AGRICULTURAL UNIVERSITY - PLOVDIV

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Influence of basic microclimatic and technological parameters on some indicators characterizing the comfort of free ranged dairy cows

DISERTATION ABSTRACT

Dissertation for the award of the educational and scientific degree "Doctor" in the scientific specialty "Zoohygiene"

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The dissertation is structured in an introduction, ten chapters including conclusions, recommendations for practice and contributions with a total length of 154 pages. 258 references were used. There are 28 tables and 34 figures and 22 author photographs.

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Note: The numbering of the tables and figures does not coincide with those in the thesis.

I. INTRODUCTION

The main problems in the rearing of cows for milk have been and remain unsatisfactory hygienic conditions and microclimate of the production environment, disturbances in technological processes, poor execution of construction works, and others, which are the cause of high mortality in calves, gynecological, hoof and musculoskeletal problems in cows. The specific prophylaxis applied is very often insufficient to limit disease incidence, increase resistance and prolong the economic use of the animals. During the lactation period, a number of physiological, biochemical and endocrine changes take place in the cow's body, due on the one hand to the growing fetus and on the other hand to lactation itself. The proper development of the fetus, the quantity and quality of the milk produced and the duration of the lactation period itself depend not only on the physiological and health status of the animal, but also on its resistance and adaptability to the factors of the production environment.

Dynamic climate change, accompanied by periods of extremely high temperatures in summer, and not only, is becoming a problem for the countries of Europe. The temperature factor, as one of the components forming the welfare and comfort of dairy cows, has been the subject of continuous discussion and evaluation in recent years. Comfort indices are widely used for this assessment.

Considering that comfort is a combination of microclimatic, technological, health, social, and other factors, its assessment by these alone is insufficient. To assess what changes occur at the cellular and organ level in the body, beyond the visible behavioral responses, one would need to include indicators of physiological adaptability as well as some complex metabolic markers of heat stress.

Revealing the specific relationship between the animal and environmental factors, which are in fact specific to each individual building and technology, will broaden the concept of comfort without neglecting the physiological characteristics and norms of the animals and the economic efficiency of the farm as a whole.

II. AIM AND OBJECTIVES:

The aim of this dissertation is to study the influence of basic microclimatic and technological parameters on some indicators characterizing the comfort of free-range dairy cows in the Upper Thracian Lowland.

In order to achieve our goal we set the following tasks:

1. Measurement and analysis of the main mesoclimatic factors of the area of the controlled buildings.
2. Constructional features and technological solutions of dairy buildings.

3. Measurement and analysis of the main elements of the microclimate of the controlled buildings. Determination of the temperature-humidity index (THI) and risk assessment of temperature stress.
4. Determination of comfort indices and the influence of THI on them.
5. Evaluate the impact of THI on some physiological and blood biochemical parameters of dairy cows.
6. Influence of production environment factors on animal health status.

III. MATERIAL AND METHODS

MATERIAL

The studies were carried out for two years in three dairy cattle farms with different capacities in one district of Central South Bulgaria: Asenovgrad town, village. Tsalapitsa and Rogosh village. The technology of cow rearing in two of them is free in individual stalls, and in the third - group stall on deep bedding. The farms are conventionally designated by numbers (F1, F2 and F3) and the buildings under study by B1, B2 and B3.

On all three farms the cows are of the Holstein breed.

Farm 1. The farm is located on the land of the town of Asenovgrad. The capacity of the farm is 307 milking cows free-range in individual pens. The building (B1) subject of our research is for 130 dairy cows divided into two groups of 65. The building is a semi-open metal structure with a roof of thermopanels and concrete walls. On each side of the feed aisle, between the two rows of manure aisles, two rows of individual stalls are located opposite each other. The fertiliser is cleaned by a delta scraper which runs at 6 hour intervals. Forage placement is Twice during the day and is done with a self-propelled mixer. Feeding is at will with whole ration mix and constant access to water. Milking is Twice during the day in a 2x12-herringbone parlour equipped with herd management software.

Farm 2. The farm is located in the village of Tsalapitsa. Its capacity is 500 milking cows, free-range in individual pens. The building under study (B2) is inhabited by 200 dairy cows divided into 4 groups. The building is enclosed with reinforced concrete construction, concrete wall and roof panels. There are no dividing partitions between the individual stalls. The feeding path is 4,3 m wide. The feed is loaded with a mechanical mixer in the morning and in the evening. The fertiliser is cleaned with a delta scraper that moves every three hours. Milking is Twice during the day in a 2x8-herringbone parlour, still without management software.

Farm 3. It is located in the land of the town of Plovdiv (Rogosh village). The capacity of the farm is 110 cows, free-range on deep bedding. The building under study (B3) is for 67 milking cows. The building is semi-open, with double brick walls, no internal or external render. The natural ventilation is close to tunnel ventilation. Additionally, 8 fans (DeLaval) with a power of 0,55 kW are installed, switching on automatically in stages at temperatures above 18 and above 25ºC. The roof structure of the building is made of galvanised sheet metal and without insulation. Feeding is at will with whole ration mix and constant access to water. Feed is set with an automatic mixer twice during the day. Fertilizer cleaning is twice during the year. Straw bedding is added periodically. Milking is Twice in a 2x5 DeLaval parlour.

METHODS

1. Outdoor and indoor air testing

Air measurements were carried out in parallel outside and inside the buildings with respect to:

Temperature (Cº) - we measured using a mercury thermometer (BDS 8451-77), an Asman aspiration psychrometer, and a weekly NOVI thermo-hygrograph.

Relative humidity (%) - measured in parallel with an Asman aspiration psychrometer and a weekly NOVI thermo-hygrograph.

Air velocity (m/s) - with a PeakTech 5170 wing anemometer,

Atmospheric pressure (hPa) - with aneroid barometer type 103, Germany.

Illuminance in buildings - with luxmeter PU 150 PRAHA,

Ammonia - with gas detector "Portable Four - In - One".

The carbon dioxide and hydrogen sulphide content in the rooms was recorded in parallel with the ammonia concentration measurement. Air samples were taken to record contamination with microorganisms from the points where the temperature and relative humidity were measured. Because no deviations from the normal values for these parameters according to Regulation No. 44 were observed, we have not included and commented on them in the Results section. We recorded these parameters in the buildings of the three farms twice each month at 10:00 h; 13:00 h and 17:00 h in the resting, feeding and fertilizer aisles, including at least five measurements at animal level (100-120 cm) for each process area.

External measurements were made bilaterally on the buildings, at least 5 m away from them at five consecutive points at a height of about 120 cm. We took all readings year-round, but because of the similarity in climatic characteristics measured outside and inside the buildings for the spring and fall seasons, this dissertation comments on the data obtained only for the spring, summer, and winter seasons.

2. Buildings:

We evaluated the air exchange and heat balance of the buildings according to the methods adopted in zoohygiene (*Iliev et al., 2008, Hristev, 2008)* and those for public buildings (*Ivanov and Krapchev, 1978).*

We measured the temperature of the internal surface of the walls and the floor with a handheld multifunction Compact infrared thermometer 105518 with a range from -50 to + 550ºC and a resolution of 0.1ºC. The reading of these values was in parallel with the recording of air environment parameters. We measured the temperature of each of the four walls at five points at 120cm. height and the floor in the bedding, feeding and torus aisle areas at a minimum of five points. We took the dimensions of individual building elements and individual boxes with a 50 m long metal tape measure and a Hitachi digital laser tape measure.

3. Comfort indices

We monitored and recorded the necessary data to calculate comfort indices *(Grant, 2009)* twice each month for one year during daylight hours at 11:00 h; 13:00 h; 15:00 h and 17:00 h. Based on the recorded and averaged data, the following indices were calculated:

Cow Comfort Index (CCI) = Number of cows lying down in stalls/number of cows standing straight or lying down x 100

Stall Utilization Index (SUI) = Number of cows lying down in stalls/number of non-feeding cows x 100

Stall Standing Index (SSI) = Number of cows standing in stalls and those with their forelimbs in the stalls/number of cows in the stall x 100.

4. Animal physiological studies

Eighteen Holstein cows aligned by calving period were included in the study. The first reading of physiological parameters and blood collection coincided with the spring season, when the cows included in the study were in the 1st lactation period (60-80-th day). For the summer season, the same cows are in their second lactation period (180-200-th day) and for the winter season, the cows are at the end of their lactation. The microclimatic parameters were recorded and the physiological parameters were monitored during the three seasons (spring, summer and winter). The cows were on different lactations from second to third. The physiological parameters were recorded in the same animals for all seasons, except for two animals that were culled for different reasons and replaced with animals of the same age and lactation.

Physiological studies were also conducted twice each month of the study period, at 11:00 h; 13:00 h; 15:00 h and 17:00 h:

Rectal temperature (ºC) - with a Kebl electronic thermometer, model 2130, *Arterial pulse rate (n/min)*, with a facial artery stopwatch, on the external surface of the mandible.

Respiratory movements (n/min), with stopwatch, by tracking the movements of the animal's rib arch and flanks.

Skin temperature (ºC), with Compact infrared thermometer 105518 with a range of -50 to + 550ºC and a resolution of 0.1ºC. The latter is presented as an average of the temperatures measured on the forehead, back and belly of the animals.

5. Biochemical blood tests - Venous blood was collected Twice a month in the morning before feeding and before the first recording of the physiological status of the animals. We obtained blood by puncture of the v. Jugularis or v. Cava cudalis. For the purpose of these analyses, we collected blood in Serum Separation Tubes (BD SST II Advance), 3 ml vacutainers. An additive-separating gel forms a barrier between the blood and serum formers after centrifugation and a silicone clotting activator injected along the sides of the vacutainer (Image 10). The resulting blood was mixed 5 - 6 times, and after clotting, the separated serum was centrifuged for 10 min /1300-2000 g. The study was carried out in the laboratory of the Department of Animal Sciences on a semi-automated biochemical analyzer with ready-made tests of the company "BIOMED" for :

blood sugar, mmol/L; total protein, g/L; urea, mmol/L; creatinine, μmol/L; cholesterol, mmol/L; cortisol, ηmol/L; alanine aminotransferase,(ALT), U/L; aspartate aminotransferase, (AST), U/L; calcium (Ca); phosphorus (P); magnesium (Mg); sodium (Na); potassium (K); and chlorine (Cl), mmol/L.

6. Animal health status survey.

The lesions on the animals were visually identified (various skin changes and lesions: abrasions, alopecia, edema, ulcers, vesicles, pustules, decubital wounds). Any animal showing deviation in movement, regardless of the degree of lameness, was recorded as having lameness. We also used the farm ambulatory diaries, tracking the data over a two-year period (the study period). The percentage of animals with relevant afflictions (mastitis and diseases under the general definition of "metabolic": ketosis, hepatic dystrophy, indigestion, hypocalcemia, metritis, etc.) per farm was recorded.

7. Temperature adaptability indicators

Benezra and Dmitriev indices were calculated:

Benezra's (1954) heat adaptability index:

$$\mathbf{I = T/38.3 + R/23,} \text{ where}$$

T is the body temperature and **R** is the respiratory rate.

Dmitriev's (1970) heat adaptability index:

$$\mathbf{HAI = T_1/T_2 + R_1/R_2}$$

where $\mathbf{T_1}$ is the daily and $\mathbf{T_2}$ the morning body temperature, $\mathbf{R_1}$ the daily and $\mathbf{R_2}$ the morning respiratory rate

To estimate temperature and relative humidity, we used the temperature-humidity index of ***Tom (1959):***

$$\mathbf{THI = 0.8 \times T_0 + (B_0/100) \times (T_0 + 14.4) + 46.4,} \text{ and}$$

Tom's adapted temperature-humidity index for cattle of the *National Research Council, 1971: (Dikmen & Hansen, 2009 and Habeeb et al., 2018a)*

$$\mathbf{THI = (1.8 \times T + 32) - \{(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)\},}$$

where **T** is the dry bulb temperature in ^{0}C and **RH** is the relative humidity in %

Many authors classify the temperature-humidity index into classes that indicate the level of heat stress *(Vitali et al., 2009; Habeeb et al., 2018; Mylostyvyi et al., 2019).* We used *Armstrong's (1994)* classification to assess comfort status: animals are in optimal comfort at an index below 72, from 72 to 80 is the zone of mild stress, from 80 to 90 is the zone of moderate stress, and at values above 90 is the zone of severe stress.

8. Statistical processing of results

For the basic statistical processing of the data, the MS Excel package was used, and for obtaining the means, errors and analysis of variance, the corresponding modules of STATISTICA by StatSoft *(Copyright 1990-1995 Microsoft Corp.)*

For better approximation, the factors are presented in classes as follows: reporting season: winter from December to February; spring from March to May; and summer from June to September. We have used the term 'season' in the sense of the astronomical concept. On this basis, the following factor classes have been formed: winter from 21 December to 21 March; spring from 22 March to 21 June; summer from 22 June to 22 September.

In this way, the actual seasonal climatic features of the temperate continental climate of the study region are distinguished - high temperatures from late June to mid-September and low temperatures from late December, January and February.

Temperature-humidity index (THI): values up to 72, from 73 to 79 and above 80 (*Armstrong 1994)*

CCI and SUI: values up to 50%, 51 to 69 and above 70% respectively

SSI respectively: values up to 20%, 21 to 30% and over 31% according to *Coock et al. (2005)*. Rearing technology: free with individual boxes and free group rearing.

The following model was used to estimate the influence of the controlled factors on the climatic performance of the farm area:

$$\mathbf{Y_{ijk} = \mu + F_i + S_j + eijk}$$

where: $\mathbf{Y_{ijk}}$ is the dependent variable (temperature, humidity and outdoor Air flow speed, m/s), **μ** is the model mean; Fi is the fixed effect of farm, $\mathbf{S_j}$ is the fixed effect of reporting season, and **eijk** is the effect of uncontrolled factors (error);

The following model was used to estimate the effect of controlled factors on THI values (indoors):

$$\mathbf{Y_{ijk} = \mu + F_i + S_j + F*S_{ij} + eijk}$$

where: $\mathbf{Y_{ijk}}$ is the dependent variable (THI temperature-humidity index), **μ** is the model mean; $\mathbf{F_i}$ is the fixed effect of farm, $\mathbf{S_j}$ is the fixed effect of season of reading, $\mathbf{F*S_{ij}}$ is the related effect of farm and season of reading and **eijk** is the effect of uncontrolled factors (error);

The following model was used to estimate the influence of the controlled factors on the values of the comfort indices (CCI, SUI and SSI):

$$\mathbf{Y_{ijklm} = \mu + F_i + Gj + S_k + THI*F_l + eijklm}$$

where: $\mathbf{Y_{ijklm}}$; is the dependent variable (CCI, SUI and SSI), **μ** is the model mean; $\mathbf{F_i}$ is the fixed effect of farm, $\mathbf{G}_j$ is the fixed effect of survey year, $\mathbf{S_k}$ is the fixed effect of survey season, $\mathbf{THI*F_l}$ is the related effect of THI and farm and **eijklm** is the effect of uncontrolled factors (error);

The following model was used to estimate the effect of controlled factors on the percentage of cows lying down:

$$\mathbf{Y_{ijklm} = \mu + T_i + G_j + T*S_k + THI*T_l + eijklm}$$

where: $\mathbf{Y_{ijklm}}$; is the dependent variable (percentage of recumbent cows), **μ** is the model mean;

$\mathbf{T_i}$ is the fixed effect of rearing technology, $\mathbf{G_j}$ is the fixed effect of year of study, $\mathbf{T*S_k}$ is the associated effect of season and rearing technology, $\mathbf{THI*T_l}$ is the associated effect of **THI** and rearing technology and **eijklm** is the effect of uncontrolled factors (error);

The following model was used to estimate the effect of controlled factors on the values of biochemical parameters:

$$\mathbf{Y_{ijk}l = \mu + F_i + G_j + S_k + eijkl}$$

where: $\mathbf{Y_{ijkl}}$; is the dependent variable (biochemical indicator), **μ** is the model mean; $\mathbf{F_i}$ is the fixed effect of farm, $\mathbf{G_j}$ is the fixed effect of survey year, $\mathbf{S_k}$ is the associated effect of season of recording, and **eijkl** is the effect of uncontrolled factors (the error);

The following model was used to estimate the effect of comfort indices on the rates of different diseases:

$$\mathbf{Y_{ijkl} = \mu + CCI_i + SUI_j + SSI_k + eijkl}$$

where: $\mathbf{Y_{ijkl}}$**;** is the dependent variable (lameness, lesions, mastitis, metabolic), **μ** is the model mean; $\mathbf{IIK_i}$ is the fixed effect of CCI (in classes**),** $\mathbf{IIB_j}$ is the fixed effect of SUI (in classes), $\mathbf{ISB_k}$ is the fixed effect of SSI (in classes), and **eijkl** is the effect of uncontrolled factors (the error);

Least squares means (LSM) were obtained by class of fixed factors using analysis of variance (ANOVA) for the model.

IV. RESULTS AND DISCUSSION

4.1. Status of the main mesoclimatic factors of the studied farms

The three cattle farms, subject of our study, are located in the region of Central South Bulgaria - the Upper Thracian Lowland. In climatic terms, the area is not homogeneous. The average annual temperature is about 18ºC. Average maximum temperatures are in the range of 30-31ºC and absolute maximum temperatures sometimes exceed 40ºC. Average minimum temperatures are in the order of 6,5ºC and annual temperatures around 10 - 12ºC.

The farms surveyed are sufficiently far apart to show some differences in the climatic conditions of the localities.

Farm 1 is located on the outskirts of the town of Asenovgrad. The municipality of Asenovgrad occupies the southeastern part of the Plovdiv region. It is situated on both banks of the river Asenitsa (Chepelaarska, Chaya). In the north it covers a small part of the Thracian Lowland, and in the south it covers large areas of the Rhodope Mountains, so a significant part of the terrain is very rugged and sloping. The town has an altitude of about 220 m. The climate of the area is temperate-continental. Average summer temperatures are between 19,5ºC and 23,7ºC, with absolute maximum temperatures mostly ranging from 36 to 40,9ºC. Total rainfall for the summer months (May - June) is 157 mm or 26.5% of the annual total. Precipitation for a year is 592 mm and for winter138 mm or 23.3% of the total. Average daily temperatures in winter are below 5ºC, i.e. winter is mild.

Farm 2 is located in the village of Tsalapitsa, Rhodope municipality of Plovdiv region. The weather in Tsalapitsa is characterised by sharper temperature fluctuations than in the municipality of Plovdiv, despite the fact that both municipalities are in the Upper Thracian Lowland. The village has an altitude of about 150 m. It is located 19.3 km west of the town of Plovdiv and 10.9 km south of the town of Sojdenie. The average duration of sunshine in summer is 73% and the average relative humidity is about 71%. The measured average annual temperatures are about 12,1ºC. The highest absolute temperatures are measured during the July-August period.

Farm 3 is located in the eastern part of the city of Plovdiv. The city is situated on both banks of the river Plovdiv. Maritza in the Upper Thracian Lowland. The climate is mainly transitional-continental, with warm summers (with average temperatures around 30-31ºC) and relatively mild winters (with average temperatures just below 0ºC). The average annual relative humidity is about 73%, with the highest in December at 86% and the lowest in August at 42%. The average annual rainfall is 540 mm, with a maximum in May-June of 69.2 mm and a minimum in September of 31 mm. The prevailing winds in the areas of the controlled farms are northwesterly, followed by northerly.

Table 1. Average values of temperature, relative humidity and air movement in the farm area during different periods of the year

Indicators	Farm 1	Farm 2	Farm 3
	X ± SE	X ± SE	X ± SE
Spring			
Temperature, ºC	20,60 ± 0,30	19,80 ± 0,35	21,00 ± 0,30
Relative humidity, %	66,00 ± 2,60	65,70 ± 2,90	67,00 ± 1,80
Air flow speed, m/s	0,31 ± 0,03	0,30 ± 0,02	0,34 ± 0,018
Summer			
Temperature, ºC	28,50 ± 0,88	30,30 ± 0,85	28,80 ± 1,02
Relative humidity, %	48,20 ± 0,35	45,10 ± 0,27	47,40 ± 0,41
Air flow speed, m/s	0,25 ± 0,025	0,21 ± 0,05	0,23 ± 0,002
Winter			
Temperature, ºC	3,70 ± 0,25	2,90 ± 0,01	4,10 ± 0,40
Relative humidity, %	70,40 ± 3,60	72,80 ± 3,40	67,90 ± 1,60
Air flow speed, m/s	0,23 ± 0,03	0,17 ± 0,01	0,19 ± 0,01

X – Average
SE - standard error of the mean

We present our measured main parameters (temperature, relative humidity and air velocity) characterizing the mesoclimate of the controlled farms in Table 1. The data in the table show that in the areas of the three farms in summer, the average temperatures are above 28ºC, with the highest in the area of farm 2 at 30.3ºC. In winter, again in the area of farm 2, the lowest average temperatures were recorded - plus 2,9ºC. In spring, the average daily temperatures were approximately the same for all three farm areas, ranging from 19,8ºC for the farm 2 area to 21ºC for the farm 3 area. Larger diurnal amplitudes are recorded for the farm 2 area in spring. The absolute maximum and minimum temperatures recorded during our study period for the three farm areas were 35ºC and minus 8.6ºC. All recorded differences in mean temperatures for the different seasons by farm area are within 1 - 2ºC, but they are statistically insignificant.

Changing temperatures as a result of global warming are having a negative impact on the comfort and productivity of dairy cows, especially those with high genetic potential. The recorded values of average relative humidity are highest during the winter season and range between 67.9% for the area of farm 3 and 72.8% for the area of farm 2. In summer, humidity is low for all three areas, ranging between 45.1% and 48.2%. Regarding air velocity, no significant difference was found for the different areas of the controlled farms. The highest velocity was recorded in spring (0.3 - 0.34 m/s) and the lowest in winter in the areas of farms 2 and 3 (0.17 and 0.19 m/s, respectively). Due to the location of all three farms in the Upper Thracian Lowland region, no statistically significant farm effect was found for the parameters temperature, relative humidity and air velocity. Only the season had an effect on all three indicators - $p < 0.001$ (Table 2).

From the results obtained, it should be summarized that the area of farm 2 is characterized by the highest temperatures in summer and the lowest in winter compared to the other two farms, as well as the lowest air velocity. Summers are cool and winters mild for the Farm 1 area. The warmest in winter is found to be the area of farm 3 (4.1oC). The areas of the three farms also recorded absolute maximum temperatures of the order of 34 - 35 oC in summer, which are above the thermoneutral zone. If sustained for a longer period of time, these temperatures can cause the development of heat stress in dairy cows.

Table 2. Average annual values of climatic factors in the areas of the surveyed farms and confidence levels

Parameters	LS	SD	±SE	Season, F,p	Farm, F,p
Temperature,^{o}C	17,74	11,30	±0,295	652,243***	0,147
Relative humidity, %	61,17	10,95	±0,692	109,313***	0,103
Air flow speed, m/s	0,25	0,058	±0,006	33,419***	3,129

LS, least squares mean; SD, standard deviation of the mean; SE, standard error of the mean; F, Fisher's criterion; P, confidence level; ***, significant difference at p < 0.001

4.2. Hygienic assessment of ventilation and architectural, structural and technological solutions of the studied dairy cow buildings.

The main differences between the buildings we studied (Table 3) are in terms of architectural and construction elements. The first building is a steel structure with concrete walls 150 cm high, the second is made entirely of reinforced concrete elements and the third is made of brick. The roof of the first building is made of thermopanels, of the second building of concrete roof elements without additional insulation, and of the third building of galvanised sheet metal without insulation.

The type of ventilation in all three buildings is mixed (natural and mechanical), feeding is at will with constant access to water. The cleaning of the manure in building 3 is done with a bulldozer shovel and in the other two with a delta scraper.

Table 3. Characteristics of the buildi**ngs**

	Building 3	Building 2	Building 1
Type of building	brickwork, semi-open	reinforced concrete structure and walls	open metal construction
Roof construction	galvanized sheet metal without thermal insulation	concrete roof panels	Thermal panels
technology of animal husbandry	group on deep bedding	free boxing, individual boxes	free boxing, individual boxes
Type of ventilation	natural + mechanical	natural + mechanical	natural + mechanical
Nutrition	at will, allotment mixtures	at will, allotment mixtures	at will, allotment mixtures
Illumination	natural + artificial	natural + artificial	natural + artificial
Cleaning the fertilizer	bulldozer shovel	scraper	scraper
Animal watering	automatic drinkers - troughs	automatic drinkers	automatic drinkers
Required air exchange, m3/h	113 900	340 000	221 000
Required exchange rate (times/h): in winter in summer	 2,8 19-28	 2,8 19-28	 2,8 19-28

According to *Regulation 44,* a minimum of 6 m² of building space must be provided for each animal. On the three farms surveyed, this requirement was met, even if the area provided exceeded this standard. The cows on farm 2 had the largest amount of personal space (11,5 m²), followed by farm 1 (9,4 m²) and farm 3 (8,06 m²). Therefore, one of the animal welfare conditions guaranteeing cosiness and comfort is met. Assuming the optimum volume recommended by *Dinev & Dimova (2006)* and *Dinev (2007*), which is between 56,3 and 72,3 m3 , it is clear that it is only achieved in the stables of farm 2. In the other two farms this volume turns out to be almost half (about 25 m3), which implies an increase in the volume of ventilation or its frequency, both in winter and in summer.

The food front provided is larger than recommended (0.8 m) for farms 2 and 3, while it is slightly smaller for farm 1 at 0.74 m. According to *Dinev (2007*) this does not limit the cow to have free and constant access to the feed even when it is reduced to 0.59 - 0.66 m for a dairy cow. In our opinion, it is possible that dominant cows push out weaker cows, violating the European requirements for animal protection and welfare based on the five freedoms.

The buildings we have studied, according to the number of animals and the height of the side walls, should provide the air exchange and exchange intensity indicated in Table 4. The air exchange requirement per animal remains the same regardless of the ventilation method and the number of fans.

Table 4. Required air exchange (m³/h) and intensity (times/h) of air exchange in buildings

Building	Height of side walls, m	Number of cows	Required air exchange (m³/h)	Intensity (rate) of exchange (times/h)
3	3	67	113900	2,8/19-28
2	4,5	200	340000	2,8/19-28
1	1,5	130	221000	2,8/19-28

To maintain the minimum temperature neutral limit (5ºC) in Building 3 in winter, the airflow needs to be increased by a factor of about 15 to remove excess heat and about 5 to remove excess moisture. This also requires an increase in the intensity of the exhaust air change itself. In fact, the existing insufficient air exchange and low replacement intensity are the cause of the retention of excess moisture in winter and of excess heat in summer.

In Building 2, the opposite is observed: there is more than twice as much air exchange in winter (with more than 115350 m³/h), thus releasing the heat to the outside. In summer, the fresh air shortage increases manifold. If additional ventilation capacity is not included during this period, the maintenance of thermal homeostasis in the animals will be disturbed. This calls into question the extent to which the changes made to the rearing technology with cosmetic adjustments to the building have had an impact on the comfort of the cows and the overall efficiency of the farm itself.

In Building 3, the actual air exchange, both for the removal of excess moisture and for the removal of excess heat, is extremely deficient in both seasons, but this deficiency is most sensitive in terms of excess heat. Assuming that each cow requires about 1700 m^3/h of fresh air according to *Gooch (2007)*, an air exchange of 115 000 m^3/h should be guaranteed in Building 3, 340 000 m^3/h in Building 2 and 221 000 m^3/h in Building 1. It can be seen that the actual air change in all three buildings is well below the required air change. The heat balance in the buildings studied is considered on the basis of the number of animals, live weight, and the differences in their architectural, construction and technological design. The incurred heat costs in winter in all three buildings are greater than the incomes. The highest costs are in the closed building 2 (639 174 kJ/h). One of the reasons for this in our opinion is the low stocking density. The lowest costs are found in the open building 1 (135359 kJ/h).

In summer, an almost equal heat balance is observed in building 2. The higher heat costs in building 3, both in winter and summer, are related to the warming of the deep litter and the evaporation of moisture from it. In Building 1 the costs are about 4 times less than the revenue. The determined Δt of the zero balance showed that maintaining a temperature of 5ºC in Building 3 would continue until outside temperatures drop below 0.7ºC, for Building 2 below minus 7.5ºC and for Building 1 below minus 1.6ºC. This temperature is not risky for cattle, but it still exists as a minimum threshold in the current *Regulation 44*, on which our judgements are based. It is noteworthy that the solid (closed) building has better thermal properties than the two open ones, making it suitable for areas with short and sharp temperature fluctuations of the outside temperature. The dimensions of the individual technological elements of the pens are respected, with the exception of the height of the chest restraint in farms 1 and 2 (30 - 35 cm), which causes discomfort when the animals stand up. The higher leading edge of the crib on farm 3 (55 cm) is in keeping with the size of the cows and we do not consider it a disadvantage.

In conclusion, we can summarize that due to the incorrect positioning of the fans and their insufficient number, the ventilation is inefficient: the required air exchange in buildings 1 and 3 is insufficient in both winter and summer, and in building 2 - in summer. The winter heat balance in all three controlled buildings is negative. The main reason for this in Building 2 is the almost doubled surface area per animal and in Building 3 the high humidity of the deep litter throughout the year.

4.3. Basic elements of microclimate in controlled buildings

Appropriate temperature and relative humidity in the production rooms ensure the comfort of the animals and are a prerequisite for good health and maximum productivity.

The average values of the investigated hygiene indicators in the controlled buildings presented in Table 5 demonstrate their own character of dynamics for each building, but also a certain dependence on the factors of the external environment. Compared with the values recommended in Regulation No 44, it follows that in winter the cows on these farms are placed in environments with temperatures around and above 5º C, and in summer in temperatures close to or more often exceeding the upper permissible limit (28º C).

Table 5: Average values of microclimate factors in controlled buildings

	Spring			Summer			Winter		
Indicators	B 1	B 2	B 3	B 1	B 2	B 3	B 1	B 2	B 3
Temperature, ºC	22,2	23	24,1	29,2	29,5	28,5	5,2	7,5	8,5
Relative humidity, %	70	75	73	67	82	81	73	89	78
Air velocity m/s	0,22	0,28	0,36	0,45	0,55	0,65	1,2	1,5	0,9
Cooling rate, mJ/cm²/s	8,8	9,5	10,2	9,7	8,5	9,9	11,2	13,9	9,8
Illumination, Lux:	400-600	350-750	200-450	400-1200	400-700	250-700	250-550	220-700	180-450
Ammonia content, mg/l	14,4	8	15,2	0,25	0,22	0,28	0,21	0,18	0,24
Bed temperature, ºC	16,8	18,5	12,5	25,9	25,7	22,6	4,3	6,3	2,5

The level of light on all three farms matches the physiological needs of the animals, even in winter, whereas in other seasons the light input is two to three times higher. Lack of sufficient light, despite good feeding of the animals, can become a leading cause of poor sexual activity and low fertility.

High ammonia values have been recorded for all three farms, in all three seasons, with logically the highest values on farm 3, where animals are kept on non-replaceable bedding. In building 1, during the spring season, ammonia values exceed the permissible values (0,02 mg/l) several times due to the low air velocity.

A high correlation dependence of air temperature, floor temperature and air movement on the architectural, structural, and technological design of production buildings was found. Relative humidity is negatively correlated not only with building type but also with the temperature maintained in them. The season of the study also has a high degree of influence on the air temperature, that of the floor, and the air movement in the production buildings, but not on the humidity in them (Table 6).

Table 6: Correlations between the studied indicators and degree of reliability

	Temperature 2, ºC	Temperature 3, ºC	Relative humidity 2, %	Air flow speed, m/s
Season	0,97***	0,91***	-0,1	0,90***
Farm	0,99***	0,93***	-0,47***	0,8***

******p<0.001; **p<0.01; *p<0.05***

Temperature 2 - indoor temperature; Temperature 3 - floor temperature; Relative humidity 2 - relative humidity of indoor air; Air flow speed - air movement in buildings

The use of averaged temperature and humidity values, usually measured during daylight hours (7, 14 and 21 hours), does not give an accurate, but rather a general picture of the temperature-humidity regime in buildings. What is the width of their amplitudes and what is their duration, most often remain hidden. In order to unleash a specific stress response, both the strength (severity) of the factor and its duration are of utmost importance. For this, we also performed a round-the-clock tracking of temperature and relative humidity dynamics to get a more accurate picture of the real state of these environmental factors during the warmest and coldest days of the year. From the recorded thermo-hygrograms it can be seen that the temperatures in summer in Building 2 fluctuate from 20 to 40ºC with amplitudes sometimes greater than 20ºC. The relative humidity at the same time varies between 30 and 90 %. In winter, the amplitudes of both temperature and relative humidity are almost twice as large. The measured temperatures varied between 0 and 10 ºC.

In the open building 1 (similarly in building 3) the temperature fluctuations in summer are in the range 18 - 33ºC with daily amplitudes of about 15ºC. The relative humidity for the most part follows the outdoor humidity fluctuations and is between 32 and 80%. Winter temperatures in these buildings are mostly around freezing (- 6 and + 8ºC). This makes the animals instinctively flock to the inside of the buildings. A similar pattern is observed in summer, with grouping mainly in areas of fresher and cooler air. In the closed building, both in summer and winter, the displayed thermo- and hygrograms show some consistency and regularity over the day, whereas in the open buildings this pattern is absent. The fluctuations are within a smaller range, following outdoor temperature and humidity or overlapping with them completely. This indicates that the temperature-humidity regime in the two open buildings is more dependent on atmospheric factors in contrast to the closed building. This is supported by the zero balance temperature difference found, which for building 1 is minus 1,6 ºC and for building 3 is 0,7 ºC, while for building 2 is minus 7,5 ºC. The time for the indoor temperature to equilibrate with the outdoor temperature (phase shift) in the closed building is on average 3,8 ± 0,5 hours against 1,5 ± 0,3 hours in the open buildings. Considering the recommendations in *Regulation No 44* on the optimum limits of relative humidity in cow housing (70-75% with a minimum of 50% and a maximum of 85%), we can conclude that most of the data, for the three buildings and for the different seasons of the year, are within the acceptable limits. The extent to which the temperature and humidity in buildings can be assumed to match the physiological needs of the animals depends on both the movement of air and its cooling capacity. The results of our investigations show that air movement and cooling magnitude in summer are quite low, in all three farms, from 0.45 to 0.65 m/s, and from 3.1 to 4.8 mcal/cm²/s. In winter, the air velocity exceeds 3 to 5 times the accepted norm, which is 0.3 m/s. In cases where this velocity is maintained for a longer period of time, especially at temperatures below 0ºC, the prerequisite for cold stress occurs. This is also supported by the higher than recommended (5-8 mcal/cm²/s) magnitude of cooling recorded in almost all seasons of the year. This makes it necessary to avoid air currents at temperatures below 10 ºC. Microclimate factors dictate animal welfare and behavior, including the amount of time spent lying down. Our topography of floor temperatures in summer and winter reflects differences in cow behavioral responses as a consequence of rearing technology. The high temperatures we measured in Buildings 1 and 2, and on the bedding areas in summer, caused cows to group mainly in the fan action area, staying upright most of the time to cool down due to the rubber bedding not being able to cool their bodies in time. At the same time, for the cows in Building 3, where moist and cool bedding is available at all times, it becomes the preferred area where they remain to spend up to 55.2% of their time lying down.

In conclusion, it can be summarized that the microclimate formed in each building is the result of the number of animals in the building, the rearing technology, their constructional characteristics and the season. The temperature and relative humidity in the controlled buildings are approximately the same during the three seasons of the year.

4.4. Temperature-humidity index and comfort

4.4.1. Temperature-humidity index

From the average daily THI values for the areas of the three farms (Figure 1), it can be seen that during the summer season, even with the possibility of outdoor rearing, values that predispose to heat stress in dairy cows are recorded, ranging from 71.15 to 74.50. Due to the fact that the studied farms are located in the region of the Upper Thracian Lowland, the THI for all three regions during summer and transition periods are approximately the same. However, the highest mean value of THI of the external environment was measured in the area of farm 1 in summer (74.5), when the highest absolute values of 35 ºC were also recorded, and in the area of farm 3 in spring (66.05). These values are below the accepted limit of temperature-humidity comfort for dairy cows, but may also be a possible risk, albeit less so, for the development of temperature stress. One of the highest average summer temperatures measured (29.2 ºC) was in the building on Farm 1.

Figure 1. Mean values and variation of THI in buildings and in the farm area and by season of reporting

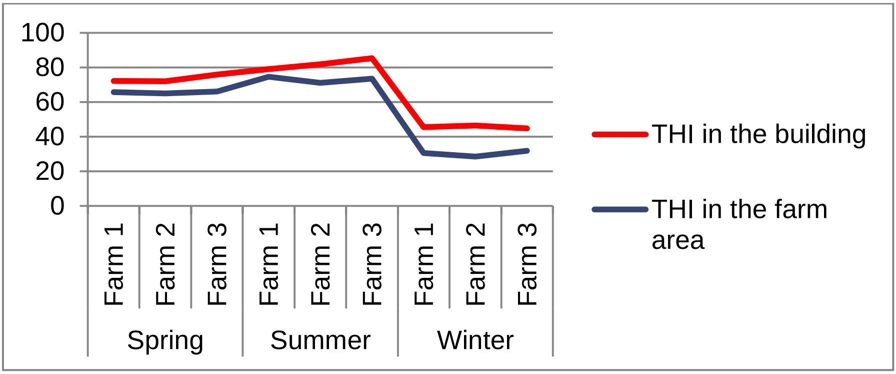


Table 7: Mean values and variation of THI by farm and season

Farm	Temperature humidity index			
	X ± SE	SD	min	max
Spring				
Farm 1	72,25 ± 0,739	2,56	69,8	74,7
Farm 2	72,05 ± 0,317	1,10	71,0	73,1
Farm 3	75,80 ± 1,327	4,60	71,4	80,2
Summer				
Farm 1	78,95 ± 0,196	0,68	78,3	79,6
Farm 2	81,80 ± 0,181	0,63	81,2	82,4
Farm 3	85,40 ± 0,121	0,42	85,0	85,8
Winter				
Farm 1	45,55 ± 0,497	1,73	43,9	47,2
Farm 2	46,40 ± 0,030	0,10	46,3	46,5
Farm 3	44,80 ± 1,146	3,97	41,0	48,6

X mean; SE - standard error of the mean; SD - standard deviation of the mean

All recorded average THI values in the controlled buildings during spring and summer exceeded those assumed to be optimal (72) temperature-humidity comfort for cows (Table 7). From all this, it can be seen that in summer, in addition to the buildings, daily THI values were recorded in the area of the three study farms that fell within or outside the comfort zone for dairy cows, defined by the different authors as mild stress or signaling the danger of heat stress.

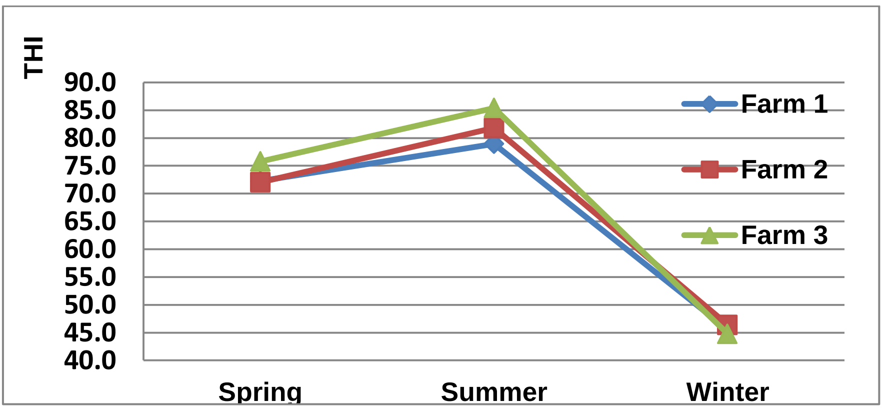
From the analysis of variance (Table 8) a significant effect ($p<0.001$) of farm, season and the associated effect of farm by season were reported, which are in fact of interest for the present study. The significant effect of season by farm found indicates that THI values differ significantly across the study farms irrespective of their location in the same climatic zone of the country.

Table 8: Variance analysis of the effect of environmental factors on THI values

Sources of variation	Degrees of freedom (n-1)	THI	
		MS	F p
Total about the model	8	3312,32	612,68 ***
Farm	2	87,3	16,15 ***
Season	2	13062,8	2417,1 ***
Farm*season	4	49,6	9,18 ***
Error	99	5,4	

MS, mean square; F, Fisher's criterion; p, significance level; *- significance at p<0.05; **- significance at p<0.01; ***- significance at p<0.001; - no significant effect

Figure 2. LS-averaged THI values by farm and reporting season



From the presented LS-mean values of THI (Figure 2) obtained from the model, it can be seen that the differences between farms are larger for the summer season, with the highest values reported in farm 3 (85.4) and the lowest in farm 1 (79.0). The mean values reported for farm 3 in summer according to the classification *of Segnalini et al. (2013)* fall under the heading of emergency or warning of a possible serious danger of temperature stress for the animals.

The results of our studies show that heat stress conditions for dairy cows in the Upper Thracian Lowland region are possible not only in summer, but also in spring when THI values reach 72-75. In this sense it can be predicted that in the future critical days of the year in Bulgaria will continue to increase against the background of general global warming.

4.4.2. Comfort indices

The influence of the controlled factors on the values of the three comfort indices is reflected in Table 9.

Table 9. Variance analysis of the influence of controlled factors on the values of the three comfort indices

Sources of variation	Degrees of freedom (n-1)	CCI		SUI		SSI	
		MS	F p	MS	F p	MS	F p
Total about the model	8	243,67	63,77 ***	734,07	21,54 ***	426,33	294,18 ***
Farm	1	195,5	41,74 ***	2107,8	61,85 ***	797,54	550,33 ***
Year	1	1,4	0,38 -	0,8	0,02 -	0,16	0,11 -
Season	2	46,9	12,26 ***	945,8	27,85 ***	274,96	189,73 ***
THI*Farm	4	435,4	113,94 ***	349,4	10,25 ***	125,79	86,80 ***
Error		3,8		34,1		1,45	

MS - mean square; F - Fisher's criterion; p - confidence level
CCI- cow comfort index; SUI- stall utilization index
SSI- index of standing in the stall; *- significance at p<0,05; **- significance at p<0,01; ***- significance at p<0,001; - no significant effect

Due to the close values for the two years, no impact of year on the reported indices was found. Significant effects of farm, season of reporting and the associated effect of farm on THI values (at p<0.001) had an effect on the three comfort indices. Worryingly, two-thirds of the comfort indices assessed using *Armstrong's (1994*) classification fell into the mild, moderate and severe stress categories (Table 10).

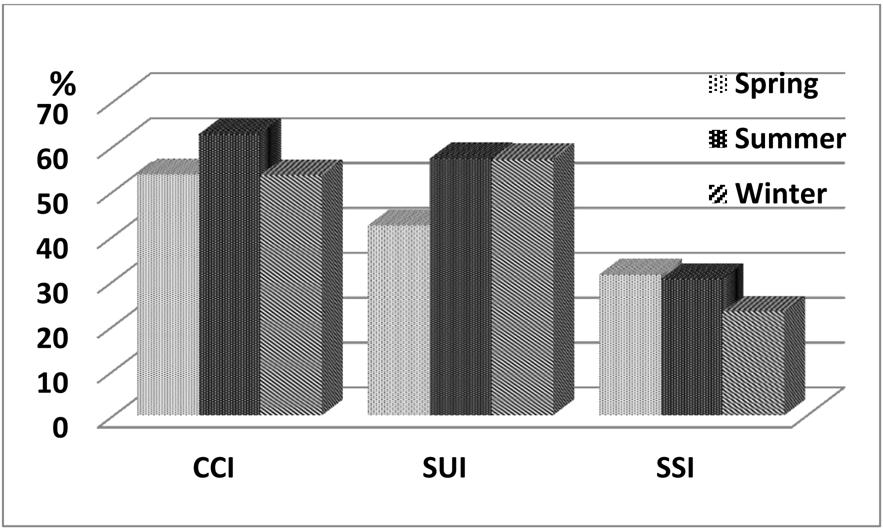
Table 10. LS-mean values of comfort indices depending on temperature-humidity index

THI-class	Number	CCI, %	SUI, %	SSI, %
≤72	54	65,82 ± 1,44	61,89 ± 1,942	20,54 ± 0,82
from 73 to 79	18	60,37 ± 0,65	50,37 ± 3,02	28,50 ± 0,84
≥80	36	63,87 ± 1,89	62,03 ± 1,86	29,37 ± 1,29

CCI - cow comfort index; SUI - stall utilization index
SSI - index of standing in stalls

Figure 3 presents the average values of the comfort indices depending on the season. Our finding is that across seasons the two indices (CCI and SUI) vary with the highest being in summer at 60 and 55% respectively. In spring they are between 40 and 50% and in winter 50-55%.

Figure 3. LS averages for comfort indices by season

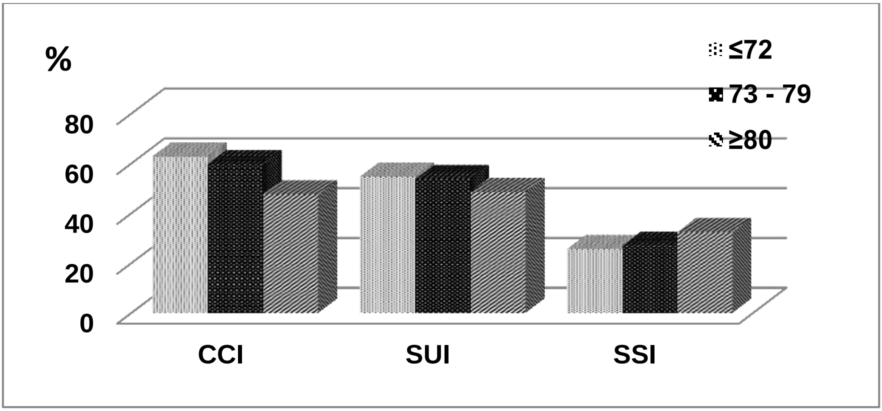


CCI - cow comfort index; SUI - stall utilization index
SSI - index of standing in stalls

There were also apparent differences in the values of the three comfort indices at the same THI values (Figure 4). These differences become even more pronounced at higher index values. The reason for this, in our opinion, is the architectural and thermal qualities of the buildings themselves, in which the building materials, the envelope, the technological equipment, the number, and the productivity of the animals themselves are all involved. While in the case of an open steel building the environmental factors are dependent on those of the external environment, following them with a phase deviation of about 1.5 hours, in the case of an enclosed building this deviation is 3.5 hours.

When tracking the comfort level of the cows according to the THI, classified according to Armstrong (1994), it is seen that the indices of CCI and SUI are in the zones of mild to moderate discomfort ranging between 50 and 60% (Figure 5 and 6). As THI increases above 80, the percentage of CCI also increases and is maintained at farm 1 while at farm 2 the percentage decreases to 42-45%.

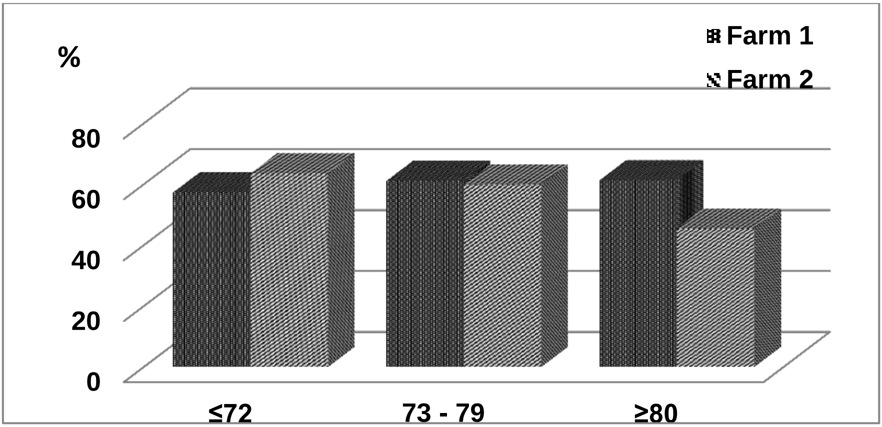
Figure 4. LS means for comfort indices versus THI values



CCI - cow comfort index; SUI - stall utilization index
SSI - index of standing in stalls

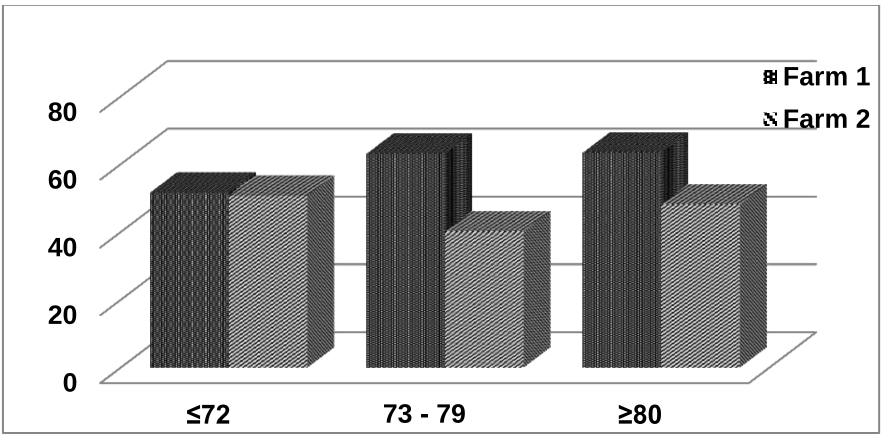
Quantification of comfort in dairy cows depends on the CCI. In fact, this index takes into account the behavior of the animal in the stall. The higher this index, the better the comfort and the higher the welfare of the animals. In the spring, the CCI on both farms is almost the same (57.7-58.8%) while in the summer on farm 2 the index decreases by 15.7%. However, in winter the index increases again compared to farm 1 by about 10%. However, very good comfort conditions for dairy cows were not reached in any of our controlled buildings.

Figure 5. LS averages for CCI by farm depending on THI



According to Rao et al. (2014) these index values should be 85-90%. Reading the diagram further proves that building 2 provides a higher percentage of optimal conditions than building 1 at THI up to 72. In building 1, the possibilities to avoid cases of moderate and severe discomfort in cows increase with increasing THI

Figure 6. LS averages for SUI by farm depending on THI



The temperature-humidity index characterizing the conditions in the individual buildings is also indicative of the behavioural responses of the animals, but the same cannot be applied to dairy cows free-housed on deep bedding. However, the data presented on the behavioural responses of free-housing and deep litter animals provide an answer to the question which of the two free-housing options provides more comfortable conditions in summer (Table 11) and in winter (Table 12).

Table 11. Main behavioural responses of cows in summer

Farm / Behaviour	Building 3		Building 1		Building 2	
	Number of animals	%	Number of animals	%	Number of animals	%
Lying	45,2±18,7	55±5,4	62,6±15,2	47,8±4,6	77±21,8	38,5±4,8
Fed	13,5±1,8	19,5±2,2	25,8±1,8	19,6±1,3	37±2,4	18,5±0,9
Stand straight or move	16,2±1,2	24,2±0,5	35±2,1	30,8±1,9	80,2±2,2	40,6±1,3
Drink water	1±0,05	1,5±0,3	3,5±1,2	2,2±0,8	5,4±1,3	2,2±0,6

In order to regulate their organismal homeostasis, cows from Building 3 preferred direct contact with the moist litter, i.e. lying and chewing - 55.0% instead of standing straight. Cows from Building 2 spent the least time lying in the stall - 38.5%. They prefer to move around or stand straight, thereby increasing the cooling surface area of their body.

Table 12. Main behavioural responses of cows in winter

Farm / Behaviour	Building 3		Building 1		Building 2	
	Number of animals	%	Number of animals	%	Number of animals	%
Lying	38±2,5	56,6±3,8	48,2±4,5	37,1±2,9	71±8,1	35,5±3,8
Fed	30,4±1,9	45,3±2,9	20±3,6	15,4±3	35±2,8	17,5±1,2
Stand straight or move	8±2,8	12±1,7	40,5±5,5	31,2±4,2	40±5,9	20±1,9
Drink water	1±0,1	1,5±0,2	-	-	-	-

A similar picture is observed in the winter period. The deep bedding provided a warmer bed, which was preferred by 56.6% of the animals compared to the colder rubber bedding of the individual stalls where the number of cows lying down was 37.1 and 35.5% respectively.

In the spring (Table 13) again the number of cows lying down was highest in the deep bedding building at 58%. Due to the higher THI measured more frequently, the number of cows lying free-chocked during this period was greater in the building with the reinforced concrete structure (Building 2) - 43.5% compared to Building 1 - 36.9%.

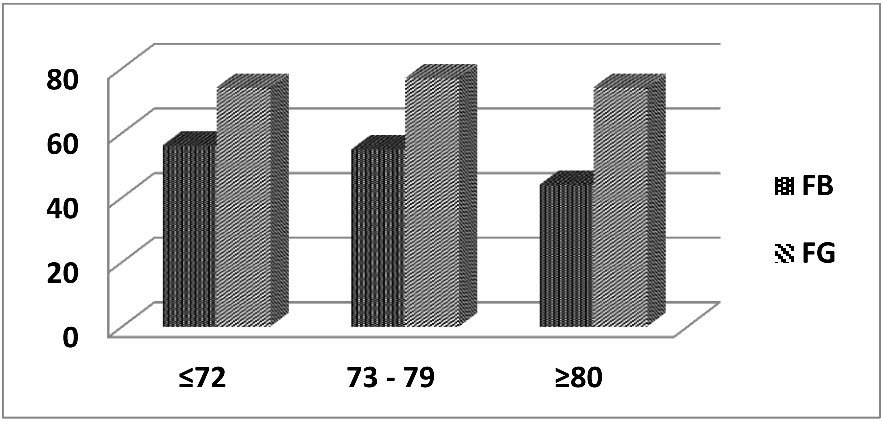
Table 13 Main behavioural responses of cows in spring

Farm / Behaviour	Building 3		Building 1		Building 2	
	Number of animals	%	Number of animals	%	Number of animals	%
Lying	39±2,2	58±2,8	48±6,5	36,9±2,5	88±9,6	43,5±2,8
Fed	12±0,9	17,9±1,7	42±3,6	32,3±3	43±2,5	21,5±1,2
Stand straight or move	14±1,8	21,9±1,4	34±3,5	26,2±2,3	65±5,1	32±1,5
Drink water	2±0,3	2,4±0,5	5±0,7	3,8±1,2	6±1,5	3±0,8

In our view, this is a result of its better thermal balance and the greater phase deviation provided by the envelope compared to that of the open building (Building 1), where internal temperatures almost overlap with external temperatures. The comfort conditions in the buildings studied can therefore be classified as 'good'. At optimum THI, the use of the boxes in both buildings is almost the same. As the THI increases, the use of stalls increases for the cows in building 1 while it decreases in building 2. At optimum THI, stall use was almost equal on both farms.

As THI values increase, the number of standing cows in stalls increases in Building 2 while it decreases in Building 1. Its relative proportion almost doubled with increasing THI in summer. Lying down is a prerequisite for comfort, higher milk yield, better general health, fewer limb problems and so on. For this, we made a comparison between the two buildings with technology free with individual rest boxes and building 3 with technology free group on deep bedding.

Figure 7: Percentage of recumbent cows for the two technologies versus THI values



FB - free box breeding, FG - free group breeding

Table 14. Analysis of variance for the effect of controlled factors on the percentage of lying cows out of the total number

Sources of variability	Degrees of freedom (n-1)	Percentage of recumbent cows	
		MS	F p
Total about the model	9	1702,32	36,10 ***
Year	1	60,6	1,28 -
Season*technology	2	15,96,9	33,87***
Technology	1	81,48,0	172,81***
THI*technology	3	162,2	3,44*
Error	98	47,2	

MS - mean square; F - Fisher's criterion
p - Significance level, *- significance at p<0, 05; **- significance at p<0, 01; ***- significance at p<0,001; - no significant effect

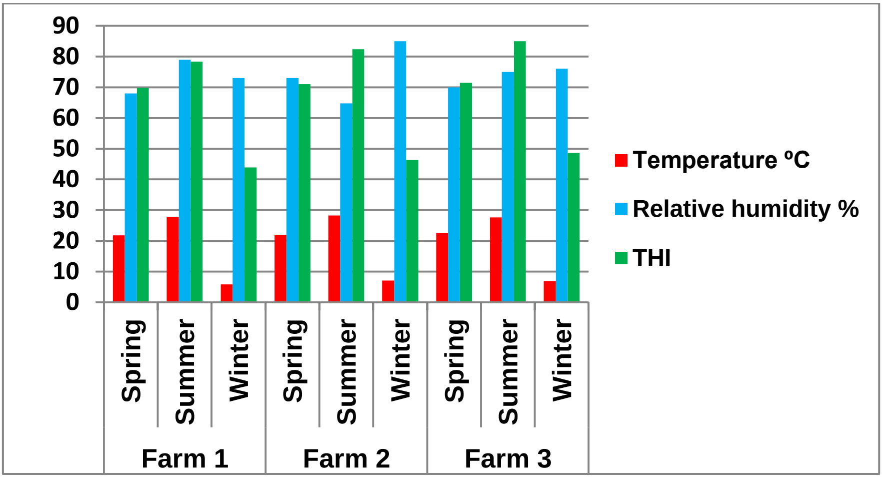
In all seasons of the year, cows lying free in groups are 15 to 30% more than those free in individual pens. Regardless of comfort zones, the percentage of cows lying down was again 15 to 30% higher for cows free-ranged in groups (Figure 7). A significant effect was observed for the percentage of cows lying down in both technologies depending on season ($p<0.001$) and THI ($p<0.05$ - Table 14).

4.5. Influence of temperature-humidity index on some physiological and biochemical parameters in animals.

4.5.1. Influence of temperature-humidity index on some physiological changes in animals.

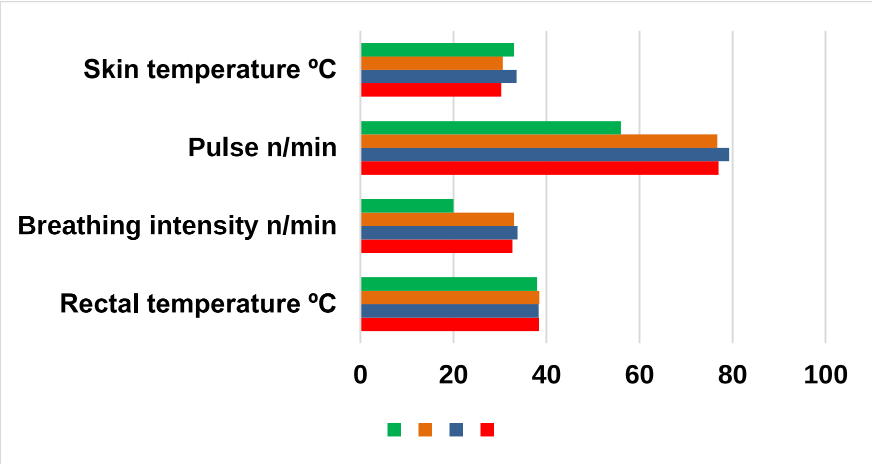
The effect of climatic changes on the physiological characteristics of cattle can be used to determine their responses to heat stress.

Figure 8. Average values of microclimate factors in controlled buildings



According to Figure 8, the mean values of temperature and relative humidity fluctuate as they pass through periods of undesirable temperature-humidity regimes, mostly in summer and spring. In winter, the lowest average temperature (5,8 ºC) was measured in building 1 and the highest (7,1 ºC) in building 2. In summer, the highest average temperature measured was in Building 2 (28.5 ºC). During the same period, 27,5 ºC was measured in building 3 and around 28 ºC in building 1. The average maximum value of the index in summer is 85. The values recorded in the spring hover around and above what is considered to be the optimum limit, namely between 70 and 75. In winter, the most frequent average values recorded are around 50. An analysis of the thermogram data reveals that in summer, even in spring, the absolute maximum values recorded have reached 35-40ºC and the index has exceeded 90. In our investigations, we found an acceleration of respiration and pulse rate, which still remained within the reference values. At THI values between 72 and 78, and more, the rectal temperature slightly exceeded the physiological limits (Figure 9). Respiration almost doubles in frequency. There is a trend for cow rectal temperature to increase relative to physiological limits at all THI levels. This is in all likelihood a response of the organism trying to restore its thermal balance.

Figure 9. Values of physiological indicators per farm



B1, 2, 3 – Building 1, 2, 3

Table 15. Analysis of variance for the effect of THI (in classes) on physiological parameter values

Physiological indicator	Degrees of freedom	THI		Error
	(n - 1)	MS	F ….p	MS
Rectal temperature, °C	2	4,2	2,81 -	1,5
Skin temperature, °C	2	151,50	60,8***	2,5
Pulse, n /min	2	1472,50	54,3***	27,1
Respiratory rate, n /min	2	401,08	33,1***	12,1

MS - mean square
F - Fisher's criterion
p - degree of reliability, ***- significance at p<0,001

From the analysis of variance (Table 15), there was a significant effect of THI values on physiological parameters such as skin temperature, heart rate and respiratory rate (p<0.001). Since the cows included in the study were equated in lactation period and were reared and fed under the same conditions the main factor appeared to be THI. The effect of study season overlapped with that of THI. Since cows are more sensitive to high temperatures we decided to also test their heat tolerance using the Dmitriev and Benezra indices (Table 16).

Table 16. Cow heat tolerance indices

Farm / Indices for heat resistance	Building 3	Building 2	Building 1
By Dmitriev	2,10	2,14	2,12
By Benezra	2,45	2,49	2,44

Both indices in the three controlled buildings are between 2 and 2.5. The lower these values, the more resistant the animals are to high temperatures. These heat tolerance indices complement the THI of the barn environment and indicate that the cows in all three barns are in conditions closer to discomfort during the summer. These temperature conditions are capable of becoming a prerequisite for the development of heat stress, especially during daylight hours during the spring and summer seasons.

The body's tolerance to heat also determines its adaptive capacity, which allows it to maintain a high level of milk production in extreme conditions. The way the Benezra and Dmitriev indices are constructed determines the inverse relationship between their values and the level of heat tolerance, i.e. a high value of these indices corresponds to the lowest heat tolerance.

4.5.2. Influence of temperature-humidity index on some biochemical changes in animals

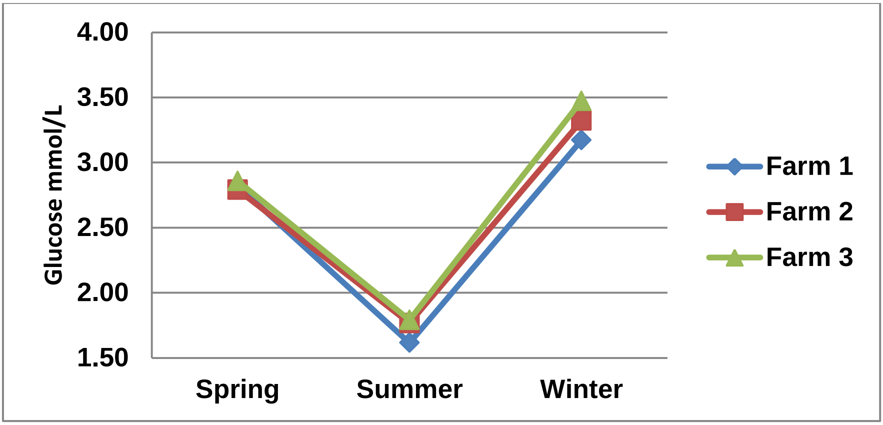
From the data in Table 17, it can be seen that on the farms we studied, blood sugar values in cows were close to the lower limits of the reference norms. The influence of season on them is particularly marked ($p<0.001$). In summer, glucose content was 61% lower than in spring and even more than in winter (Figure 10).

Blood sugar is not a major energy source for ruminants, but in late pregnancy and early lactation much of it is used for lactose and milk fat synthesis. For this, its alteration can be a signal of some pre-pathological and pathological conditions.

Analysis of variance (Table 18) for the influence of the main environmental factors (farm, year, and season) revealed that season had a marked effect on the values of all biochemical parameters except Ca and Mg. The values of these two macronutrients were not significantly influenced by other environmental factors such as farm and year of study.

In this case, THI was not included as a factor due to its effect overlapping with that of season (p<0.001).

Figure 10. Variation of blood glucose level by farm and season



To evaluate the influence of THI (in classes) on the values of biochemical parameters we applied a univariate analysis of variance, the results of which we present in Table 19. High THI values (above 80) caused a decrease not only in blood glucose levels but also in total protein, cholesterol and slightly in sodium and potassium. The decrease in sodium may be the result of sweating and subsequent electrolyte loss or the result of respiratory alkalosis developed as a result of acid-base disturbances leading to accelerated urinary Na excretion and K retention. Heat stress did not affect calcium, magnesium and chlorine metabolism.

The values of urea, ACAT, cortisol and phosphorus increased, with the most pronounced increase in creatinine (more than twice) and cortisol (37%). The increased creatinine level may also be the result of increased locomotor activity of the animals. The high urea values recorded at the highest THI confirm our view of the likely use of amino acids as an energy source.

Blood plasma analysis proved that cholesterol concentration was strongly influenced by THI and season (p<0.001), while the influence of farm was insignificant. This assumption of ours we also relate it to the higher levels of ACAT and ALT due to disturbances in the energy metabolism of the body in summer and partly in spring. ACAT and ALT activities were significantly affected by both farm and season (p<0.001). In our studies, we found that season (and high THI) reliably influenced blood cortisol content (p<0.001) (Figure 11).

Table 17. Average values of biochemical indicators by farm and season

Biochemical indicators	number	Farm 1			Farm 2			Farm 3		
		spring	summer	winter	spring	summer	winter	spring	summer	winter
		LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE
Glucose, mmol/L	12	2,82±0,07	1,62±0,11	3,17±0,09	2,79±0,06	1,77±0,11	3,32±0,11	2,86±0,03	1,79±0,09	3,47±0,13
Total protein, g/L	12	77,65±1,90	69,61±2,03	72,51±0,27	72,98±2,03	64,87±2,34	72,31±0,57	73,79±1,20	70,43±2,71	71,98±0,64
Urea, mmol/L	12	5,09±0,28	7,85±0,28	4,25±0,15	4,71±0,28	6,56±0,18	3,95±0,12	5,17±0,23	4,34±0,20	5,47±0,16
Creatinine, μmol/L	12	78,92±3,02	210,58±5,43	82,50±1,71	75,50±1,86	195,17±4,83	78,33±2,56	68,00±2,98	180,25±5,87	73,67±2,80
Cholesterol, mmol/L	12	3,41±0,09	1,53±0,11	3,08±0,18	3,54±0,11	1,39±0,07	2,88±0,14	3,48±0,13	1,72±0,10	2,98±0,21
АСАТ, U/L	12	92,68±7,30	130,95±11,37	85,02±2,60	104,49±9,54	110,08±11,52	80,64±1,97	78,73±3,90	118,59±20,24	73,38±2,53
АЛАТ, U/L	12	20,78±1,68	26,62±1,39	19,31±0,94	27,69±2,98	30,83±2,07	19,70±0,99	16,22±0,88	21,08±1,90	16,78±1,07
Cortisol, ηmol/L	12	38,85±0,79	95,36±2,08	54,36±3,80	39,89±0,34	72,69±4,31	55,17±3,60	40,97±0,72	77,65±2,73	55,21±4,14
Ca, mmol/L	12	3,99±1,66	2,38±0,06	2,35±0,06	2,32±0,05	2,32±0,03	2,30±0,05	2,25±0,04	2,20±0,03	2,31±0,07
P, mmol/L	12	1,67±0,06	2,10±0,13	1,94±0,06	1,90±0,07	2,03±0,09	1,91±0,08	1,82±0,06	1,97±0,06	1,81±0,05
Mg, mmol/L	12	0,99±0,03	0,89±0,04	0,97±0,02	0,89±0,02	0,95±0,03	0,98±0,02	0,99±0,02	0,96±0,04	0,96±0,02
Na, mmol/L	12	150,42±0,99	137,92±0,91	142,92±2,46	148,00±074	133,83±0,92	142,83±1,96	146,42±1,67	132,08±1,06	139,25±2,64
К, mmol/L	12	5,24±0,10	4,29±0,03	4,69±0,05	5,20±0,09	4,31±0,03	4,73±0,05	5,09±0,07	4,23±0,04	4,91±0,04
C, mmol/L	12	106,58±0,23	103,67±0,36	104,25±0,71	106,67±0,38	104,42±0,79	104,50±0,71	106,17±0,63	103,17±0,84	103,92±0,51

LSM - least squares mean
SE - standard error of the mean

Table 18. Analysis of variance for the influence of environmental factors on the values of biochemical parameters

Biochemical indicators	Farm		Year		Season		Error
	MS	F .p	MS	F .p	MS	F .p	MS
Glucose, mmol/L	0,26	2,49-	0,09	0,89-	24,06	227,6***	0,11
Total protein, g/L	104,1	3,52*	683,6	23,08***	400,4	13,52***	29,6
Urea, mmol/L	4,39	8,25***	5,21	9,79**	90,17	169,36***	0,53
Creatinine, μmol/L	2514	15,33***	1626	9,91**	170594	1040,09***	164
Cholesterol, mmol/L	0,12	0,74 -	5,60	33,76***	36,06	217,06***	0,17
АСАТ, U/L	1481	1,30-	33	0,03-	15273	13,38***	1142
АЛАТ, U/L	582,83	16,68***	41,44	1,19-	524,92	15,02***	34,94
Cortisol, ηmol/L	449,7	3,69*	183,8	1,51-	16313,3	133,97***	121,8
Ca, mmol/L	4,74	1,28-	1,81	0,49	3,54	0,96-	3,69
P, mmol/L	0,06	0,84-	0,02	0,21-	0,49	6,69**	0,07
Mg, mmol/L	0,01	0,79-	0,05	5,60*	0,01	1,30-	0,01
Na, mmol/L	182	7,69**	800	33,75***	1682	70,91***	24
К, mmol/L	0,001	0,03-	0,014	0,29-	7,33	154,48***	0,05
Cl, mmol/L	5,0	1,3-	23	5,7*	76	18,6***	4

MS - mean square; F - Fisher's criterion; p - confidence level, *- significance at p<0.05; **- significance at p<0.01; ***- significance at p<0.001; - no significant effect

Three minerals were influenced by season and high THI of the barn medium ($p<0.01$ for phosphorus and $p<0.001$ for sodium and potassium). The probable reason for the decrease in K and Na is the animals' desire to compensate for high THI values by enhancing fluid evaporation through accelerated respiration and profuse sweating.

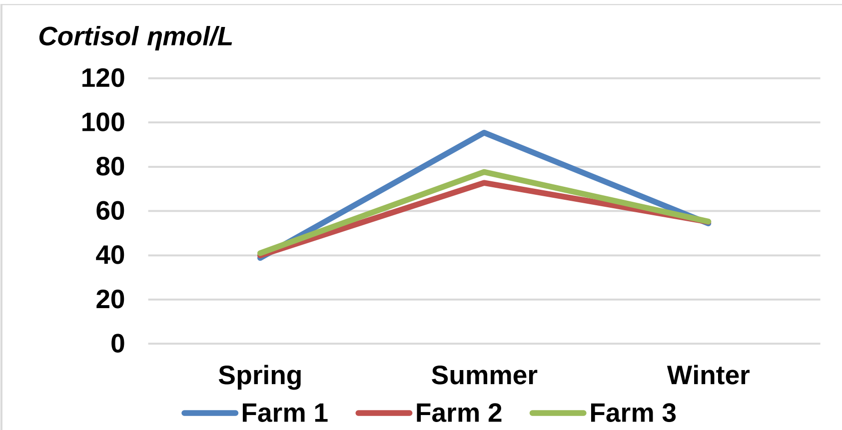
In conclusion, we can summarize that the main environmental factors (farm, year, season) had a marked effect on the values of all biochemical parameters ($p < 0.001$) except Ca and Mg. The effect of THI in this case overlaps with that of season. Cultivation technology significantly affected the values of cortisol ($p < 0.001$), urea and cholesterol ($p < 0.01$).

Building type does not significantly affect blood glucose, creatinine, phosphorus, magnesium, sodium, potassium and chlorine and these remain within physiological norms. High THI values (above 80) in summer and part of spring are responsible for decreasing blood sugar, total protein, cholesterol and slightly sodium and potassium levels while increasing those of creatinine (more than twice) and cortisol (37%).

Table 19. LS averages for biochemical indicators by THI influence (in classes)

Biochemical indicators	THI (class)		
	≤72 (n=54)	from 73 to 79 (n=24)	≥80 (n=30)
	LSM ± SE	LSM ± SE	LSM ± SE
Glucose, mmol/L	3,16±0,07	2,21±0,11	1,99±0,09
Total protein, g/L	72,65±0,86	74,45±1,30	68,80±1,16
Urea, mmol/L	4,28±0,13	6,75±0,20	6,58±0,18
Creatinine, µmol/L	74,63±5,58	147,38±8,37	164,93±7,49
Cholesterol, mmol/L	3,18±0,10	2,49±0,16	1,88±0,14
ACAT, U/L	82,04±4,64	120,53±6,97	105,72±6,23
АЛАТ, U/L	18,39±0,85	28,14±1,27	23,99±1,14
Cortisol, ηmol/L	49,89±2,57	67,07±3,85	68,53±3,44
Ca, mmol/L	2,32±0,26	3,18±0,39	2,25±0,35
P, mmol/L	1,83±0,04	1,96±0,06	2,03±0,05
Mg, mmol/L	0,97±0,013	0,91±0,02	0,97±0,02
Na, mmol/L	144,15±0,98	142,96±1,48	135,63±1,32
К, mmol/L	4,93±0,05	4,74±0,08	4,42±0,07
Cl, mmol/L	104,87±0,32	105,13±0,56	104,47±0,44

Figure 11. Variation of cortisol values depending on according to season by farm



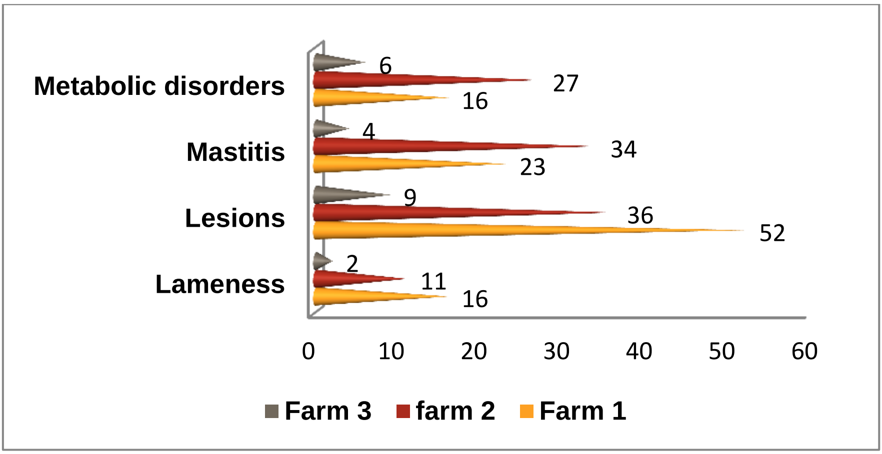
All this gives reason to assume that high daytime temperatures act as a depressant factor that increases the functional strain on cardiovascular, neuromuscular, immunological, i.e. on the overall organism metabolism.

4.6. Influence of production environmental factors on animal health status.

The number of cows with overt mastitis was found to be highest on farm 2 (34%) and lowest on farm 3 (4%) (Figure 12). The mastitis problem on farm 2 is largely subjective in nature: incompetent staff, high turnover and inadequate management decisions. In our research, we found a low rate of lameness in the building with deep bedding (3%) while in the other two buildings where rubber bedding is used on the beds it was 11-16%. Free-range indoor cows spend almost half of the day standing (10-12 hours) and moving around, which is mainly in the exercise and feeding area, and the floor is cement. For the time being, rubber matting has not yet found a use in the exercise and feeding areas of our barns. The hoof horn abrasion is uneven and the hoof takes on an irregular shape (barn hoof).

Cows with such hooves put excessive strain on the joints and because of the pain they prefer to lie down. As can be seen from Figure 13, about 52% of the cows on Farm 1 had scabs, sores or swellings on the limbs with 16% showing obvious lameness. The most commonly affected joints are the hock and knee joints. The most common cause of these injuries appeared to be the replacement of the original rubber armrests with wooden ones.

Figure 12. Percentage distribution of technopathies per farm



Selection over the years to increase the size of the animals on Farm 1 has caused the beds to be short for these animals with both hind limbs falling in and out of the rear edges of the bed. Our observations suggest that leaner animals are at higher risk of lesions due to less fat in the joint area.

Figure 13. Lesions, superficial injuries and local inflammation in the hind limbs (original)

Comparing our results with the data of other authors, it follows that urgent measures to improve comfort conditions are needed to reduce this health problem from 52% for farm 1 and 32% for farm 2 to the acceptable 5%. As a result of a complex of reasons, a large proportion of animals are culled during their first or second lactation. This problem is particularly pronounced for cows on Farm 2, which rarely reach the age at which they are expected to reach their full potential, i.e. the fourth lactation for the Holstein breed.

Table 20. Analysis of the influence of the values of the three indices of comfort on the reported rates of various diseases

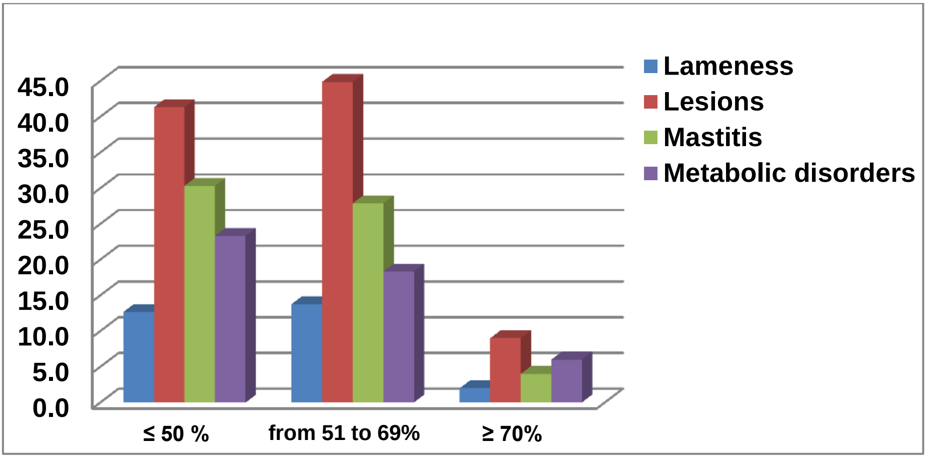
variation	Lameness		Lesions		Mastitis		Metab. dis.	
	MS	F p	MS	F p	MS	F p	MS	F p
CCI	265,89	55,22 ***	2464,22	49,98 ***	1207,22	51,80 ***	397,14	11,06**
SUI	283,25	113,30 ***	2641,00	103,20 **	1291,25	106,71 ***	534,04	30,24***
SSI	19,14	0,51 -	166,85	0,47 -	487,85	4,09*	329,21	7,32**

MS - mean square; F - Fisher's criterion; p - confidence level
****- significance at p<0.05; **- significance at p<0.01; ***- significance at p<0.001; - no significant effect***

To determine the extent to which the three comfort indices (CCI, SUI and SSI) were related to the rates of lameness, mastitis and metabolic disorders we performed a univariate analysis of variance. From the data in Table 20, it can be seen that the values of CCI and SUI were related to the percentage of all diseases included in the study ($p \leq 0.01$ and 0.001). The cow stall index (SSI) had a significant effect mostly on the percentage of metabolic diseases ($p \leq 0.01$) and a weak effect on different forms of mastitis ($p \leq 0.1$).

For a more visual representation of the percentage variations of the different diseases according to the values of the tracked comfort indices, we present them in classes in Figures 14, 15 and 16.

Figure 14. Percentage of diseases according to CCI values



In CCI, up to 70% of lesions and mastitis cases reach 40-45%. Only when this index rises above 70% the cases of lesions and mastitis decrease almost tenfold. And this is because this index on the farms we studied only reaches 60% in summer instead of 85-90%. A similar trend can be observed with regard to the SUI, which in our studies reaches a maximum of 60% instead of 70-80% or more.

Figure 15. Percentage of diseases according to the values of the SUI

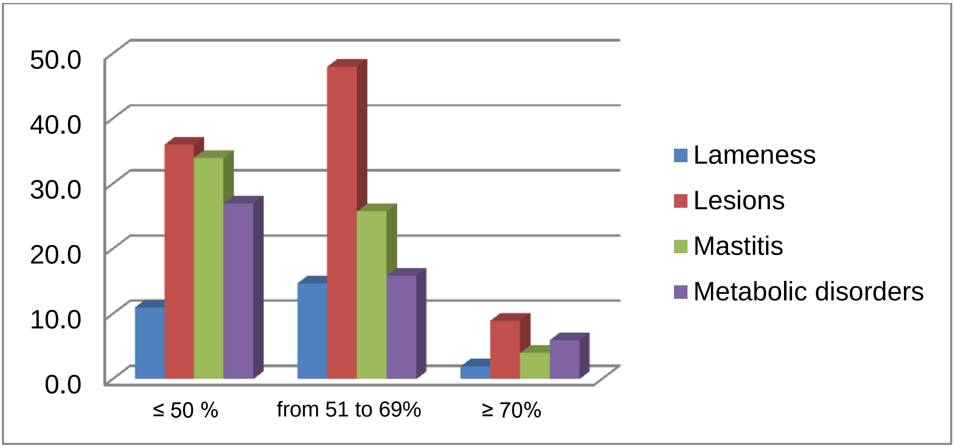
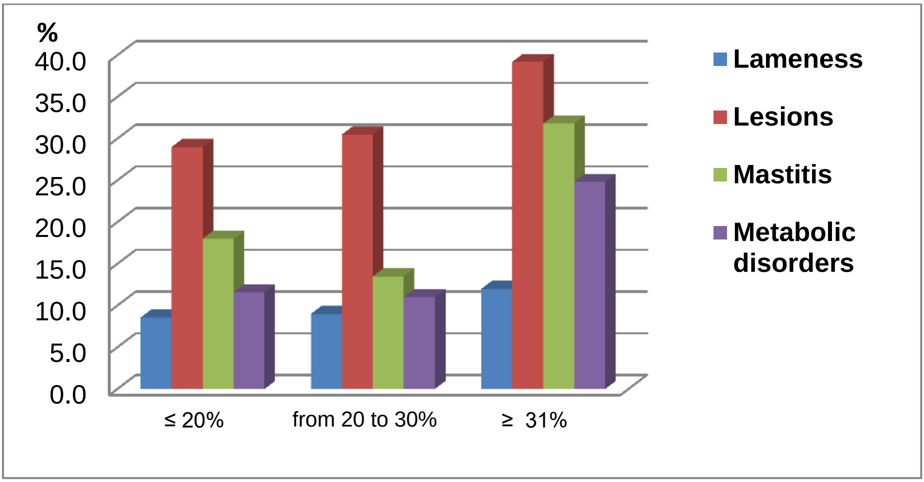


Figure 16. Percentage of diseases according to the SSI values



Our reported index values remain higher than 15% in all seasons of the year, with its relative share almost doubling as the THI increases in the summer to over 30%. Hence, the comfort indices give an accurate picture of the development of the different groups of technopathy in intensively reared cows.

V. CONCLUSIONS:

On the basis of the studies carried out and the results obtained, the following conclusions can be drawn:

1. In summer, the areas of the three farms recorded risky values of air temperature exceeding the thermoneutral zone for dairy cows, but no significant differences were found between the THI of the areas in different seasons of the year.

2. In technological terms, all three buildings provide the necessary space for an individual animal. The required relative room volume is only met in building 2 whereas it is 50% for buildings 1 and 3. The feeding front corresponds to the accepted norms in buildings 1 and 2 while in building 3 it is reduced by 6,5%.

3. The ventilation and thermal balance of the buildings depends on the number and productivity of animals, the building materials, the type, architectural and construction and technological design of the buildings. The air exchange in buildings 1 and 3 appears to be insufficient in both winter and summer while in building 2 the same in winter is about 2 times higher and in summer insufficient.

4. The heat balance in winter is negative in all three buildings. The phase deviation in the open buildings 1 and 3 is short - only 1,5 hours while in the closed building 2 it is 3,5 hours. Maintaining a minimum temperature of 5 ºC in winter is possible up to minus 1,6 ºC in building 1, minus 7,5 ºC in building 2 and plus 0,7 ºC in building 3. This gives the advantage of the closed building to be used in areas with short and sharp temperature fluctuations of the outside temperature.

5. There was a high correlation between air temperature, floor temperature and air movement and the architectural and technological design of the production buildings ($p<0.001$), and a negative correlation between relative humidity and building type and between relative humidity and building temperature ($p<0.001$).

6. There was statistically significant effect ($p<0.001$) of the factors farm, season and the associated effect of farm by season. This significant effect of season by truss indicates that differences in THI values are dependent not only on external factors but also on the architectural, structural and technological features of each building.

7. The cow stall utilization index increases as the THI increases from 72 to and above 80, in the open building, while it decreases in the closed building.

8. No maximum indices of welfare and comfort were found in any of the buildings studied (CCI; SUI; SSI). Significant effect of truss, season of reporting and associated effect of truss on THI values ($p < 0.001$) influenced the three indices.

9. It was found that when THI increased, the number of cows lying free grouped was highest in all seasons of the year compared to cows free boxed, i.e. the effect of significance on season and technology was high ($p<0.001$), and on THI significantly lower ($p<0.05$).

10. There was a significant effect of THI on physiological parameters - skin temperature, pulse rate and respiratory rate increased ($p < 0.001$). At THI values above 72, rectal temperature increased by almost 1 °C (from 37.6 to 38.4 °C), but remained within the physiological normal range.

11. Analysis of variance for the influence of the main environmental factors (farm, year, season) revealed that season had a marked effect on the values of all biochemical parameters, which changed their values beyond the species reference ($p < 0.001$), except for Ca and Mg.

12. The significant negative influence on the percentage of the studied diseases was exerted by the CCI and the SUI ($p \leq 0.01$ and 0.001), while the index for standing cows in the stall (SSI) mainly influenced the percentage of metabolic diseases ($p \leq 0.01$) and less on the different forms of mastitis ($p \leq 0.1$).

VI. RECOMMENDATIONS FOR PRACTICE

1. In order to obtain higher production results, to ensure the comfort and preserve the health of the animals, we suggest that both in the newly built and in the older production facilities it is mandatory to carry out an analysis of the architectural, constructional, technological and thermal features of the buildings. The complex nature of the assessment should also include a comprehensive analysis of the barn environment and the area in terms of the main microclimatic factors such as temperature, humidity, air movement, illumination.

2. Use comfort indices as indicators for assessing the welfare, health and comfort of cows, together with temperature tolerance coefficients, which are non-invasive and easy to apply, and use basic biochemical markers for a more in-depth analysis.

3. We recommend that an adjustment be made in the length of the stall as well as replacing the wooden armrests with the original rubber ones in Farm 1.

4. To minimize the number of standing cows in the walking areas during the summer, we recommend a two-stage ventilation system: one for general ventilation of the building and the other for direct ventilation in the lying area, not as before in the walking and feeding area.

VII. CONTRIBUTIONS OF THE THESIS

1. A complex methodology is applied for the assessment of comfort conditions in dairy cows, including the study of the influence of the natural-climatic conditions of the area, the constructional and technical-technological features of the buildings, their thermal capabilities, the quality and efficiency of the ventilation system, the physical, biological and mental state of the animals and the possibilities for the prevention of major diseases.

2. The assessment of the barn environment has been extended by comparing and complementing the THI data with the Benezra and Dmitriev temperature tolerance coefficients.

3. A relationship between THI and indices of comfort in dairy cow buildings was established.

4. Changes in biochemical parameters and metabolic processes associated with seasonal variations in temperature and THI were found.

5. Relationship between comfort indices and percentage of cows diseased by technopathy was found. In the highest degree on all the diseases included in the study affect the indices of CCI and SUI ($p \leq 0.01$ and 0.001), while the index of standing cows in the stall (SSI) has a marked effect on the percentage of metabolic diseases ($p \leq 0.01$/ and little on the different forms of mastitis ($p \leq 0.1$).

VIII. ARTICLES RELATED TO THE DISSERTATION

1.Hristev H., Ivanova R., ***Tasheva Sm.***, (2018), „Hygiene evaluation of the ventilation and thermo-technical properties of buildings used for free-bred dairy cows", Scientific Papers-Animal Science Series: Lucrări Ştiinţifice - Seria Zootehnie, vol. 70, p 45-49, Romania, ISSN 2067-2330

2. Ivanova R., Hristev H., ***Tasheva Sm.***, (2019), „The impact of summer temperatures on certain hematolotgical indicators in diary cows", International Journal Knowledge, Scientific papers, Natural sciences, Vol. 30.3, pp. 649-653, ISSN 2545-4439

3. ***Смиляна Ташева***, Христо Христев, Румяна Иванова, (2020), «Особености при формиране на микроклимата и условията на комфорт в сгради за млечни крави през лятото», Научни трудове на АУ, ILXII, 1, 147-156, ISSN 1312-6318, **Web of sciense**

4. H. Hristev, R. Ivanova, ***S. Tasheva***, (2020), A Study of the farm factors in buildings used for farming dairy cows, Scientific Papers. Series D. Animal Science. Romania, Vol. LXIII, No. 1, pp279-286**, Web of sciense**

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5.***Tasheva, S***., & Ivanova, R. (2021). STUDI ON THE ENVIRONMENT IN HOUSING FOR DAIRY CATTLE. Agricultural Sciences/Agrarni Nauki, 13(28), 110-118, DOI: 10.22620/agrisci.2021.28.012, **Web of sciense**