	D9 Recovery	D11 Recovery	P value
C14:0	96.9^{a}	93.2^{a}	86.1^{b}	78.4^{c}	83.1^{bc}	< 0.0001
C16:0	347.8^{a}	301.2^{d}	309.9^{cd}	316.7^{c}	334.4^{b}	< 0.0001
C18:0	84.1^{b}	83.8^{b}	89.5^{a}	90.4^{a}	88.4^{ab}	< 0.0001
C18:1	198.7^{d}	223.8^{bc}	232.6^{ab}	236.1^{a}	220.8^{c}	< 0.0001
Saturated FA	681.1^{a}	619.7^{bc}	611.2^{c}	606.1^{c}	633.2^{b}	< 0.0001
Unsaturated FA	234.7^{c}	262.8^{b}	278.4^{a}	275.2^{a}	254.1^{b}	< 0.0001
Mono-unsaturated FA	213.1^{d}	242.5^{b}	253.5^{a}	249.6^{ab}	229.3^{c}	< 0.0001
Poly-unsaturated FA	21.7^{b}	20.3^{b}	24.9^{a}	25.6^{a}	24.8^{a}	< 0.0001
SCFA	83.2^{a}	72.5^{b}	71.7^{b}	71.7^{b}	76.4^{b}	< 0.0001
MCFA	525.7^{a}	452.6^{c}	453.9^{c}	457.5^{c}	488.9^{b}	< 0.0001
LCFA	299.9^{c}	322.7^{b}	337.9^{a}	346.5^{a}	319.6^{b}	< 0.0001
Total de novo FA	216.1^{a}	169.0^{c}	165.2^{c}	172.0^{c}	190.8^{b}	< 0.0001
Mixed FA	385.4^{a}	357.1^{c}	366.3^{bc}	366.9^{bc}	378.5^{ab}	0.008
Preformed FA	331.9^{c}	384.6^{a}	387.9^{a}	386.5^{a}	355.8^{b}	< 0.0001

Each value is the mean of 77 samples.
Total de novo FA = sum of C4:0 to C14:1 fatty acids; total mixed FA = sum of C16:0 and C16:1 fatty acids; total preformed = sum of all fatty acids with more than 15 carbon atoms.
$^{a-d}$ Least squares means with different superscripts within a row are significantly different (P < 0.05).

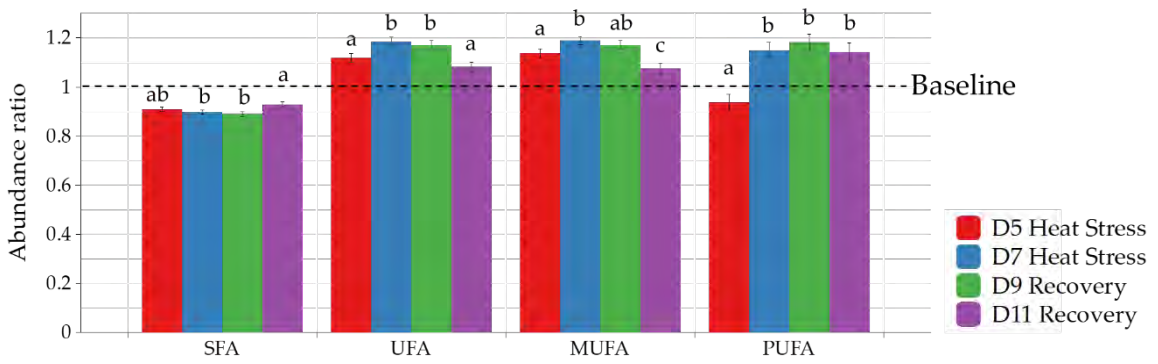

Figure 1. Effect of heat stress on the relative abundance of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and unsaturated fatty acids (UFA) in milk. Abundance ratios for SFA, MUFA, PUFA, and UFA during days 5 and 7 of heat stress (HS) and days 9 and 11 of recovery are presented in comparison to the baseline levels observed on days 1 to 3. Error bars represent standard error (n = 77). Statistically significant differences among different HS and recovery phases are indicated by different superscripts (P < 0.05).

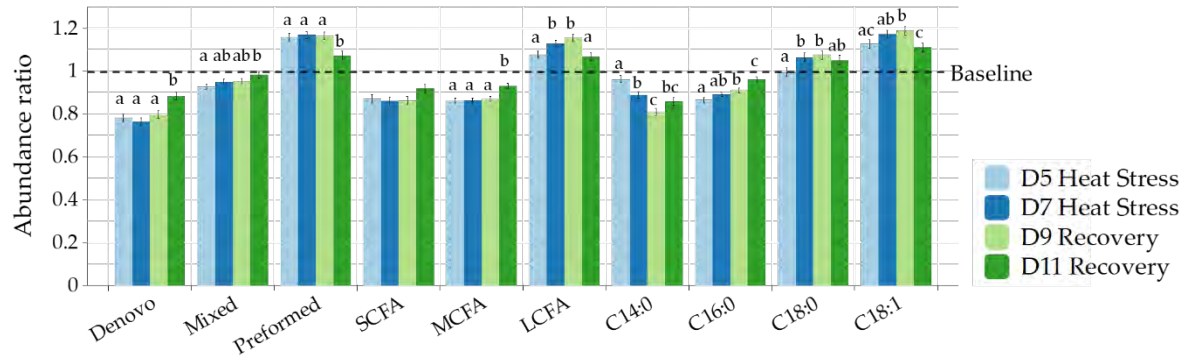

Figure 2. Effect of heat stress on the relative abundance of de-novo fatty acids (de-novo FA), mixed fatty acids (mixed FA), preformed fatty acid (preformed FA), short chain fatty acid (SCFA), medium chain fatty acid (MCFA), long chain fatty acid (LCFA), C14:0 fatty acid (FA), C16:0 FA, C18:0 FA, and C18:1 FA during days 5 and 7 of heat stress (HS) and days 9 and 11 of recovery are presented in comparison to the baseline levels observed on days 1 to 3. Error bars represent standard error (n = 77). Statistically significant differences among different HS and recovery phases are indicated by different superscripts (P < 0.05).

Discussion

It is well established that most of the C4:0 to C14:0 and almost half of the C16:0 FA in milk are synthesized de novo in the mammary gland, whereas the rest of the C16:0 and approximately all LCFA originate from blood lipids (Knudsen *et al*, 1986). HS can significantly alter the synthesis and composition of these FAs in bovine milk (Li *et al*, 2016). Our study's findings highlight the metabolic adaptations of lactating cattle in response to HS. The significant alterations in the FA profiles, particularly the shift towards increased LCFA, are indicative of the physiological changes that occur in dairy cows during HS and subsequent recovery periods.

Understanding these changes in the FA profile is crucial for evaluating the impact of HS on the nutritional composition of milk and its potential implications for human consumption. Furthermore, the results suggest that the altered FA profiles observed during HS may have implications for dairy product quality (Jenkins and McGuire, 2006). These findings have important implications for the dairy industry, as they demonstrate that HS can significantly impact the FA composition of milk. HS affects the quality of dairy products primarily by altering the composition of FAs in cow's milk, which can have implications for the nutritional value, taste, and processing properties of the products (Liu *et al*, 2017). Research indicates that HS causes changes in the triacylglycerol (TAG) profile of milk, with a reduction in TAG groups containing SCFA and MCFA and an increase in those containing LCFA. This change in TAG composition could modify the physical properties of milk fat. Additionally, HS was shown to significantly reduce the levels of certain polar lipid classes, which are main structural constituents of the milk fat globule membrane and play a critical role in stabilizing the milk emulsion system (McManaman, 2014).

The reduction in SCFA and the increase in LCFA during HS is a clear indication of the metabolic adaptations of lactating cattle to high-temperature conditions. Hammami *et al* (2015) also showed similar results, indicating that the rise in THI between seasons correlated with a reduction in the content of SCFA and MCFA, and an increase in LCFA. HS might also lead to a decline in total protein and total fat content in milk, which can affect the texture and flavor of dairy products (Chandan, 1997). Furthermore, the reduction in milk fat globule membrane polar lipids, such as sphingomyelin which has beneficial effects on human health, could have implications for the nutritional value of milk and its health benefits (Lopez *et al*, 2008). Consequently, HS not only presents challenges for maintaining the welfare and productivity of dairy cattle but also for preserving the quality of milk and dairy-related foods.

The findings of this study underscore the need for effective HS management strategies in dairy farming, especially in regions like Taiwan that are projected to experience more frequent high-temperature days due to climate change. Adaptive measures such as improved ventilation, access to shade, and cooling systems can help mitigate the adverse effects of HS on dairy cattle, thereby preserving milk quality and quantity. Furthermore, the observed partial recovery of FA profiles during the recovery phase suggests that interventions to support lactating cows during and after HS periods can aid in restoring milk composition to some extent. This highlights the potential for targeted nutritional and management interventions to minimize the long-term impact of HS on milk FA profiles and overall dairy production.

The study also underscores the potential use of the observed changes in FA profiles as biomarkers for HS in dairy cattle. The relatively rapid change of these markers made it possible for routine monitoring and early detection of heat stress in dairy cows. This suggests that monitoring the FA profiles of milk could provide a valuable tool for daily assessment and management of HS in dairy farming. Identifying and implementing effective heat alleviation strategies based on these biomarkers could help minimize the impact of HS on dairy production and product quality.

Conclusion

The study provides important insights into how heat stress affects lactating cattle's metabolism and milk composition. It highlights the increase in long-chain fatty acids in bovine milk and emphasizes the need for proactive measures to protect dairy production from environmental challenges. Understanding these biochemical changes can help dairy farmers implement strategies to maintain animal well-being and milk quality in changing climate conditions, while also serving as valuable biomarkers for monitoring heat stress effects on milk production.

List of references

Chandan, R.C., 1997. Chapter 1: Properties of Milk and Its Components. In: Properties of Milk and Its Components, R.C. Chandan (author), American Dairy Science Association, Champaign, IL, p 1-25.

Bernabucci, U., Basiricò, L., Morera, P., Dipasquale, D., Vitali, A., Cappelli, F.P., and Calamari, L.U.I.G.I., 2015. Effect of summer season on milk protein fractions in Holstein cows. J. Dairy Sci. 98(3), 1815-1827.

Hammami, H., Vandenplas, J., Vanrobays, M. L., Rekik, B., Bastin, C., and Gengler, N., 2015. Genetic analysis of heat stress effects on yield traits, udder health, and fatty acids of Walloon Holstein cows. J. Dairy Sci. 98(7), 4956-4968.

Hill, D. L. and Wall, E., 2015. Dairy cattle in a temperate climate: the effects of weather on milk yield and composition depend on management. Animal. 9, 138-49.

Ho, C., Chen, J., Nobuyuki, Y., Lur, H., and Lu, H., 2016. Mitigating uncertainty and enhancing resilience to climate change in the fisheries sector in Taiwan: Policy implications for food security. Ocean and Coastal Management 129: 50-65.

Jenkins, T. and McGuire, M.A., 2006. Major Advances in Nutrition: Impact on Milk Composition. J. Dairy Sci. 89(4): 1302-1310.

Kadzere, C., Murphy, M.R., Silanikove, N., and Maltz, E., 2002. Heat stress in lactating dairy cows: a review. Livest. Prod. Sci. 77(1): 59-91.

Knudsen, J., Hansen, H.O., and Mikkelsen, J., 1986. Medium-chain fatty acid synthesis. Biochem. Soc. Trans. 14(3): 575-599.

Lacetera, N., Bernabucci, U., Ronchi, B., and Nardone, A., 2003. Physiological and productive consequences of heat stress. The case of dairy ruminants. Interaction between climate and animal production, 45-59.

Li, M., Zhao, S., Tian, H., Zhou, X., Zhang, Y., Li, S., Yang, H., Zheng, N., and Wang, J., 2016. Metabolic responses and "omics" technologies for elucidating the effects of heat stress in dairy cows. Int. J. Biometeorol. 61(6): 1149-1158.

Liu, J., Li, L., Chen, X., Lu, Y., and Wang, D., 2019. Effects of heat stress on body temperature, milk production, and reproduction in dairy cows: a novel idea for monitoring and evaluation of heat stress. Asian-Australas. J. Anim. Sci. 32(9): 1332-1339.

Liu, Z., Ezernieks, V., Wang, J., Arachchillage, N.W., Garner, J.B., Wales, W.J., Cocks, B.G., and Rochfort, S., 2017. Heat Stress in Dairy Cattle Alters Lipid Composition of Milk. Sci. Rep. 7: Article 11209.

Lopez, C., Briard-Bion, V., Ménard, O., Rousseau, F., Pradel, P., and Besle, J., 2008. Phospholipid, Sphingolipid, and Fatty Acid Compositions of the Milk Fat Globule Membrane are Modified by Diet. J. Agric. Food Chem. 56(10): 5226-5236.

McManaman, J.L., 2014. Lipid Transport in the Lactating Mammary Gland. J. Mammary Gland Biol. Neoplasia 19(1): 35-42.

Polsky, L. and Keyserlingk, M.V., 2017. Invited review: Effects of heat stress on dairy cattle welfare. J. Dairy Sci. 100(11): 8645-8657.

Summer, A., Lora, I., Formaggioni, P., and Gottardo, F., 2019. Impact of heat stress on milk and meat production. Anim. Front. 9(1): 39-46.