THE EFFECT OF ENERGY BALANCE AND NON-NUTRITIONAL FACTORS ON PREGNANCY SUCCESS AFTER THE 1ST INSEMINATION IN DAIRY COWS

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ABSTRACT

This study aimed to evaluate energy balance and climatic conditions on pregnancy success after 1st insemination in Holstein cows. Successful conception rate, including the hypothalamic–pituitary–ovarian axis is most sensitive to energy balance and the availability of metabolic fuels. The analyzed negative energy balance of cows with successful insemination was confirmed at a lower level of -14.4 MJ/d, while in 74% of dairy cows with unsuccessful insemination NEB was at the level of -19.4 MJ/day. The level of NEB at the level - 47 MJ/day resp. -53 MJ/d corresponds to a weight loss of 2.3 kg resp. 2.7 kg in the 1st month after parturition. The pregnancy success after artificial insemination in summer is reduced and affected by reduced dry matter intake, the function of the endocrine system's level of hormones and the level of negative energy.

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INTRODUCTION

The milk yield and reproductive performance are the standard economic barometers of dairy production, which are affected by nutritional factors (composition of feed ration, dry matter intake) and non-nutritional factors. The most important non-nutritional factors include age, breed, lactation order and, last but not least, the temperature of the external environment (M'hamdi et al. 2012). The high temperature of the environment, together with a change in humidity and airflow, causes heat stress with a direct effect on the thermoregulation of animals (Laporta et al. 2017). High production of metabolic heat created during digestion in the rumen (Mader, Davis 2004), along with high temperature, directly proportionally increases the body temperature and frequency of breathing, with a negative effect on milk production and reproductive performance in dairy cows (Domingues et al. 2005).

Up to 65% of the proportion to reduced productions with the impact on the fertility of dairy cows is related to metabolic changes and overall load of metabolism (disruption of digestion in the rumen, change acid-base balance as a result of the heat stress of dairy cows) Up to 65% of the proportion to reduced productions with the impact on the fertility of dairy cows is related to metabolic conversion and overall burden of metabolism (disruption of digestion in the rumen, change acid-base balance as a result of the heat stress of dairy cows) (Vujanac et al. 2012; De Rensis et al. 2017; Liu et al. 2018). A significant impact on the changes in reproductive response during heat stress has a reduction in dry matter intake, leading to deepening of the negative energy balance (NEB) in the peripartal period.

When significant metabolic changes occur directly, a state of negative energy balance (NEB) with high energy loss affects the recovery of the estrous cycle and the success of further inseminations. NEB leads to lipomobilisation and thus burdens the liver. Recovery of the estrous cycle after calving and subsequent successful insemination depends on several factors associated with the function of organs that undergo a significant load in the peripartum period (Wathes et al., 2007). Important metabolic processes take place in the liver that affects liver function (gluconeogenesis, oxidation of fatty acids, production of insulin-like growth factor (IGF-I) (Butler 2005).

Problems associated with NEB include, for example, delayed first ovulation, prolonged reproductive cycles compared to physiological standard (persistent corpus luteum), long intervals between other luteal phases, when dairy cows do not come into ovulation and others. Various stress factors, diseases (uterine infections), and reduced feed intake before the expected ovulation after luteolysis (Wathes 2007) may also affect cycles without ovulation.

Changing the temperature of the environment mainly elevated temperature during the summer months, as well as deficient intake of nutrients have a direct effect on the endocrine dairy system (Ronchi et al. 2001), causing a reduction concentration of IGF-1 and glucose (O'Callaghan, Boland 1999), and effects the development of follicles and glucose as the main source of energy for the ovaries (Rabiee et al. 1997) with the impact on the manifestation of the heat (estrus) and the conception rate in dairy cows. During the summer months, the effect of increasing temperature on the hypothalamus-pituitary-ovarian axis, which affects the secretion of hormones, with the manifestation of reduced GnRH secretion, reduced ovarian activity and lower dominance of follicles, is described.

The goals of the work are based on the hypothesis of a published negative effect of energy balance and climatic conditions on milk synthesis and reproduction performance in dairy cows. In this intention the aim of the work was to verify the influence of milk production, the energy balance and heat stress on success pregnancy after the 1st insemination in multiparous dairy cows in breeding conditions on a farm with milk production over 10,000 kg.

MATERIAL AND METHODS

The evaluations were carried out on the selected farm with a controlled nutritional level system and an average annual production over 10 000 kg per cow. Cows were housed in a freestall barn in a separate group and fed a TMR that was formulated to meet NRC (2001) recommendations for 1^{st} phase of lactation of multiparous dairy cows. Multiparous dairy cows (n = 359) were evaluated for the efficiency of utilizing N and energy balance in relation to pregnancy success at first insemination. Samples of prepared TMR in the monitored farm were taken from the feed manger on the control day and there were analysed for dry matter (DM), crude protein (CP), acid and neutral detergent fibre (ADF, NDF), starch and ether extract (EE) contents according to conventional methods according to the Commission Regulation (EC) no. 691/2013. NEL and non-fibrous carbohydrate (NFC) values were calculated using regression equations (NRC 2001).

All cows were enrolled in a Presynch Ovsynch protocol. Controlled reproduction was applied from the 70^{th} day after parturition. The success of reproduction was evaluated in dairy cows by transrectal ultrasonography of the ovaries and uterus.

Analysis of production parameters on the control day on individually collected milk samples was evaluated for milk production levels in dairy cows, milk components and milk urea. Milk samples were analysed the total protein content, fat, lactose, and urea concentration by near infrared spectrophotometric assay using MilkoScan FT$^+$ and BENTLEY FTS at the Central Analytical Laboratory of Milk with accreditation under registration number 096/5878/2015/2.

The energy balance of the nutrients in dairy cows was evaluated by the balance of the amount of actually received energy in TMR in relation to the excreted energy in the nutrients produced by milk, as a positive or negative balance of NEL MJ per day, and determined by calculation according to Daniel et al. (2017).

Efficiency of Nitrogen Utilization (EUN) for group of dairy cows was estimated according to the analysed content of MUN and the amount of milk produced by using regression equation by Huhtanen et al (2015) from meta-analytical assessments of the balance experiments.

The influence of climatic conditions by measuring air temperatures during the year seasons was evaluated using daily measurements from a weather station located approximately 13 km from the farm. Average, maximum and minimum daily temperatures were recorded in a climatological booth at a standard height of 2 m above the ground for 24 hours. Maximum daily temperature was generally recorded with a mercury thermometer 2 hours after peak and minimum temperature was recorded with an alcohol thermometer before sunrise. Information on daily temperatures was provided to us by the Slovak Hydrometeorological Institute of the Slovak Republic.

The achieved results were processed by the statistical program GraphPad Prism9. Each parameter was presented as its mean (x), and standard deviation (SD).

RESULTS AND DISCUSSION

1. Evaluation of nutritional composition

The average concentration of nutrients in TMR at the evaluated farm are presented in Tables 1. The feed ration based on corn, clover and grass silage with the addition of concentrated feed was formulated for multiparous dairy cows for milk production 39.3 kg (3.28% protein and 3.6% fat).

Table 1. Nutritional composition of TMR

		Postpartum phase X ± SD	1st Lactation phase X ± SD
CP	g/kg DM	170.1 ± 2.5	157.2 ± 5.8
Fat	g/kg DM	50.6 ± 3.1	43.6 ± 2.3
ADF	g/kg DM	209.2 ± 5.8	215.9 ± 7.8
NDF	g/kg DM	224.4 ± 9.0	348.2 ± 10.4
Starch	g/kg DM	230.1 ± 8.8	272.2 ± 18.4
NFC	g/kg DM	361.7 ± 11.3	385.9 ± 2.8
NEL	MJ/kg DM	6.60 ± 0.2	6.63 ± 0.3
Dry matter intake	kg	19.0 ± 0.6	24.5 ± 0.74
NEL intake	MJ/d	125.4 ± 2.5	162.4 ± 3.0

Composition: *corn silage 19-33 kg, legume silage 6-17 kg, grass (oat) silage 2-3 kg, grass/alfalfa hay 0.7–1 kg, wheat straw 0.5-0.8 kg, DDGS 4 kg, wheat 0.8–3.5 kg, rapeseed meal 2.2 – 3.8 kg, Feed mixture 4.2 kg*

2. Evaluation of milk production, energy balance and N utilization on reproduction

In the annual assessment, the impact of nutrient intake, production and milk components was analyzed in relation to the success of conception of dairy cows after the 1st insemination. The results of the examinations are summarized in Table 2. From the total number of 359 evaluated dairy cows with an average daily production of 43.5±7.1 kg and a total milk production of 3003.2 kg until insemination, 25.6% of cows successfully became pregnant after the 1st insemination. In dairy cows that did not conceive at the first insemination, a 1.9 kg higher daily milk production and a 4.5 kg higher milk fat production by the date of insemination was confirmed.

The analysis of the energy balance assessed by the energy intake in the ration and the excretion of nutrients in milk (Daniel et al. 2017) confirmed for the period of the first two months of lactation until the 1st insemination a negative energy balance (NEB) on average -18.2 MJ/day with a lower level of -14.4 MJ/d in dairy cows with successful insemination, while in 74% of dairy cows with unsuccessful insemination NEB was at the level of -19.4 MJ/day.

A higher level of NEB was confirmed at the 1st test control, on average 43 days after calving, where NEB was on average - 47 MJ/day in the group with successful insemination or – 53 MJ/day in the group with unsuccessful insemination. Such a level of NEB corresponds to an average weight loss of 2.3 kg or 2.7 kg in the 1st month after birth. The higher level of the NEB was confirmed in the 1st test of control on average until the 43rd day after calving, where the NEB was on average - 47 MJ/day in the group of dairy cows with successful insemination, resp. - 53 MJ/day in the group with unsuccessful insemination. Such a level of NEB corresponds to a weight loss of 2.3 kg resp. 2.7 kg in the 1st month after parturition. The difference in the level of the NEB within the groups at the 1st resp. 2nd day of the testing is 6.2 resp. 5.0 MJ NEL/day, which corresponds to the energy requirements for synthesis 1.8 - 2.0 kg of milk, and correlates with confirmed average milk production within groups. The studies by authors (Garverick et al., 2013, Patton et al., 2007) showed that the pregnancy rate at the first insemination is higher in proportion to the decrease in negative energy balance in dairy cows in the postpartum period.

Table 2. Production, milk components and level of energy balance in relation to pregnancy success of dairy cows at the 1st insemination

		multiparous cows (n 359) X ± SD	1st insemination positive (n 92) X ± SD	1st insemination negative (n 267) X ± SD
Pregnancy rate		**25.6 %**	**-**	**-**
DIM at insemination		69 ± 19	69 ± 17	69 ± 15
Milk production	kg/d	43.5 ± 7.1	42.1 ± 7.9	44.0 ± 6.8
Total yield	kg	3003.2 ± 14.1	**2906.9 ± 14.4**	**3034.6 ± 13.9**
Milk fat	%	3.50 ± 0.6	3.50 ± 0.6	3.50 ± 0.7
Total yield	kg	105.1	101.7	106.2
Milk protein	%	3.05 ± 0.3	3.11 ± 0.3	3.02 ± 0.3
Fat/Protein		1.15 ± 0.2	1.13 ± 0.2	1.16 ± 0.2
Lactose	%	4.90 ± 0.1	4.92 ± 0.1	4.90 ± 0.2
Energy Balance	MJ/d	**-18.2± 7.2**	**-14,4 ± 7.0**	**-19.4 ± 6.3**
EUN[1]	%	32.3 ± 0.7	32.1 ± 0.2	32.1 ± 0.4
Milk Urea	mg/dl	26.9 ± 5.6	26.3 ± 4.4 (↑ 21 %)	27.1 ± 5.9 **(↑ 29 %)**
DIM in 1st test day		43 ± 25	43 ± 22	43 ± 27
Milk production	kg/d	41.9 ± 8.3	**40.3 ± 8.2**	**42.4 ± 8.3**
Total yield	kg	1800.4 ± 20.2	**1733.3 ± 17.4**	**1823.2 ± 20.3**
Milk fat	%	3.74 ± 0.9	3.73 ± 0.8	3.74 ± 1.0
Total yield	kg	67.3	64.7	68.2
Milk protein	%	3.12 ± 0.4	3.20 ± 0.4	3.10 ± 0.4
Fat/Protein		1.20 ± 0.3	1.17 ± 0.2	1.21 ± 0.3
Energy Balance	MJ NEL/d	**-51.5 ± 7.2**	**-46.9 ± 7.0**	**-53.1 ± 6.3**
EUN[1]	%	32.1 ± 0.7	31.9 ± 0.2	32.1 ± 0.4
Milk Urea	mg/dl	26.2 ± 7.7	25.7 ± 6.7 (↑ 22 %)	26.4 ± 7.9 **(↑ 29 %)**

DIM – days in milk

EUN – Efficiency of nitrogen utilisation (Huhtanen et al.2015)

3. Evaluation of energy balance and climatic condition on reproduction

The analysis of the effect of the seasonal dependence of production and milk components in relation to the conception rate is summarized in Table 3.

Table 3. Seasonal dependence of production, milk components and the level of energy balance in relation to the success of conception of dairy cows at the 1st insemination

	Spring-Summer (IV. – IX.)		**Autumn-Winter (X. – III.)**	
	1st insemination		**1st insemination**	
	positive (n 38) X ± SD	**negative (n 122) X ± SD**	**positive (n 54) X ± SD**	**negative (n 145) X ± SD**
Pregnancy rate	**24%**		**27%**	
DIM at insemination	70 ± 23	70 ± 17	67 ± 12	67 ± 13
Milk production kg/d	**43.3 ± 6.6**	**44.1 ± 7.4**	**41.3 ± 7.5**	**43.9 ± 6.6**
Total yield kg	3029.6±13.2	**3087 ± 10.3**	2767.1±15.1	**2941.3± 9.1**
Milk fat %	3.36 ± 0.9	**3.43 ± 0.6**	3.60 ± 0.6	3.56 ± 0.7
Total yield kg	101.8	**105.9**	99.6	**104.7**
Milk protein %	2.97 ± 0.3	2.89 ± 0.3	3.22 ± 0.2	3.13 ± 0.2
Fat/Protein	1.14 ± 0.3	1.19 ± 0.2	1.12 ± 0.2	1.14 ± 0.2
Lactose %	4.91 ± 0.1	4.89 ± 0.2	4.92 ± 0.1	4.90 ± 0.1
Energy Balance MJ/d	**-19.1± 8.7**	**-22.0 ± 6.3**	**-15.6± 7.7**	**-23.2 ± 6.3**
EUN[1] %	32.1 ± 0.3	32.0 ± 0.3	32.2 ± 0.2	32.5 ± 0.4
Milk Urea mg/dl	28.2±4.1 (↑34%)	29.5±5.3 **(↑43%)**	24.9±4.1 (↑11%)	25.1 ± 5.6 **(↑17 %)**
DIM in 1st test day	41 ± 26	44 ± 36	43 ± 18	42 ± 15
Milk production kg/d	41.4 ± 8.1	**42.1 ± 8.5**	39.6 ± 8.2	**42.6 ± 8.3**
Total yield kg	1697.4±10.2	**1852.4±10.2**	1702.8±10.2	**1789.2±8.2**
Milk fat %	3.60 ± 0.9	**3.55 ± 0.9**	3.82 ± 0.7	**3.91 ± 1.0**
Total yield kg	61.9	**65.8**	65.0	**70.0**
Milk protein %	3.10 ± 0.4	2.98 ± 0.4	3.27 ± 0.3	3.19 ± 0.3
Fat/Protein	1.17 ± 0.3	1.19 ± 0.3	1.17 ± 0.2	1.23 ± 0.3
Energy Balance MJ/d	**-47.0 ± 8.7**	**-47.1± 10.8**	**-46.9 ± 7.7**	**-58.1 ± 8.7**
EUN[1] %	32.0 ± 0.2	31.8 ± 0.3	31.8 ± 0.2	32.4 ± 0.3
Milk Urea mg/dl	26.2±8.2 (↑34%)	28.6±8.2 (↑42%)	25.3±5.6 (↑13%)	24.6±7.3 (↑18%)

DIM – days in milk
EUN – Efficiency of nitrogen utilisation (Huhtanen et al.2015)

The seasonal evaluation of the analyzed relationships confirmed in the colder months of the year (Autumn-Winter X. - III.) a higher proportion of inseminated animals (55.4%) together with a higher rate of conception (27%) with a higher NEB difference in the group of dairy cows with unsuccessful insemination. In the warmer months of the year (Spring-Summer (IV. - IX.)) with a higher average production of milk and milk fat together with a lower proportion of inseminated animals (44.6%), a lower acceleration rate (24%) was confirmed.

The results can be given in relation to the influence of heat stress with a confirmed share of 64 days with daily temperatures above the thermal optimum level (above 25ºC). The effect of summer heat on the level of conception with a significant decrease in the summer months of 27.7 % compared to the winter months of 42.6 % was confirmed by Wolfenson and Roth (2019). The results of climatological information on seasonal dependence are presented in Table 4. The upper critical temperature for Holstein dairy cows is 25 to 26 °C (Yang et al. 2010). Maximum and minimum temperatures in summer were 35.5 and 14.6 °C, respectively. The average temperature exceeded 25 °C on 70% of test days (64 days out of 92 testing days) during this period, indicating that cows were exposed to high temperatures.

Table 4. Climatological information in seasonal dependence

Items	Period Spring	 Summer	 Autumn	 Winter
	X ± SD			
Temperature, minimum (°C)	3.2	14.6	-1.7	-14.5
Temperature, maximum (°C)	21.0	35.5	21.0	6.7
Average temperature (°C)	11.0±4.8	20.4±3.0	9.3±5.0	-2.2±4.9
Days temperatures > 25 °C	-	64	-	-

CONCLUSION

The results of the examination confirmed the tendency of a negative dependence between the amount of milk and milk fat production, as well as the level of NEB at the same time on the first test day of production as well as at the time of the first insemination. The obtained dependencies according to the evaluation of production markers are currently experimentally verified by analyzing the rate of lipomobilization, ketogenesis and the functional load of liver metabolism in dairy cows after parturition and in the insemination phase.

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