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Variations in milk production based on the temperature-humidity index and blood metabolic parameters in cows during exposure to heat stress*

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The temperature-humidity index (THI) is conventionally used to measure heat stress. The aim of this paper is to determine the influence of THI and THI+metabolic parameters in prediction of milk production. A total of thirty Holstein-Friesian cows were exposed to heat stress and the THI values, milk production and metabolite concentrations were measured on days 0 (in the thermoneutral period), 7 and 14 after the exposure. The average daily THI values obtained were 65±1.05 (day 0), 75±1.1 (day 7) and 77±1.4 (day 14). Heat-stressed cows were found to exhibit a decrease in milk production, contents of glucose and non-esterified fatty acids (NEFA) and the glucose-to-insulin ratio (G:I), whereas the levels of insulin, tumor necrosis factor-α (TNF-α) and the revised quantitative insulin sensitivity check index (RQUICKI) turned out to be elevated. THI can explain 37% of the milk production variance. The percent variance explained is significantly higher after the addition of metabolic parameters in the THI+glucose (58%) and THI+glucose+TNF-α (65%) models and non-significantly higher after the addition of other metabolic parameters. Partial correlation analysis showed that a correlation between milk production and THI is significantly dependent on glucose. TNF-α showed a tendency to regulate the above-mentioned correlation, while other metabolic parameters showed a non-significant effect on the correlation between THI and

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milk production. It was concluded that variation in milk production during heat stress could be better predicted when THI is used in combination with glucose and TNF-α as a metabolic predictor. The effect of glucose and TNF-α in milk production during heat stress would be investigated in the next stage of our research.

KEY WORDS: cows / heat stress / milk production / metabolism

The climate of Europe has changed markedly, as indicated by an overall temperature increase of 1°C, increased precipitation in Northern Europe,as well as a greater number of hot, sunny days in Sothern Europe, accompanied by occasional heat waves. Vranic and Milutinovic [2016] stated that Serbia will likely experience an increase in temperature of up to 4°C and a decrease in summer precipitation of up to 50%. Heat stress is an important factor, which decreases sustainability of milk production. The main indicator of thermal stress is the temperature-humidity index (THI), which represents the relathionship between air temperature and relative humidity. Heat stress in dairy cows occurs when THI exceeds 72 [Kadzere *et al*. 2002]. In the Vojvodina region (north of Serbia) maximum daily THI values showed a positive trend from 2005 to 2016 for all months except for January, October and November [Cincović *et al.* 2017]. Cincović *et al*. [2017] presented a negative correlation between THI and milk production and a positive correlation with udder skin temperature.

Blood parameters and metabolic adaptation are related to milk production in dairy cows [Jóźwik *et al*. 2012, Puppel and Kuczyńska 2016]. Metabolic changes and differences in nutrient metabolism during heat stress exposure are markedly enhanced [Belhadj Slimen *et al*. 2017]. Heat stress causes a decreased dry mater intake and glucose concentration, and an increased insulin concentration, with reduced lipid mobilisation from adipose tissue. The NEFA values remained relatively constant during heat stress, or turned out to be slightly lower owing to elevated insulin concentrations [Wheelock *et al.* 2010, O'Brain *et al.* 2010]. Cows with a considerable drop in milk production (over 18%) during heat stress showed a great reduction in glucose and NEFA concentrations and increasedinsulin concentration and insulin sensitivity indexes in relation to cows with a moderate or low decrease in milk production [Majkić *et al*. 2017]. In their study Min *et al*. [2016] argued that long-term moderate heat stress results in an increased plasma TNF-α (Tumor necrosis factor-α) level and induces inflammation in cows.

We have hypothesised that milk production during heat stress depends on the THI index, but also on the metabolic adaptation of cows. The aim of this study is to determine an influence of THI and THI+metabolic parameters in prediction of milk production.

Material and methods

Cows and Management. A total of thirty Holstein-Friesian cows were enrolled in the study. The average milk yield was 7,995 L per cow in the last lactation considered.

All the cows were in mid lactation and in optimal body condition. They were managed in the same manner and fed the same total mixed rations containing alfalfa hay, corn silage (30% dry matter, DM) and concentrate (18% crude protein, CP). The chemical composition of the TMR diet offered was in accordance with the Nutrient Requirements of Dairy Cattle.

Measurements of THI (temperature-humidity index). The stress load testing of cows in this study was based on the THI index, expressed by the following formula (Kibler, 1964): *THI= 1.8Ta - (1 - RH) (Ta - 14.3) + 32*; where *Ta* is the measured ambient temperature in °C and *RH* is relative humidity as a fraction of the unit. *THI* was calculated on days 0 (in the thermoneutral period), 7 and 14 after heat stress exposure.

Milk production measurements. The farm in this study features a herringbone milking parlour with automatic milk quantity measuring devices. Cows were milked twice a day. The amount of milk was expressed as cow/liter/day.

Blood and milk sampling: Blood samples were taken on days 0, 7 and 14 after heat stress exposure by coccygeal venipuncture. Blood was taken before morning feeding to minimize the postprandial effect on metabolic values. Upon laboratory analysis, the following parameter values were determined: glucose (Biosystem, Es), NEFA (Randox, UK), insulin (Cusabio, Ch) and the TNF-α (Cloud-Clone Corp., US). Blood analysis for biochemistry parameters was performed on automatic biochemistry analyzer (Chemray 120) and for insulin and TNF-α on the ELISA reader (RT-2100C) manufactured by Rayto (Ch). The values of the insulin sensitivity/resistance index was determined using the RQUICKI index and the glucose-to-insulin ratio (G:I) ratio. The RQUICKI is calculated according to the following formula: RQUICKI = 1/ [log (glucose mg/dL) + log (insulin μU/mL) + log (NEFA mmol/l)].

Data Analysis. The effects of heat stress on milk production and metabolic adaptations were determined by ANOVA and by comparing the parameter values recorded at days 0, 7 and 14 after heat stress exposure. Moreover, the applicability of multiple regression models in assessing milk production was also investigated. Regression analysis was performed using the pool of samples collected at days 0, 7 and 14 of the measurement (N=30×3=90). The independent variables for regression analysis initially included THI, whereas 6 types of multiple regression models used subsequently involved the following metabolic variables: a) THI+glucose, b) THI+NEFA, c) THI+insulin, d) THI+RQUICKI, e) THI+G:I, f) THI+TNF-α, and g) THI+significant metabolic parameters from models a-f. The overall statistical significance was determined for the models employed, as well as each variable separately and the milk production variance. Significance of the difference between two correlation coefficients was calculated using the Fisher r-to-z transformation. The multiple regression results are displayed using scatter plots, indicating the predicted values of milk production on the x-axis and the measured values of milk production on the y-axis. The effect of metabolic parameters as a control factor on the relationship between milk production and THI was determined by partial correlation. The scatter

diagrams show the linear regression lines between the parameter values before and after the exclusion of metabolic parameters as a control factor (as a residual correlation). The SPSS (IBM) statistical software was used for data analysis.

Results and discussion

The average daily THI values were significantly higher in the cows exposed to heat stress. The heat-stressed cows were found to exhibit a decrease in milk production, glucose and NEFA concentrations, whereas the concentrations of insulin and TNF-α were higher in comparison to the control. The RQUICKI values increased over the time of the experiment, whereas the G:I index decreased (Tab. 1).

Table 1. Influence of heat stress on milk production and metabolic parameters

Item	Thermoneutral control	Heat stress		P-value	LSD at minimum p<0.05
Measurement day	0	7	14		
Daily THI	65±1.05	75±1.1	77±1.4	0.0001	0:7, 0:14, 7:14
Milk (L/day)	27.01±2.5	23.95±2.1	21.97±2.05	0.0001	0:7, 0:14, 7:14
GLU (mmol/L)	3.14±0.36	2.13±0.31	1.98±0.2	0.0004	0:7, 0:14
NEFA (mmol/L)	0.19±0.05	0.17±0.05	0.13±0.03	0.0002	0:14
Insulin (ng/mL)	8.35±1.53	9.26±1.7	10.03±1.83	0.0015	0:14
RQUICKI	0.52±0.04	0.57±0.05	0.62±0.06	0.0003	0:7, 0:14, 7:14
G:I	0.39±0.06	0.24±0.05	0.2±0.04	0.0004	0:7, 0:14, 7:14
TNF-α (ng/mL)	4.35±0.59	5.15±0.61	5.68±0.55	0.0002	0:7, 0:14, 7:14

THI can explain 37% of the milk production variance. The percent variance explained is significantly higher after the addition of metabolic parameters in the THI+glucose (58%) and THI+glucose+TNFalpha (65%) models and non-significantly higher after the incorporation of other metabolic parameters. Results are presented in Table 2, Figs. 1 and 2. Heat stress significantly reduces milk productivity. Milk production per cow was negatively correlated with an increase in maximum, minimum and average THI (-0.11, -0.08, and -0.15 kg/THI unit increase, respectively) up to 3 days before milking (Wildridge et al. 2018). THI is a good predictor of milk production and it has to be measured at different locations in the barn [Herbut and Angrecka 2012]. In our previous study we found that 52% of milk variation is determined by the THI index with a negative correlation between THI and milk production [Cincović *et al.* 2017]. A lower coefficient of determination in this experiment in relation to the above-mentioned is a consequence of the smaller number of animals in the experiment and a lower number of THI measurement points and a smaller number of milk production data.

Dependent varibles included in each multiple regression model employed greatly affect the overall accuracy of the model (Tab. 2). In the THI+NEFA, THI+insulin, THI+RQUICKI and THI+G:I models, THI was statistically significant, whereas other variables were not. Both variables were statistically significant in the THI+TNF-α

Table 2. Predictions of milk production using different models (Model 1 – THI as an independent variable. Model 2 – THI+individual metabolites. Model 3 – THI+statistically significant metabolites from model 2)

Model number	Independent variables	Unstandardised coefficients		Significance of variable – p value	R^2 % of the variance explained	Significance of model – p value
		B	standard error			
1	THI	-0.38	0.05	0.001*	0.37[a]	<0.001
2	THI	-0.14	0.074	0.049*	0.59[b]	<0.001
	Glucose	2.39	0.66	0.001*		
	THI	-0.39	0.043	0.001*	0.51[a]	<0.001
	NEFA	-1.91	4.3	0.66		
	THI	-0.38	0.042	0.001*	0.51[a]	<0.001
	Insulin	0.053	0.121	0.63		
	THI	-0.33	0.047	0.001*	0.52[a]	<0.001
	RQUICKI	-6.47	3.78	0.091		
	THI	-0.30	0.062	0.001*	0.52[a]	<0.001
	G:I	4.89	3.15	0.124		
	THI	-0.30	0.049	0.001*	0.56[a]	<0.001
	TNF-α	-0.72	0.304	0.02*		
3	THI	-0.09	0.08	0.27	0.65[b]	<0.001
	Glucose	2.29	0.64	0.001*		
	TNF-α	-0.66	0.29	0.03*		

[ab]Means bearing different superscript differ significantly at min p<0.05 from model 1.

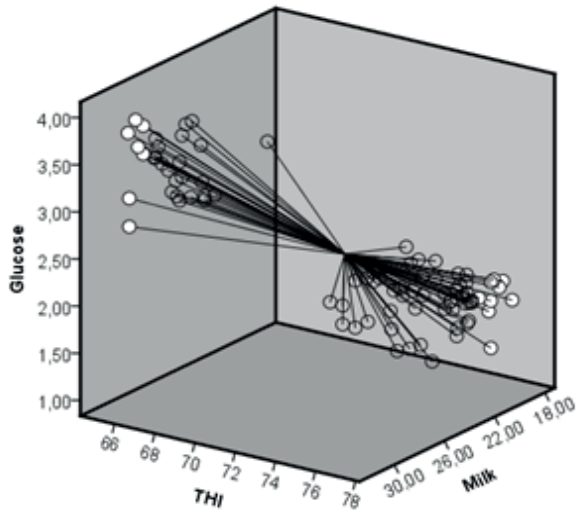

Fig. 1. Milk production in model the THI+glucose model (white circle – cows with higher milk production in the thermoneutral period and lowest milk production in heat stress).

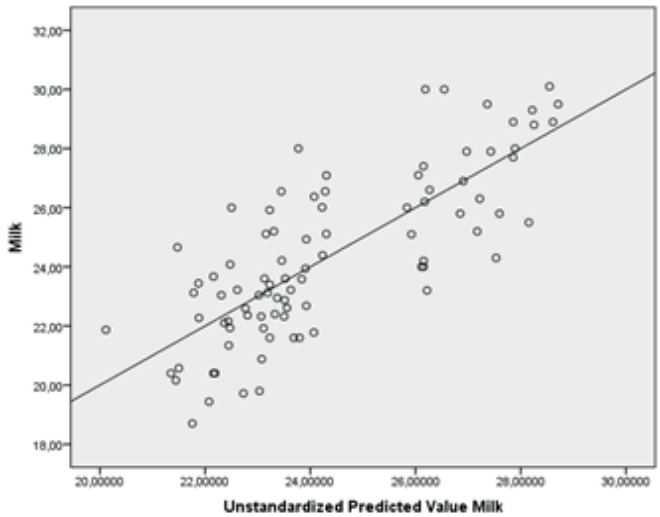

Fig. 2. The regression line between observed (Milk) and predicted milk production by model the mMilk production=THI+glucose+TNF-α model.

model. In the THI+glucose model the glucose variable was statistically significant, whereas the THI variable proved non-significant. The THI+glucose+ TNF-α model showed the best prediction of milk variability. Analysis of regression parameters B showed that blood glucose and TNF-α predicted variability in milk prodution in cows during heat stress even after the exclusion of THI from the model. Regression coefficients B indicate that a negative correlation was found between milk production and the THI, NEFA, TNF-α and RQUICKI values, compared to a positive correlation between milk production and glucose, insulin and G:I values.

Partial correlation analysis showed that the correlation between milk production and THI is significantly dependent on glucose levels. TNF-alpha showed a tendency to regulate the above-mentioned correlation, while the other metabolic parameters had a non-significant efect on the correlation between THI and milk production. Results are presented in Table 3 and Figure 3.

Table 3. Partial correlation between milk production, THI and metabolic factors when glucose is a control variable

	Zero- order	Partial correlation with THI after exclusion of					
	THI	Glucose	NEFA	Insulin	G:I	RQUICKI	TNFalfa
Milk	-0.61[a]	-0.052[b]	0.54[a]	-0.51[a]	0.58[a]	-0.59[a]	-0.47[c]
	p<0.01	NS	p<0.01	p<0.05	p<0.01	p<0.01	p<0.01

[ab]Means bearing different superscript differ significantly at: b – p<0.001; c – p<0.1 from the zero order correlation.

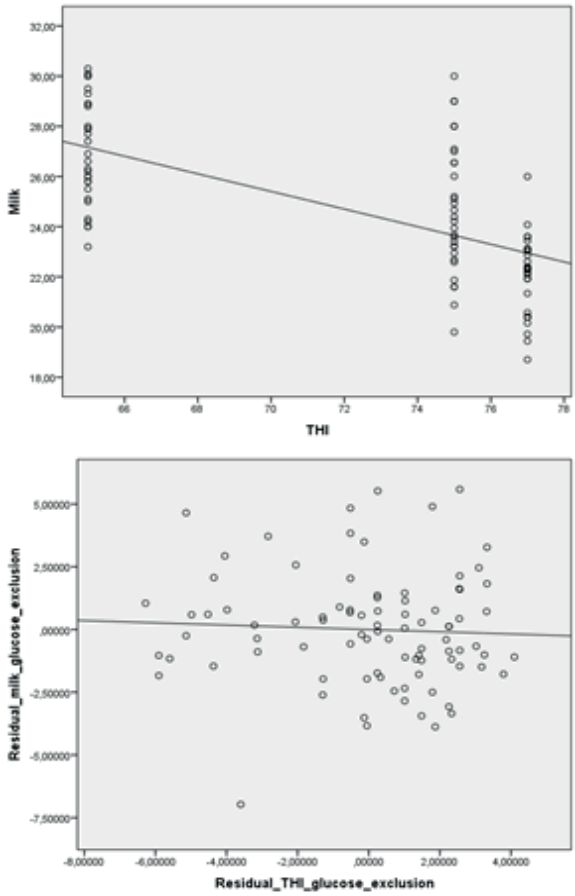

Fig. 3. The regression line between THI and milk production before (zero-order) and after exclusion of glucose as the control factor (residual THI and milk production). The sSignificant effect of glucose as the controlf factor is presented in the regression line towards to zero.

Garner *et al*. [2017] reported that dairy cows reduced their milk yield by 53% and their dry-matter intake by 48% during a 4-day exposure to heat stress in a well-controlled climate chamber. Under heat stress conditions a negative energy balance leads to decreased glucose and NEFA concentrations [Shwartz *et al*. 2009], i.e. decreased milk yields in lactating dairy cows. Glucose is an important factor, which greatly affects the production of milk as it represents the main energy source during heat stress. Tian *et al*. [2015] showed that glucose is a potent biomarker for the assesment of heat stress in dairy cows. Furthermore, low levels of plasma glucose in hot weather may be explained by a decreased energy intake, i.e. dry matter intake, increased thermoregulation energy requirements and adverse heat effects on

gluconeogenesis [Calamari *et al*. 2007]. O'Brien *et al*. [2010] reported that the glucose concentration of cows in heat stress was 93.4 mg/dl compared to 100.7 mg/dl in the thermoneutral period. These findings are consistent with our research results. Lower glucose concentrations occur due to augmented insulin concentrations in heat stress, which facilitates the glucose use and entry into cells. Moreover, using glucose as the main energy source requires less energy than utilising fats as primary energy sources [Bernabucci *et al*. 2002]. The G:I index values in our study were lower during heat stress, which means that a unit of insulin better regulates glycemia at high ambient temperatures than in the thermoneutral period. Cincović *et al*. [2012] showed that cows with increased lipomobilisation exhibit a lower tolerance to glucose than cows with decreased lipomobilisation (lower NEFA concentrations) during heat stress. Lower glucose concentrations with higher glucose clearance during heat stress are a consequence of elevated insulin concentrations and increased insulin sensitivity [O'Brien *et al*. 2010].

Higher insulin concentrations result from an increase in the intestinal glucose absorptive capacity, enhanced renal glucose re-absorptive capacity and elevated hepatic glucose output during heat stress [Settivari *et al*. 2007]. Increased insulin concentrations may also be caused by endotoxemia and the presence of proinflammatory cytokines [Nelson *et al*. 2004]. Wheelock *et al*. [2010] found that insulin concentrations were higher (0.70 ng/ml) in heat stress than in the thermoneutral period (0.44 ng/ml). Similar results were reported by O'Brain *et al.* [2010] (insulin concentrations during heat stress of 0.59 ng/ml and in the thermoneutral period of 0.47ng/ml). This may be explained by increased pancreatic sensitivity to glucose, which is one of the key metabolic adaptations of animals to thermal stress [Niu *et al*. 2003].

Heat stress leads to lower NEFA concentrations. During heat stress higher adipose tissue lipoprotein lipase levels were found in cows due to the increased capacity for intestinal and hepatic triglyceride uptake [Rhoads *et al*. 2009]. A low NEFA response indicates a decrease in fatty acid oxidation during heat stress [Febbraio 2001]. During heat stress lipid mobilisation in adipose tissue is inactivated and the energy requirements are met by glucose oxidation. Decreased NEFA mobilisation during heat stress could be due to increased insulin levels. A decrease in appetite is associated with heat stress resulting in reduced levels of volatile fatty acids (primarily acetate) in the gastrointestinal tract. The lack of precursors for fatty acid synthesis is implicated in the deficiency of available NEFAs used as energy sources. Therefore, glucose becomes a favoured fuel for heat-stressed animals [Moore *et al*. 2005]. Metabolic adaptations to heat stress in lactating dairy cows lead to a significant decrease in milk production. However, heat-stressed cows have lower NEFA concentrations and a higher rate of peripheral glucose utilisation, suggesting that glucose uptake by other tissues reduces the amount of glucose available for milk synthesis [Spires *et al.* 2004]. There is a negative correlation between NEFA values and milk production. A decrease in NEFA concentrations could protect the mammary gland from overheating during heat stress. Belić *et al*. [2011] found that the mammary gland NEFA and BHB uptake

during heat stress is markedly lower than in the thermoneutral period, resulting in lower milk yields.

TNF-α is another important predictor for milk production in heat-stressed cows. Heat stress causes an increased TNF-α concentration [Tian *et al*. 2016]. Endotoxemia, which is presented during heat stress, facilitates the synthesis of most pro-inflammatory cytokines as well as TNF-α [Mani *et al*. 2012]. Endotoxemia in heat stress could be a consequence of changes in the intestinal tissue. In ruminants heat stress can lead to rumen acidosis, causing permanent changes in the morphology of the intestine (splicing and shortening epithelial villas) as well as disorders in nutrient digestion and absorption [Inbaraj *et al*. 2016]. During heat stress the vasodilatation of skin blood vessels occurs in order to accelerate the cooling process (the lungs and heart also exhibit increased function in the cooling process), at a simultaneous vasoconstriction in the gastrointestinal tract blood vessels reducing the intestinal permeability [Lambert *et al*. 2009]. During endotoxemia insulin concentrations are augmented by LPS-induced proinflamatory cytokines [Waldron *et al*. 2006]. High insulin levels facilitate the entry of glucose into cells and its utilisation to eliminate toxins. During endotoxemia in heat-stressed cows glucose is used to meet the energy requirements of the body, resulting in the low glucose supply to the mammary gland and decreased milk production [Rhoads *et al*. 2009].

The use of metabolic parameters in combination with THI facilitates increasingly reliable predictions of milk production in heat-stressed cows compared to the model using solely THI. Changes in glucose and TNF-α values could prove to be the underlying mechanism regulating the production of milk in cows exposed to high ambient temperatures; nevertheless, this hypothesis requires further research.

REFERENCES

1. ABENI F., CALAMARI L., STEFANINI L., 2007 – Metabolic conditions of lactating Friesian cows during the hot season in the Po valley. 1. Blood indicators of heat stress. ***International Journal of Biometeorology*** 52, 87-96.
2. BAUMGARD H., WHEELOCK B., SANDERS R., MOORE E., GREEN B., WALDRON R., RHOADS P. 2011 – Postabsorptive carbohydrate adaptations to heat stress and monensin supplementation in lactating Holstein cows. ***Journal of Dairy Science*** 94, 5620-5633.
3. BELIĆ B., CINCOVIĆ M., POPOVIĆ-VRANJEŠ A., PEJANOVIĆ R., KRAJINOVIĆ M. 2011 – Metaboličke promjene i iskorištavanje metabolita u proizvodnji mlijeka kod krava u toplinskom stresu. ***Mljekarstvo*** 61, 309-318.
4. BERNABUCCI U., RONCHI B., LACETERA N., BAUMGARD H., RHOADS P., RONCHI B., NARDONE A. 2002 – Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. ***Journal of Dairy Science*** 85, 2173-2179.
5. BELHADJ SLIMEN I., NAJAR T., GHRAM A., ABDRRABBA M. 2016 – Heat stress effects on livestock: molecular, cellular and metabolic aspects, a review. ***Journal of Animal Physiology and Animal Nutrition*** 100, 401-412.
6. CALAMARI L., ABENI F., CALEGARI F., STEFANINI L. 2007 – Metabolic conditions of lactating Friesian cows during the hot season in the Po valley. 2. Blood minerals and acid-base chemistry. ***International Journal of Biometeorology*** 52, 97-107.

7. CINCOVIĆ M., BELIĆ B., STEVANČEVIĆ M., TOHOLJ B., LAKO B. 2012 – Uticaj stepena lipomobilizacije na rezultate testa opterećenja glukozom kod mlečnih krava u toplotnom stresu. ***Veterinarski Glasnik*** 70, 3-13.
8. CINCOVIĆ MR., MAJKIĆ M., BELIĆ B., PLAVŠA N., LAKIĆ I., RADINOVIĆ M. 2017 – Thermal comfort of cows and temperature humidity index in period of 2005-2016 in Vojvodina region (Serbia). ***Acta Agriculturae Serbica*** 22, 133-145.
9. FEBBRAIO MA. 2001 – Alterations in energy metabolism during exercise and heat stress. ***Sports Medicine*** 31, 47-59.
10. VRANIC P., MILUTINOVIC S. 2016 – From local sustainable development towards climate change adaptation: a case study of Serbia. ***International Journal of Sustainable Development World Ecology*** 23, 71-82.
11. GARNER JB., DOUGLAS M., WILLIAMS SRO., WALES WJ., MARETT LC., DIGIACOMO K., LEURY JB., HAYES JB. 2017 – Responses of dairy cows to short-term heat stress in controlled-climate chambers. ***Animal Production Science*** 57, 1233-1241.
12. HERBUT P., ANGRECKA S. 2012 – Forming of temperature-humidity index (THI) and milk production of cows in the free-stall barn during the period of summer heat. ***Animal Science Papers and Reports*** 30, 363-372.
13. INBARAJ S., SEJIAN V., BAGATH M., BHATTA R. 2016 – Impact of Heat Stress on Immune Responses of Livestock: A Review. ***Pertanika Journal Tropical Agricultural Science*** 39, 459-482.
14. JÓŹWIK A., STRZAŁKOWSKA N., BAGNICKA E., GRZYBEK W., KRZYŻEWSKI J., POŁAWSKA E., KOŁATAJ A., HORBAŃCZUK J.O. 2012 – Relationship between milk yield, stage of lactation, and some blood serum metabolic parameters of dairy cows. ***Czech Journal of Animal Science*** 57, 353-60
15. KADZERE C., MURPHY M., SILANIKOVE N., MALTZ E. 2002 – Heat stress in lactating dairy cows: a review. ***Livestock Production Science*** 77, 59-91.
16. LAMBERT G. 2009 – Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. ***Journal of Animal Science*** 87, 101-108.
17. MAJKIĆ M., CINCOVIĆ MR., BELIĆ B., PLAVŠA N., LAKIĆ I., RADINOVIĆ M. 2017 – Relationship between milk production and metabolic adaptation in dairy cows during heat stress. ***Acta Agriculturae Serbica*** 22, 123-131.
18. MANI V., WEBER T., BAUMGARD L., GABLER N. 2012 – Invited review: endotoxin, inflammation and intestinal function. ***Journal of Animal Science*** 90, 1452–65
19. MIN L., ZHENG N., ZHAO S., CHENG J., YANG Y., ZHANG Y., YANG H., WANG J. 2016 – Long-term heat stress induces the inflammatory response in dairy cows revealed by plasma proteome analysis. ***Biochemical and Biophysical Research Communications*** 471, 296-302.
20. MOORE C., KAY J., COLLIER R., VANBAALE M., BAUMGARD L. 2005 – Effect of supplemental conjugated linoleic acids on heat-stressed Brown Swiss and Holstein cows. ***Journal of Dairy Science*** 88, 1732-1740.
21. NELSON D., IHEKWABA A., ELLIOTT M., JOHNSON J., GIBNEY C., FOREMAN B., EDWARDS S. 2004 – Oscillations in NF-κB signaling control the dynamics of gene expression. ***Science*** 306, 704-708.
22. NIU C., LIN M., LIU I., CHENG L. 2003 – Role of striatal glutamate in heatstroke-induced damage in streptozotocin-induced diabetic rats. ***Neuroscience Letters*** 348, 77-80.
23. O'BRIEN M., RHOADS RP., SANDERS S., DUFF G., BAUMGARD L. 2010 – Metabolic adaptations to heat stress in growing cattle. ***Domestic Animal Endocrinology*** 38, 86-94.
24. PUPPEL K, KUCZYŃSKA B. 2016 – Metabolic profiles of cow's blood; a review. ***Journal of the Science of Food and Agriculture*** 96, 4321-4328.

25. RHOADS M., RHOADS R., VANBAALE M., COLLIER R., SANDERS S., WEBER W., BAUMGARD L. 2009 – Effects of heat stress and plane of nutrition on lactating Holstein cows. Production, metabolism, and aspects of circulating somatotropin. ***Journal of Dairy Science*** 92, 1986-1997.
26. SETTIVARI R., SPAIN J., ELLERSIECK M., BYATT J., COLLIER R., SPIERS D. 2007 – Relationship of thermal status to productivity in heat-stressed dairy cows given recombinant bovine somatotropin. ***Journal of Dairy Science*** 90, 1265-1280.
27. SHWARTZ G., RHOADS M., VANBAALE M., RHOADS R., BAUMGARD L. 2009 – Effects of a supplemental yeast culture on heat-stressed lactating Holstein cows. ***Journal of Dairy Science*** 92, 935-942.
28. TIAN H., WANG W., ZHENG N., CHENG J., LI S., ZHANG Y., WANG J. 2015 – Identification of diagnostic biomarkers and metabolic pathway shifts of heat-stressed lactating dairy cows. ***Journal of Proteomics*** 125, 17-28.
29. TIAN H., ZHENG N., WANG W., CHENG J., LI S., ZHANG Y., WANG J. 2016 – Integrated metabolomics study of the milk of heat-stressed lactating dairy cows. ***Scientific Reports*** 6, 24208.
30. WALDRON M., KULICK E., BELL W., OVERTON R. 2006 – Acute experimental mastitis is not causal toward the development of energy-related metabolic disorders in early postpartum dairy cows. ***Journal of Dairy Science*** 89, 596–610.
31. WHEELOCK B., RHOADS P., VANBAALE J., SANDERS S., BAUMGARD L. 2010 – Effects of heat stress on energetic metabolism in lactating Holstein cows. ***Journal of Dairy Science*** 93, 644-655.
32. WILDRIDGE A.M., THOMSON P.C., GARCIA S.C., JOHN A.J., JONGMAN E.C., CLARK E.F., KERRISK L.K. 2018 – The effect of temperature-humidity index on milk yield and milking frequency of dairy cows in pasture-based automatic milking systems. ***Journal of Dairy Science*** doi: 10.3168/jds.2017-13867.