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Influence of the level of daily milk yield on some blood biochemical parameters in dairy cows reared under the same temperature and humidity conditions

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Abstract

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The present study examines the relationship between the milk productivity and some biochemical parameters in dairy cows of the Holstein cattle breed reared under the same temperature-humidity conditions. The tests were performed in the course of one year in three cattle-breeding farms with a different capacity. The farms are located in the central part of Southern Bulgaria, in the region around Plovdiv. The region itself is characterized by a temperate continental climate with high temperatures during the summer and moderately cold ones during the winter.

The conditions in the cow-rearing premises of the three farms examined ranged from mild to moderate heat stress zone during the summer and partially – during the spring. Based on the daily milk yield and THI (by classes) values reported, a model for assessment of their effect on the biochemical indicators of the cows: protein and energy metabolism, enzymes, liver markers and cortisol was applied.

The highest glucose levels were reported for the cows with an average daily milk yield between 30 and 40 kg ($p < 0.001$), and the lowest- for the highly productive animals (≤ 40 kg) – 2.46 mmol/L which is even below the reference value for the breed (2.50 – 4.16 mmol/L). With the THI values increase, the glucose levels of the cows from all daily milk yield level groups decreased and this dependence is high ($p < 0.001$).

The protein levels were the lowest in the cows with the lowest milk yield (up to 30 kg per day)- with approximately 7.5% when compared with the other two milk yield groups. The blood urea levels increase was more considerable in the cows with higher daily milk yield from 30 to 40 kg and over 40 kg- from 3.25 mmol/L, and 3.33 mmol/L to 7.72 mmol/L, and 7.62 mmol/L respectively. The creatinine LS means of the cows from all productivity groups increased with the increase of the THI values ($p < 0.001$) with the elevation being more significant in the cows with higher daily milk yield ($p < 0.001$). The cholesterol levels increased along with the milk yield ($p < 0.001$) but a considerable decrease in this parameter was reported for the cows of the three milk yield groups ($p < 0.001$) upon increase in the THI values over 80.

The LS means of the two transaminases in the cows blood were in an inverse correlation with their daily milk yield. The cows with lower milk yield of up to 30 kg had lower ASAT levels (92 U/L) and higher ALAT levels (23.38 U/L) when compared with the cows with high daily milk yield – 106.6 U/L and 19.74 U/L respectively. The present study displays a significant increase in the two enzymes activity upon increase of the THI values: ASAT – $p < 0.001$ and ALAT – $p < 0.01$. A high significance ($p < 0.001$) was ascertained in the related effect of the daily milk yield and the cortisol levels.

Keywords: dairy cows, THI, daily milk yield, protein and energy metabolism, ASAT, ALAT, cortisol

Introduction

The blood biochemical parameters provide the opportunity to accurately evaluate the health, physiological condition and productivity of the dairy cows (Nowroozi-Asl et al., 2016). Most of the studies in this field are focused on factors such as feeding (Huhtanen et al., 2002), technology (Wright et al., 2013) or health (Fan et al., 2018). It is known that healthy cows of the same breed have different levels of milk yield even when they are under the same feeding and rearing conditions. Genetic selection application for the purpose of a higher milk yield increases the heat stress susceptibility and decreases the lactation curve during the warm periods of the year (Wheelock et al., 2010). Rarely is close attention paid to these studies due to the manifestly stable condition of the animals (Wu et al., 2019). However, a wide and careful analysis of the serum parameters in cows with different milk yield levels which are under the same conditions would explain and diagnose already existing physiological changes. Therefore, Behera et al. (2018) indicate that the correlations between the environmental parameters and the physiological responses of the animals are an indicator for the cows comfort and the milk production.

The blood biochemical parameters provide an objective information about the main biophysical and biochemical processes in the organism. These tests are valuable because they provide data on the condition of the cows even before the debut of any clinical signs of a certain disease or type of stress and can be used for diagnosis, treatment or prevention (Ndlovu et al., 2007). Studies carried out by Dikmen & Hansen (2009) show that THI can be used as an indicator for heat stress levels in cows because the appropriate temperature and humidity in the production premises are a guarantee not only for the comfort but also for the health and the productivity of the animals. According to Regulation 44, the optimal temperature zone is 10-15°C, with a minimum of 5 and maximum- 28°C. Temperatures above 18-20°C are capable of causing heat stress in the highly productive cows. Mylostyvyi & Sejian (2019) prove that the environmental conditions are major stress factors influencing the hematology and chemical parameters of the cows. Taking the above mentioned into account, we set ourselves the goal to ascertain whether, and to what extent, there is a relationship between the milk productivity and some biochemical indicators in Holstein dairy cows reared under the same temperature-humidity conditions.

Materials and Methods

The study was carried out in three cattle-rearing farms in the course of one year. They are located in Southern Bulgaria, in the region of Plovdiv which is characterized by a temperate continental climate with high temperatures during the summer and moderately cold ones during the winter.

The cow-rearing technology in two of the farms was free in groups with individual boxes for resting, and in the third one- free in groups living on deep litter bedding. The buildings of the first farm were semi-open. Each cow was provided with space of 9.4 m². The open side spaces of the building had a total area of 170 m². The buildings of the second farm were reinforced concrete constructions with concrete wall and roof panels. Each cow was provided space of 11.5 m². During the winter, the windows and the skylights were covered with polyethylene sheets. The ventilation in farm 1 was automatically turned on upon temperature of 25°C. The cows in the third farm were reared on a deep litter bedding in a semi-open building with brick walls. Each animal was provided space of 8.06 m². The cleaning of the manure was performed twice a year along with a periodic addition of hay. The feeding of the cows in all three farms was unlimited with a total mixed ration and a permanent access to water.

The study includes 18 cows of the Holstein cattle breed with the same period of calving/3-6 weeks after calving, second – third lactation. The first reporting of both the milk yield and the blood indicators coincided with the spring season when the cows examined were in I^{st} lactation period (day 60-80). The same cows were in their II^{nd} lactation period (day 180-200) during the summer, and in the winter they were at the end of their lactation. The reporting of the micro-climatic indexes (temperature and relative humidity of the air) and the monitoring of the blood biochemical parameters were performed during the three seasons. The blood parameters testing was carried out on the same animals in all seasons, with the exception of two, which fell out due to different reasons and were replaced by animals which were analogous with them in terms of period of lactation, milk yield and age. The data about the daily milk yield of the cows part of the study was taken from the official milk productivity control systems of the respective farm.

The reporting of the temperature and the relative air humidity was performed on the level of the animals at 2 o'clock pm by means of an aspiration psychrometer by Assmann. Blood samples were taken from the tail vein of the controlled cows in the morning on the same day after a fast. The serum obtained was tested for: glucose, total protein, urea, cholesterol, creatinine, cortisol, aspartataminotransferase (ASAT), alaninaminotransferase (ALAT) with a semi-automatic analyzer and 'BIOMED' tests. Taking the close temperature and relative humidity of the air values during the spring and the autumn into account, no data about the autumn were displayed. The temperature humidity index was calculated in accordance with the formula (Kelly & Bond (1971):

THI = T – (0.55 – 0.0055 × RH) × (T – 58),

where T is the temperature measured in °F; RH – relative humidity, %.

For the purpose of a better approximation during the analysis of variance, the two factors examined – daily milk yield and THI are displayed in classes as follows: daily milk yield of up to 30 kg, from 30 to 40 kg and over 40 kg, and THI – up to 72, from 73 to 80 and above 80 (Armstrong, 1994).

The data were statistically processed by MS Excel, and the statistical indexes and the analysis of variance were obtained through the respective modules of STATISTICA of Stat Soft (Copyright 1990-1995 Microsoft Corp.).

The following model was used for the evaluation of the influence of the factors on the values of the biochemical parameters examined:

$$Y_{ijkl} = \mu + DM_i + THI_j + DM*THI_k + e_{ijkl},$$

where Y_{ijkl} dependable variable (biochemical parameter); **μ** is the model average; DM_i is the effect of the daily milk yield (classes), THI_j is the effect of the temperature-humidity index (classes), $DM*THI_k$ is the cluster effect of the daily milk yield and the THI, and e_{ijkl} is the effect of the non-controlled factors (error). The average of the least square mean (LSM) per the model by classes of fixed factors was established by means of the analysis of variance (ANOVA).

Results and Discussion

The daily milk yield average values and variation by seasons and farms are displayed in Table 1. The information regarding the milk yield was obtained by the farm data on the day of reporting the temperature and the relative humidity of the air and taking the biochemical analysis blood samples. As it can be seen in the table, the highest average daily milk yield was that of the cows in farm 3 – between 34.33 kg and 50.17 kg. Close, but lower, was the milk yield of the cows in farm 1 – from 34.03 to 43.37 and the lowest was that of the cows in farm 2 – from 19.83 kg to 22.05 kg. Under the study scheme, the first reporting of both the milk yield and the biochemical parameters coincided with the spring when the cows subject to examination were in their I[st] period of lactation. The same cows were in their II[nd] lactation period during the summer, and in the winter, they were at the end of their lactation. It can be seen that the highest daily milk yield was reported during the spring, followed by the summer and the winter which correlated with the milk yield changes during the entire lactation.

The table also shows the average THI values. The highest values of the index were reported in farm 3 during the summer – 85, followed by farm 2 – 82.4 and farm 1 – 78.3. This index exhibits the presence of periods with undesirable micro-climate, especially during the summer and part of the spring. According to the classification of Armstrong (1994), these are values laying the conditions for a moderate heat stress in the dairy cows. During the spring the THI did not exceed the limit of 72 (from 69.8 to 71.4), and during the winter it was even below 50. Under the same classification, these THI values characterize the barn environment as comfortable in terms of temperature. The high THI values in farm 3 during the summer were most probably due to the type of building and the deep litter bedding which caused the higher temperatures and relative humidity. Low average values of the index during the spring and the summer were reported in farm 1 where the ventilators were set to turn on upon lower temperatures. The ventilation turning on in the

Table 1. Daily milk yield and THI average values and variation by farms and season of reporting

Season	Number	Daily milk yield, kg				THI values
		X ± SE	SD	min	max	
Farm 1						
Spring	6	43.37 ± 1.31	3.20	38.4	47.6	69.8
Summer	6	37.65 ± 1.44	3.52	33.8	43.5	78.3
Winter	6	34.03 ± 0.75	1.85	31.2	36.5	43.9
Farm 2						
Spring	6	22.05 ± 0.27	0.66	21.3	22.8	71.0
Summer	6	20.95 ± 0.34	0.82	20.2	22.3	82.4
Winter	6	19.83 ± 0.15	0.37	19.2	20.3	46.3
Farm 3						
Spring	6	50.17 ± 3.59	8.79	43.0	67.0	71.4
Summer	6	43.83 ± 2.48	6.08	37.0	53.0	85.0
Winter	6	34.33 ± 0.71	1.75	32.0	37.0	48.6

Note: The THI values were measured upon taking the blood samples

Table 2. Effect of the daily milk yield on the blood biochemical indicators in the respective THI class

Biochemical indicator	Model total		Daily milk yield		THI		Daily milk yield /THI		error
	MS	F .P	MS	F .P	MS	F .P	MS	F .P	MS
Glucose	4.52	43.54***	0.78	7.57**	11.76	113.35***	1.31	12.63***	0.11
Total protein	193.77	8.06***	75.1	3.12*	285.1	11.85***	86.7	3.61*	24.1
Urea	13.31	23.54***	9.19	16.25***	35.88	63.46***	2.91	5.15**	0.57
Creatinine	17732.8	39.97***	4501.6	10.15**	30575.6	68.91***	11411.2	25.72***	443.7
Cholesterol	4.93	26.66***	1.96	10.63***	9.44	51.04***	2.96	16.01***	0.18
ASAT	3758.3	2.66*	2899.2	2.05-	11501.5	8.12***	1393.6	0.98-	1416.2
ALAT	127.40	3.85**	51.92	1.57-	194.8	5.88**	71.41	2.16 -	33.12
Cortisol	1319.3	15.45***	1816.8	21.28***	303.5	3.56*	1266.8	14.84***	85.4

Note: * – significance upon p < 0.05; ** – significance upon p < 0.01; *** – significance upon p < 0.001; – no significant effect

other two farms was performed manually by the caretakers, which is definitely not effective.

Segnalini et al. (2013) propose an even lower threshold for the assessment of the THI values effect on the dairy cows- $68 \leq$ THI < 72 (slight discomfort). Huhnke et al. (2001) classify THI in 2 categories: from $79 \leq$ THI ≤ 83 dangerous situation and THI ≥ 84 emergency situation.

A model for the assessment of the daily milk yield and THI effect on the blood biochemical parameters of the cows subject to the study was applied based on their values reported (in classes) (Table 2). The set of parameters tested is routinely used for blood profiling of lactating cows: protein and energy metabolism parameters (total protein, urea, creatinine, glucose); enzymes and liver markers (alanine aminotransferase, aspartate aminotransferase , cholesterol), and the steroid hormone cortisol.

Independently, the factors daily milk yield and THI levels had a significant effect (from $p < 0.05$ to $p < 0.001$) on almost all blood indicators examined with the exception of the transaminases. It is also indicated that there was a related effect of the daily milk yield upon a certain THI value (class). The related effect was significant with reference to most of the biochemical parameters, again with the exception of the transaminases ASAT and ALAT regarding which a significant effect ($p < 0.001$ and $p < 0.01$) was reported only with reference to the THI values.

For the purpose of a better clarity, the effects of the daily milk yield under the different THI values represented by the LS means of the different biochemical parameters, calculated based on the model, are displayed in diagrams. Figure 1 A displays the LS means of the glucose depending on the daily milk yield level. The highest glucose levels were reported for the cows with average daily milk yield between 30 and 40 kg ($p < 0.001$), followed by the cows with milk yield of up to 30 kg. The lowest levels of blood sugar were ascertained for the highly productive cows – 2.46 mmol/L which is even below the reference value for the breed (2.50-4.16 mmol/L). Bickerstaffe et al. (1974) provide the same results and claim that they are due to the milk gland tissues use of glucose for the production of milk.

It is observed that the THI levels increase led to glucose levels decrease in the cows of all milk yield levels (Figure

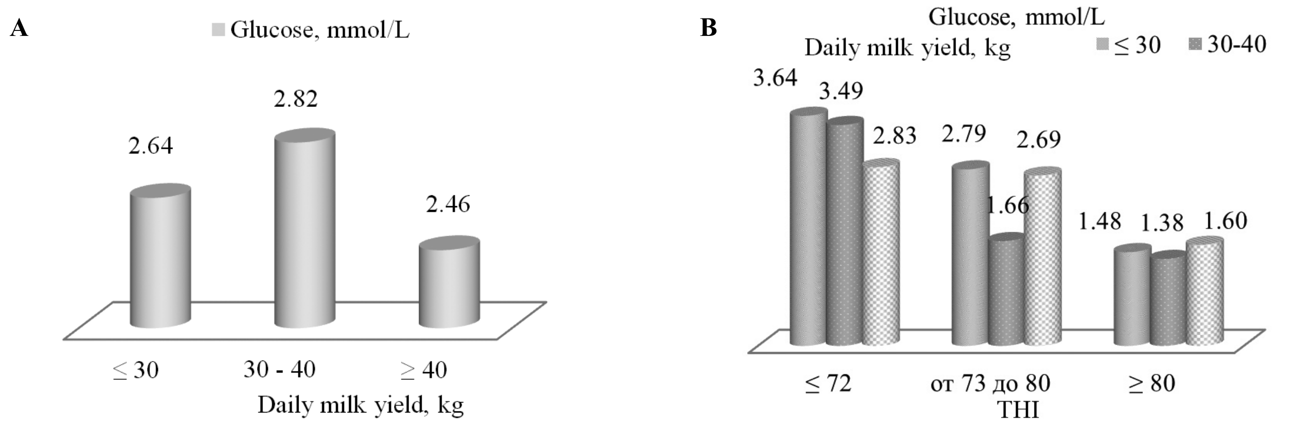

Fig. 1. A – glucose levels depending on the daily milk yield; B – glucose levels depending on the milk yield and THI

1B) and this relation was high ($p < 0.001$). The glucose levels decrease in the groups with different daily milk yield was more significant than that upon temperature comfort conditions (THI up to 72) and moderate heat stress (THI over 80) – with 40 to 60%, reaching values from 1.38 mmol/L to 1.60 mmol/L. The cows with high daily milk yield had lower glucose levels under comfort conditions than the cows with lower daily milk yield. However, the decrease in the glucose levels of the cows with lower milk yield upon change of the temperature humidity conditions in the environment was more considerable- from 3.64 mmol/L and 3.49 mmol/L under temperature comfort to 1.48 mmol/L and 1.38 mmol/L upon moderate heat stress for the cows with milk yield of up to 30 and from 30 to 40 kg respectively. In our opinion, and also according to Abeni et al. (2007), this is a result of the decreased energy intake, the increased thermoregulation expenditure and the decreased gluconeogenesis in the process of acclimatization. Graber et al. (2010) consider that gluconeogenesis and glycogenolysis processes are accelerated during lactation so that glucose is provided for the production of lactose. Shahzad et al. (2015) add that the gluconeogenesis in the lactating cows is much more intense during the hot period of the year when compared with the cold and transitional one. The high THI forces the cows to speed up their main metabolism but at the same time they decrease the food intake and respectively the presence of nutrients in the milk gland. In order to function normally, the gland needs more glucose to synthesize lactose but at the same time it needs to control the metabolic heat generation in its body.

The cows with the lowest milk yield (up to 30 kg per day) had the lowest blood protein levels – about 7.5% lower when compared with the other two milk yield groups (Figure 2A). Upon THI levels increase of up to and over 80, the blood protein levels decreased, regardless of the daily milk yield of the cows, but the respective decline was different in the different groups- from 10 to 19% respectively when compared with the levels under the conditions of temperature comfort (Figure 2B),. Even after a decrease in the blood protein levels under THI above 80, the tendency for higher levels in the cows with higher daily milk yield remained. We suppose that the higher barn environment temperatures triggered changes in the amino acid metabolism increase in the protein synthesis in the liver during diminished total catabolism and increased amino acids peripheral mobilization. According to Milaeva et al. (2017), the higher total protein serum concentration in the milk of the high-milk-yield cows is due to the intense milk synthesis which is directly connected with the protein metabolism of their entire body.

The urea LS means (Figure 3A) increased in parallel with the daily milk yield increase from 4.8 mmol/L with reference to the cows with daily milk yield of up to 30 kg to 5.7 mmol/L with reference to those with milk yield of over 40 kg, which is almost 19% difference. Such elevation in the urea levels was also observed in the three milk-yield cow groups upon THI values increase (Figure 3B). The urea blood levels increase was more considerable in the cows with higher daily milk yield from 30 to 40 kg and over 40 kg- from 3.25 mmol/L and 3.33 mmol/L to 7.72 mmol/L and 7.62 mmol/L respectively. Increase in the urea blood levels upon THI of over 80 was also reported regarding the cows with low milk yield of up to 30 kg per day but the values remained low when compared with those of the other two milk-yield groups (6.75 mmol/L). Our results concur with the tests carried out by Rasooli (2004). The reason for the

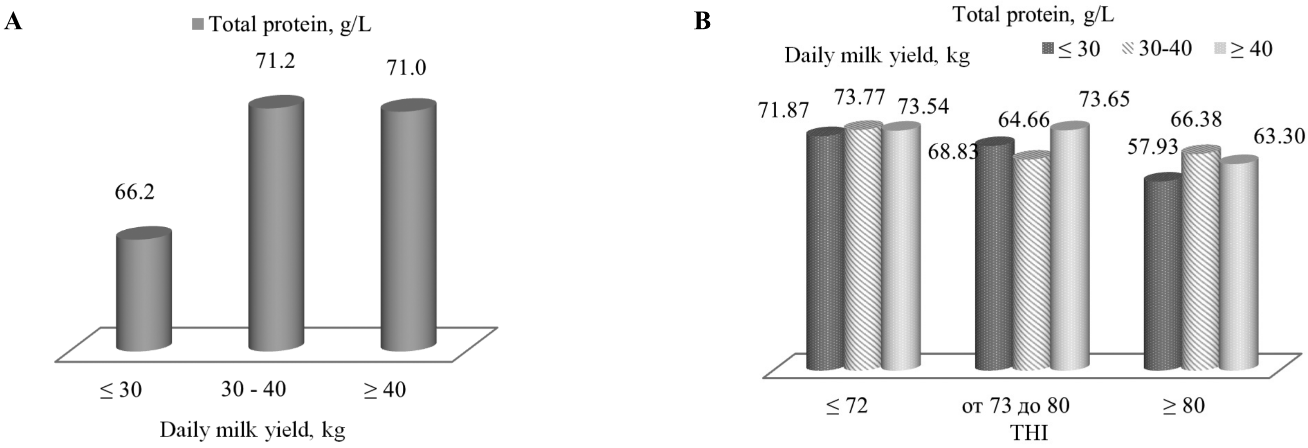

Fig. 2. A – protein LS means depending on the daily milk yield;
B – protein LS means depending on the milk yield and THI

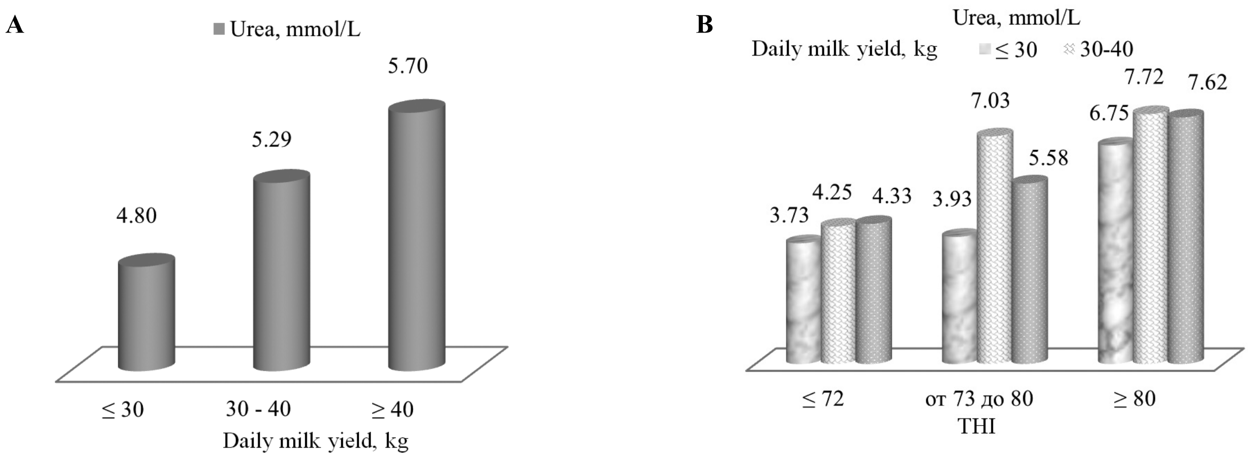

Fig. 3. A – urea LS means depending on the daily milk yield; B – urea LS means depending on the milk yield and THI

changes mentioned above is probably the increased amino acids use (and their mobilization by the muscles) as a source of energy as well as the loss of extracellular fluid due to the high temperatures. Wheelock et al. (2010) even report almost doubled urea plasma concentrations in lactating cows reared under the conditions of high THI. They suppose that they are caused by the elevated liver deamination of the amino acids.

The creatinine LS means for the cows with high milk yield of over 40 kg per day were the lowest, from 10 to 12% (Figure 4A), when compared with those of the other two productivity groups. The creatinine LS means of the cows from all productivity groups increased (Figure 4B) upon THI values increase with the elevation being more significant in those cows which had a higher daily milk yield. When the THI values were over 80, the creatinine levels increase in the blood of the cows with daily milk yield of over 40 kg was 2.5 times bigger when compared with the creatinine levels upon temperature comfort. The creatinine synthesis in healthy animals is stable and depends on the muscle mass and protein intake volume. According to Lamp et al. (2015), the decreased nutrients intake in hot weather is compensated by the intense protein oxidation necessary for meeting the organism demands for milk production. This oxidation leads to muscle proteolysis and increase of the urea and creatinine plasma concentrations. The increased creatinine levels, Cowley et al. (2015), are also linked to an increased renal function.

The total cholesterol and the glucose are main metabolites of the energy metabolism. Our studies ascertained higher cholesterol levels in the blood of the cows with higher daily milk yield (Figure 5A) which increased from 2.76 mmol/L

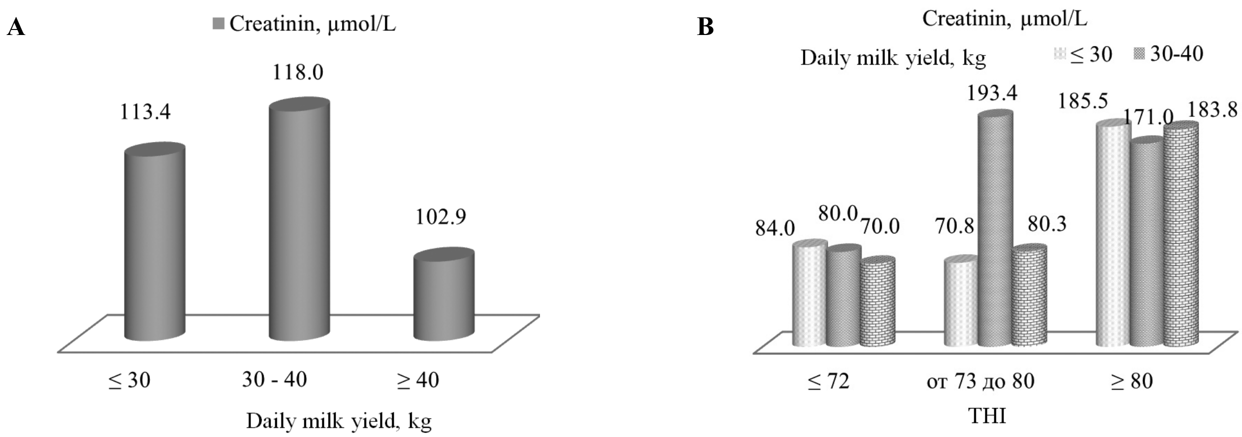

Fig. 4. A – creatinine LS means depending on the daily milk yield;
B – creatinine LS means depending on the milk yield and THI

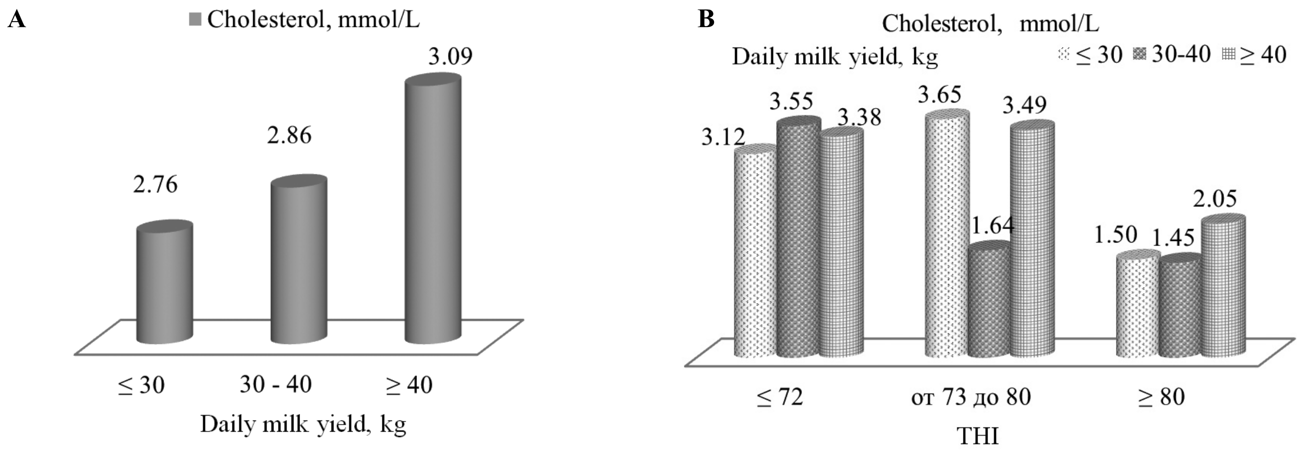

Fig. 5. A – cholesterol LS means depending on the daily milk yield;
B – cholesterol LS means depending on the milk yield and THI

in the cows with milk yield of up to 30 kg to 3.09 mmol/L in the cows with daily milk yield of over 40 kg. Upon THI of over 80, there was a serious decrease in the cholesterol levels in the cows from the three milk-yield groups. The cholesterol concentration in the blood of the cows from the three groups fell from values of over 3 mmol/L under the conditions of temperature comfort to levels from 1.45 mmol/L to 2.05 mmol/L under the conditions of moderate heat stress (THI over 80). Even under such conditions, the cows with high daily milk yield of over 40 kg had higher cholesterol levels (2.05 mmol/L) when compared with the low-productive ones (1.50 mmol/L) (Figure 5B). Similar decrease of the total cholesterol is also observed by Abeni et al. (2007) during the hotter period of the year and is associated with an increased lipid use from the peripheral tissues. Verma et al. (2000) also report that the cholesterol levels in the blood of lactating buffaloes are lower during the summer than in the winter.

The blood aminotransferases are the catalysts of the amino-acid and carbohydrate metabolism. A major change in their levels is observed upon their increased cellular activity or in the event of damage in the structures of their cells (Milinkovic-Tur et al., 2005).

The LS means of the two transaminases blood levels in the cows had an inverse correlation with the daily milk yield. The cows with lower milk yield of up to 30 kg had lower ASAT (92 U/L) and higher ALAT (23.38 U/L) levels when compared with the cows with high daily milk yield which had values of respectively 106.6 U/L and 19.74 U/L (Figure 6A).

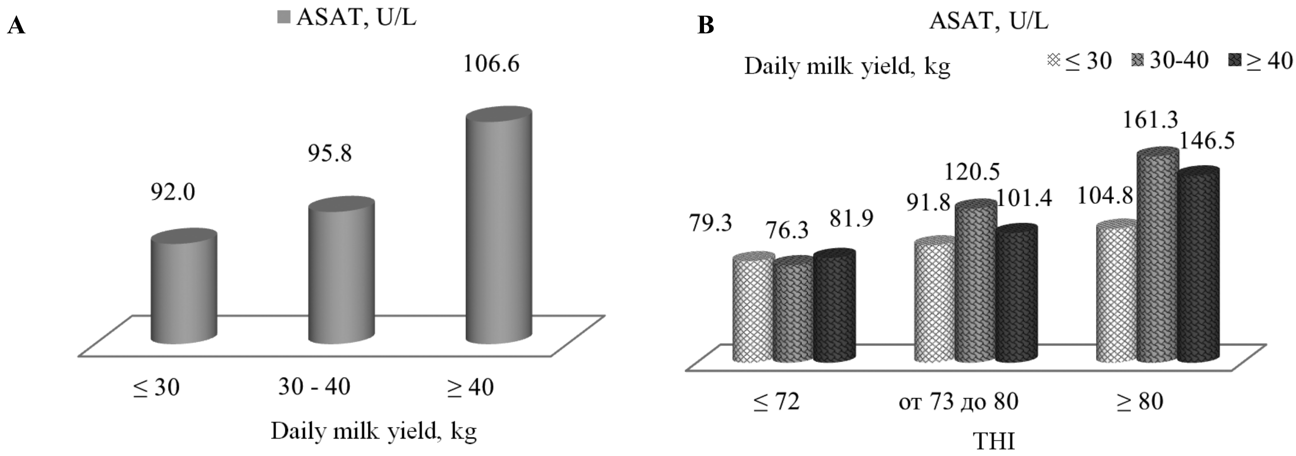

Fig. 6. A – ASAT LS means depending on the daily milk yield;
B – ASAT LS means depending on the milk yield and THI

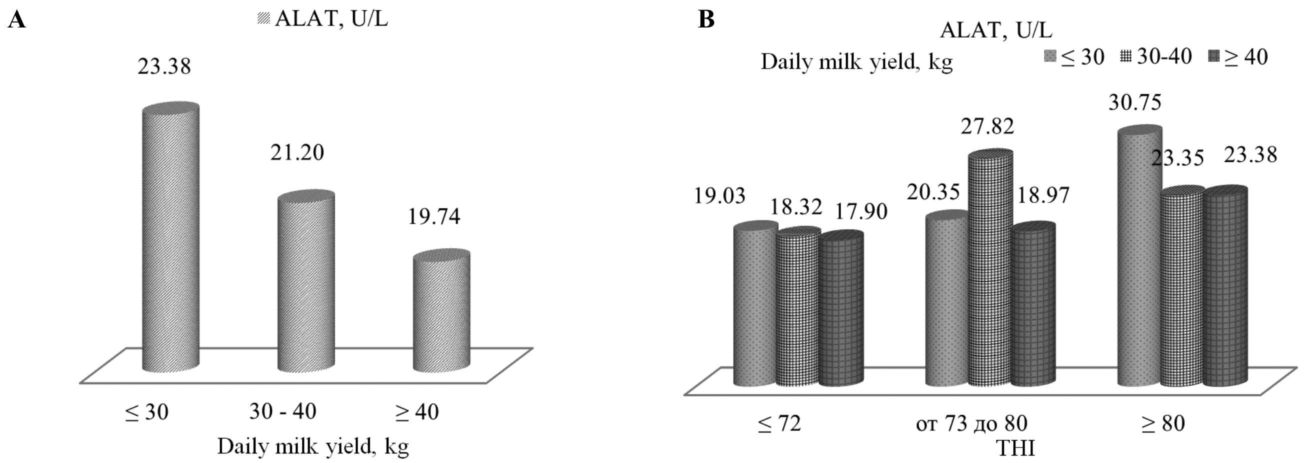

Fig. 7. A. ALAT LS means depending on the daily milk yield;
B. ALAT LS means depending on the milk yield and THI

The present study indicates that upon THI values increase (Figure 6B), especially above 80, there was a significant increase, which was above the reference values for the breed, in the activity of the both enzymes. Unlike the ALAT (Figure 7A) enzyme activity, that of ASAT increased in parallel with the increase of the daily milk yield. In our opinion, the higher ASAT and ALAT in the cows are related to the more intense metabolic activities caused by the lactation itself. Cozzi et al. (2011) specify that this effect is more commonly observed in cows during their third or following lactation.

Moore, (1997) comes to the conclusion that the elevated ALAT serum levels point to a liver pathology in dairy cows caused by degenerative processes of the hepatocytes. The ALAT activity increase is rarely a clinically important indicator except for the cases when it is twice as much or more than the upper reference values (3-10 U/L) which we also report.

According to one of our previous studies, (Ivanova & Tasheva, 2020), the reason for the high transaminases values may be found in the high productivity of these animals or the so called productive stress. The intense lactation and accelerated metabolism increase the ASAT and ALAT activities even under the conditions of moderate heat stress Gorski and Saba, (2012).

A significant increase of the cortisol (Figure 8) blood levels was observed upon elevation of the THI values and was more clearly displayed by the groups with daily milk yield of 30-40 kg and over 40 kg (Figure 8). It is the first hormone

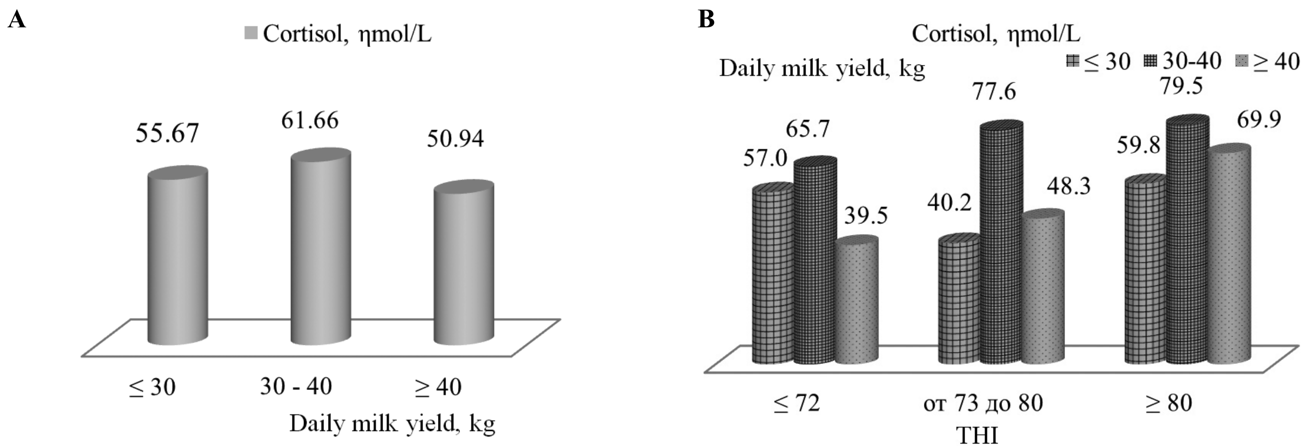

Fig. 8. A: Cortisol LS means depending on the daily milk yield;
B: Cortisol LS means depending on the milk yield and the THI

whose plasma concentration increases upon stress. According to Sejian et al. (2018) the circulating cortisol is a highly sensitive heat stress indicator. The high THI levels accelerate its synthesis and release from the suprarenal glands as a result of the hypothalamus-hypophysis- suprarenal glands system activation. This leads to a glucose increase which in turn provides the necessary source of energy for the body. (Jang-Hoon et al., 2021).

Our tests ascertained a high significance ($p < 0.001$) with reference to the related effect of the daily milk yield and the cortisol levels. Similar results are also reported by Sgorlon et al. (2015).

Conclusion

Based on the results obtained, the following conclusions can be made: the blood serum biochemical parameters directly reflect the physiological condition by modulating the milk characteristics of the lactating cows.

The blood glucose and protein levels decrease with the increase of the daily milk yield and the THI values. The urea, creatinine, cholesterol and cortisol in blood increase in parallel with the milk yield and the THI increase. The THI increase has a statistically significant effect on the ASAT ($p < 0.001$) and ALAT ($p < 0.01$) concentration. The activity of the ASAT enzyme increases in parallel with the daily milk yield while that of ALAT decreases.

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