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EFFECT OF MICROCLIMATE ON BACTERIAL COUNT AND AIRBORNE EMISSION FROM DAIRY BARNS ON THE ENVIRONMENT

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Abstract: The main microclimate parameters, i.e. bacterial count and airborne emission to the immediate environment, were analyzed in a dairy barn. Air temperature, relative humidity and air flow velocity were measured on an attested Testo 400 device (Testo Inc., Germany). Air samples were collected by use of a Merck MAS-100 device (Merck KgaA, Darmstadt, Germany) onto a commercially available nutrient Columbia agar (Biolife, Milan, Italy) and incubated for 24 h in an incubator at 37ºC work temperature. Measurements were carried out once a week in the morning, at noon, and in the evening during October and November 2002. In the barn, measurements were performed in the animal housing area along the feedlot, and outside the barn at a distance of 5 m, 25 m and 50 m eastward and westward from the barn. The measured dairy barn temperature ranged from 11.2ºC to 13.1ºC, relative humidity from 71.3-78.6%, and air flow velocity from 0.09-0.11 m/s. The mean value of total bacterial count in the barn air ranged from 2.82×10^4 cfu/m^3 at noon to 7.76×10^4 cfu/m^3 in the evening. Bacterial count decreased at particular measuring sites outside the barn, with Wilcoxon matched pair test showing statistical significance ($p<0.05$) at a distance of 5 m eastward and 5 m westward of the barn.

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INTRODUCTION

Bacteria normally occur in barn air, irrespective of the type of animal, production category, or mode of keeping/housing. Bacteria are only one of the many groups of air pollutants. Bacterial count depends on the construction and technical characteristics of the housing, number of animals kept in the housing, mode of keeping animals, temperature and humidity in the housing, and feeding, grooming, milking, and other activities [15, 22]. The quality of barn air influences the health of livestock and humans working there however, it has also been considered as a risk factor for the immediate environment [19, 25, 27].

The level of environmental pollution with airborne emissions from the barns can be assessed by comparing the bacterial count in the barn air with the bacterial pattern in the immediate environmental atmosphere. Therefore, the findings recorded in a dairy barn, the main microclimate parameters, and the bacterial count and aerial emission to the immediate environment are reported. The values obtained in the study are

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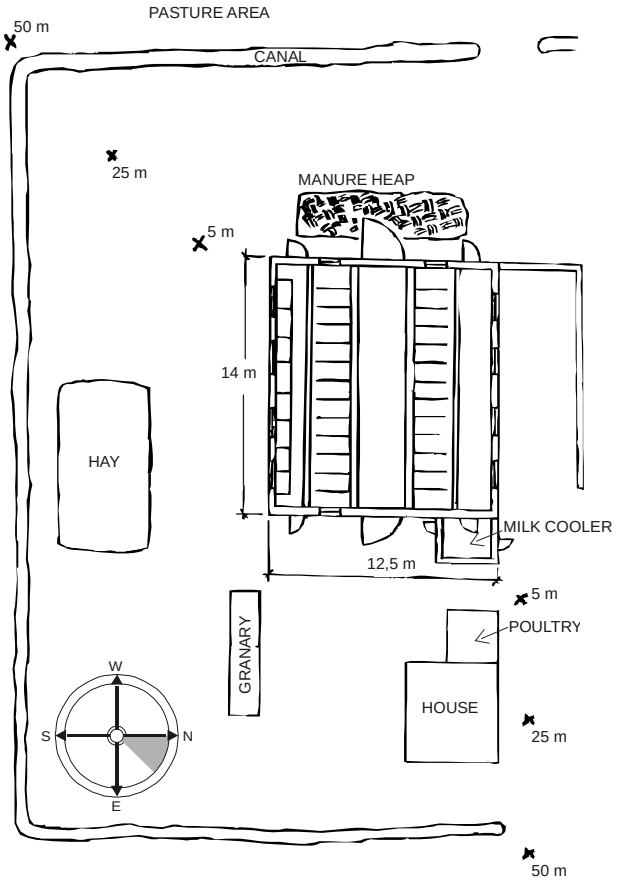

Figure 1. Plan of the dairy farm, surrounding area and main wind directions.

recommended as a criterion for the development of reference indicators of barn air quality and of the potential risk of environmental contamination with this type of pollutants.

MATERIAL AND METHODS

The study was conducted in a dairy barn on a family farm near Zagreb (Croatia). The barn dimensions are 14 m × 12.5 m × 3 m, with usual area distribution to accommodate 30 head of cattle. The barn is built on a house lot, 50 m south of the house and from the nearest neighbouring dwelling (Fig. 1).

During the study, there were 21 black-mottled lactating cows in the barn. Milking was carried out in the morning and in the evening by use of a portable automatic milking machine. The cows were tied along the feedlot supplied with the usual fodder (hay, haylage, concentrate). Water was supplied from the local waterworks via appropriate automatic watering troughs. Cow dung was manually removed from the barn and the bedding was replaced with fresh straw on a daily basis.

Measurements were carried out once a week, in the morning, at noon and in the evening during October and November 2002. Measurements were performed in the barn, in the area of animal location along the feedlot, and outside the barn at a distance of 5 m, 25 m and 50 m east and west of the barn. Air temperature (t ºC), relative humidity (rh %) and air flow velocity (w m/s) were determined by use of a Testo 400 device (Testo Inc., Germany). Bacterial count in air samples was determined by use of a Merck MAS-100 device (Merck KgaA, Darmstadt, Germany) on a commercially available nutrient Columbia agar (Biolife, Milan, Italy) incubated for 24 h in an incubator at a work temperature of 37ºC. The grown colonies (cfu/m^3) were calculated by a mechanical optic colony counter, and results were corrected by use of the respective table and mathematical equation [2]. The temperature, relative humidity and air flow velocity values, and bacterial count in the air were

Table 1. Statistical analysis of bacterial count and main microclimate parameters inside and outside dairy barn on morning measurement.

		n	Arithmetic mean	Minimum	Maximum	Variance	Standard deviation	Standard error
Bacteria (cfu/m^3)								
Inside		8	3.66×10^4	1.40×10^4	1.20×10^5	1.17×10^9	3.42×10^4	1.21×10^4
West	5 m	8	1.94×10^4	2.33×10^2	1.47×10^5	2.68×10^9	5.17×10^4	1.83×10^4
	25 m	8	3.00×10^3	1.33×10^2	1.86×10^4	4.03×10^7	6.35×10^3	2.24×10^3
	50 m	8	9.00×10^2	1.00×10^2	2.60×10^3	6.08×10^5	7.80×10^2	2.76×10^2
East	5 m	8	1.25×10^3	1.33×10^2	3.63×10^3	1.30×10^6	1.14×10^3	4.03×10^2
	25 m	8	1.07×10^3	1.00×10^2	4.25×10^3	1.83×10^6	1.35×10^3	4.78×10^2
	50 m	8	6.12×10^2	2.33×10^2	1.43×10^3	1.62×10^5	4.02×10^2	1.42×10^2
Microclimate								
Inside	t ºC	8	11.20	5.26	15.80	11.50	3.39	1.20
	rh %	8	78.60	74.40	82.3	6.06	2.46	0.87
	w m/s	8	0.11	0.07	0.15	0.00	0.02	0.01
Outside	t ºC	8	9.44	2.50	14.10	16.70	4.08	1.44
	rh %	8	77.10	73.30	80.80	10.10	3.19	1.13
	w m/s	8	0.22	0.06	0.59	0.03	0.17	0.06

n=number of measurements; cfu=colony forming unit; t=air temperature; rh=relative humidity; w=air flow velocity

analysed by use of the Microsoft Excel and Statistica 6 softwares including descriptive statistics analysis, statistical significance at the level of 5% ($p<0.05$), and Wilcoxon matched pair test [1, 20].

RESULTS AND DISCUSSION

Results presented in Tables 1-4 call for discussion about the effect of microclimate on bacterial count in barn air and their emission to the environment. However, as the issue cannot be observed separately from the technology, keeping and utilization of dairy cows, barn characteristics and general procedures of cleaning, feeding, milking and other activities, these parameters are taken into consideration along with the microclimate complex.

The measured values of the main microclimate parameters met the required ranges [13, 16, 23, 24]. Optimal temperature for dairy cows is between 4-16ºC in combination with relative humidity of 60-80% [4, 14], and air flow velocity preferably exceeding 0.30 m/s.

In addition to the measured values meeting the recommended levels, the results presented in Tables 1-3 show that there was no major diurnal fluctuation, thus ensuring a comfortable setting. This was in part attributed to the proper choice of construction material, positioning of barn openings, and barn positioning in the area, and in part to the favourable outdoor weather conditions during the study period. The latter in turn points to the conclusion that the microclimate complex is also influenced by weather conditions. The same was observed in other animal housings with natural microclimate conditioning.

The presence of bacteria in barn air is a natural phenomenon, their primary source being the animals themselves, then the fodder and humans. Bacteria are a constituent of solid and liquid bioaerosols [15, 26]. This mostly refers to saprophytes; however, pathogenic bacteria may also be found, their sources being the animals, feed, litter and dung. The airborne bacterial count depends on the type of feeding, bedding, milking, cleaning, and microclimate conditions. Aerial count of pathogenic bacteria greatly depends on the health condition of animals kept in the barn. Determination of bacterial count in the air of a dairy barn provides appropriate data on the hygienic condition of the site wherefrom milk starts its way to the consumer. In addition, bacterial count in the barn air and monitoring of its emission from the barn to the adjacent environment are important parameters for the assessment of the effect of dairy barns on the local environment.

It is by no means easy to determine the exact bacterial count in a barn, because aerial microorganisms are liable to numerous stressors that influence their concentration and survival, such as: sedimentation, aggregation, ventilation, dehydration, radiation, etc. [6, 27, 28]. The action of these stressors results in the presence of both live and dead bacteria and their bioactive components, i.e. endotoxins, in the air [22].

The bacterial count recorded at a particular site depends on the sampling technique, with a note that the methods used to date and the results thus obtained, including the present study, refer to live bacterial count. The number of viable bacteria depends on the microclimate, as relative humidity is known to play a major role in bacterial survival, so most bacteria can survive in the environment for a short period of time at a relative humidity of 55-75% [3].

The method of air sampling usually employed in similar studies worldwide was also used in this study

Table 2. Statistical analysis of bacterial count and main microclimate parameters inside and outside dairy barn on noon measurement.

		n	Arithmetic mean	Minimum	Maximum	Variance	Standard deviation	Standard error
Bacteria (cfu/m^3)								
Inside		8	2.82×10^4	7.17×10^3	5.01×10^4	1.63×10^8	1.28×10^4	4.52×10^3
West	5 m	8	1.00×10^3	0.00	3.13×10^3	1.03×10^6	1.01×10^3	3.58×10^2
	25 m	8	7.39×10^2	2.66×10^2	1.63×10^3	2.67×10^5	5.16×10^2	1.83×10^2
	50 m	8	1.44×10^3	1.66×10^2	6.13×10^3	3.93×10^6	1.98×10^3	7.01×10^2
East	5 m	8	2.47×10^3	3.33×10^2	5.97×10^3	4.42×10^6	2.10×10^3	7.43×10^2
	25 m	8	7.68×10^2	2.66×10^2	1.50×10^3	2.23×10^5	4.72×10^2	1.67×10^2
	50 m	8	4.46×10^2	3.30×10^1	1.00×10^3	1.21×10^5	3.48×10^2	1.23×10^2
Microclimate								
Inside	t ºC	8	13.10	8.23	18.20	17.40	4.17	1.47
	rh %	8	74.70	64.00	84.30	69.30	8.32	2.94
	w m/s	8	0.10	0.04	0.17	0.00	0.04	0.01
Outside	t ºC	8	12.00	6.95	19.20	23.90	4.89	1.73
	rh %	8	73.70	57.00	85.20	124.00	11.10	3.94
	w m/s	8	0.48	0.11	1.49	0.19	0.44	0.15

n=number of measurements; cfu=colony forming unit; t=air temperature; rh=relative humidity; w=air flow velocity

Table 3. Statistical analysis of bacterial count and main microclimate parameters inside and outside dairy barn on evening measurement.

		n	Arithmetic mean	Minimum	Maximum	Variance	Standard deviation	Standard error
Bacteria (cfu/m^3)								
Inside		8	7.76×10^4	2.38×10^4	2.11×10^5	4.22×10^9	6.49×10^4	2.30×10^4
West	5 m	8	3.77×10^3	1.33×10^2	1.41×10^4	1.99×10^7	4.46×10^3	1.58×10^3
	25 m	8	4.71×10^3	2.33×10^2	2.07×10^4	4.89×10^7	6.99×10^3	2.47×10^3
	50 m	8	3.63×10^3	4.00×10^2	1.89×10^4	4.02×10^7	6.34×10^3	2.24×10^3
East	5 m	8	2.24×10^3	2.00×10^2	7.67×10^3	5.84×10^6	2.42×10^3	8.54×10^2
	25 m	8	8.14×10^2	1.00×10^2	2.15×10^3	4.60×10^5	6.78×10^2	2.40×10^2
	50 m	8	7.70×10^2	1.33×10^2	1.53×10^3	2.44×10^5	4.94×10^2	1.75×10^2
Microclimate								
Inside	t ºC	8	12.90	5.50	22.50	33.40	5.78	2.04
	rh %	8	71.30	54.50	88.00	140.00	11.80	4.18
	w m/s	8	0.12	0.03	0.25	0.01	0.08	0.03
Outside	t ºC	8	11.40	3.29	22.20	36.90	6.08	2.15
	rh %	8	69.80	54.00	85.20	103.00	10.10	3.59
	w m/s	8	0.36	0.12	0.70	0.05	0.22	0.08

n=number of measurements; cfu=colony forming unit; t=air temperature; rh=relative humidity; w=air flow velocity

[26]. A disadvantage was that a reduced air volume had to be collected due to the high bacterial burden in the barn air, and even then the number of grown colonies was difficult to correct according to the manufacturer's tables, as it occasionally exceeded maximal values. According to literature data, total bacterial count in livestock housing is within the range of 10^4-10^6 cfu/m^3 [8, 10, 22, 25], while Eduard [9] reports 10^8-10^9 cfu/m^3 air.

In the present study, total bacterial count measured in the barn air according to time of day was 1.40×10^4-1.20×10^5 cfu/m^3 in the morning, 7.17×10^3-5.01×10^4 cfu/m^3 at noon, and 2.38×10^4-2.11×10^5 cfu/m^3 in the evening (Tab. 1-3), which is consistent with the literature data [8, 9, 10, 22, 25]. The mean values of total bacterial count were generally comparable, except on a few occasions when the mean value was somewhat higher, irrespective of timing. On these occasions, the elevated bacterial count was most likely associated with microclimate conditions, especially air flow velocity, which then reached the lowest values recorded throughout the study (0.03-0.10 m/s). This observation appears to confirm the hypothesis according to which air flow velocity, i.e. ventilation as a real process of air dilution, is most important for barn air bacterial count reduction [17].

Comparison of maximal values of total bacterial counts reveals the highest values were recorded on evening measurements, which could be explained as a consequence of diurnal animal and barn activities. Wilcoxon matched pair test demonstrated the effect of microclimate parameters on total bacterial count in the barn atmosphere at the level of $p<0.05$ (Tab. 4).

On regular air exchange, airborne bacteria are disseminated to the barn environment, thus acting as a potential environmental pollutant. Bacterial concentration in the outdoor air mostly depends on their indoor count, survival ability influenced by dehydration, radiation, oxygen and pollutants present in the atmosphere [6, 28]. The distance the bacteria will cross from the source and the trajectory of their migration to their temporary or permanent sedimentation depends on a number of factors, including the source of contamination, position of air outlet on the barn roof or wall, ground configuration, atmospheric events, air flow, temperature, humidity, sunlight and bacterial tenacity [5, 11, 17, 18, 21, 22].

Airborne bacteria are part of the bioaerosol and are bound to solid or liquid carriers (dust or drops) [7, 13]. The rate of bacterial sedimentation and the distance the bacteria will reach depend on the carrier size.

In the present study, bacterial emission and count were determined at 3 sites at a distance of 5 m, 25 m and 50 m east and west from the barn. As small family farms with barns located within the farmyard and neighboring farms at a distance of about 50 m are most common in Croatia, it appeared quite reasonable to choose these measuring sites, the greatest distance being 50 m from the dairy barn. For this reason, the potential mixing and cumulation of bacteria from several barns at particular locations should be presumed, thus being quite difficult to determine the utmost range of migration and count of bacteria from a single barn. On determination of airborne bacterial count and emission from the dairy barn to the adjacent environment, mesurements were carried out at 2 opposite cardinal points (East and West). It should be noted that a pasturing area used in summer is located to the west, and a house to the east, with a local road at a distance of 50 m.

The results of morning total bacterial count measurements outside the barn at a 5-m distance showed it to be about 20 times lower than the total bacterial count measured in the barn at all but 1 measurement. On this particular measurement, the bacterial count showed

Table 4. Wilcoxon matched pair test inside and outside dairy barn

Matched pairs	n	T	Z	p
Bacteria (cfu/m^3) morning				
Barn – 5 m westward	8	7.00	1.54	0.12
5 m westward – 25 m westward	8	11.00	0.98	0.32
25 m westward – 50 m westward	8	12.00	0.84	0.40
Barn – 5 m eastward	8	0.00	0.52	0.01
5 m eastward – 25 m eastward	8	13.50	0.63	0.52
25 m eastward – 50 m eastward	8	13.00	0.70	0.48
Bacteria (cfu/m^3) noon				
Barn – 5 m westward	8	0	2.52	0.01
5 m westward – 25 m westward	8	12.00	0.84	0.40
25 m westward – 50 m westward	8	14.50	0.49	0.62
Barn – 5 m eastward	8	0	2.52	0.01
5 m eastward – 25 m eastward	8	0	2.52	0.01
25 m eastward – 50 m eastward	8	3.00	1.86	0.06
Bacteria (cfu/m^3) evening				
Barn – 5 m westward	8	0	2.52	0.01
5 m westward – 25 m westward	8	13.00	0.70	0.48
25 m westward – 50 m westward	8	7.00	1.54	0.12
Barn – 5 m eastward	8	0	2.52	0.01
5 m eastward – 25 m eastward	8	7.00	1.54	0.12
25 m eastward – 50 m eastward	8	15.00	0.42	0.67
Bacteria/microclimate				
Morning				
Barn – t (°C)	8	0	2.52	0.01
Barn – rh (%)	8	0	2.52	0.01
Barn – w (m/s)	8	0	2.52	0.01
Noon				
Barn – t (°C)	8	0	2.52	0.01
Barn – rh (%)	8	0	2.52	0.01
Barn – w (m/s)	8	0	2.52	0.01
Evening				
Barn – t (°C)	8	0	2.52	0.01
Barn – rh (%)	8	0	2.52	0.01
Barn – w (m/s)	8	0	2.52	0.01

n=number of measurements; T, Z=test coefficients; t=air temperature; rh=relative humidity; w=air flow velocity; $p<0.05$

deviation, being several times higher than other values recorded in the barn, to further increase at 5-m distance from the barn, from where it gradually decreased to reach the lowest value at 50-m distance. This deviation was associated with lowest air temperature and highest air flow velocity measured on the same occasion. Wilcoxon matched pair test demonstrated a statistically significant difference ($p<0.05$) between the bacterial count measured in the barn and at 5-m distance eastward (Tab. 4). A somewhat higher bacterial count was recorded west of the barn.

Total bacterial count measured at noon in and outside the barn showed the greatest decline at 5-m distance from the barn (about 25-fold), the declining tendency being less pronounced with increasing distance. Wilcoxon matched pair test demonstrated a statistically significant correlation ($p<0.05$) between total bacterial count in the barn and at 5-m distance from the barn, both westward and eastward (Tab. 4). A correlation of the same level of significance ($p<0.05$) was recorded between total bacterial count at 5-m and 25-m distance east of the barn. Identical to the morning measurements, a somewhat higher bacterial count was recorded westward from the barn.

Total bacterial count measured in the evening outside the barn was higher than those recorded at other measurement times. A 20-fold decrease in total bacterial count was observed at 5-m distance from the barn, both eastward and westward, as also verified by Wilcoxon matched pair test at the level of $p<0.05$ (Tab. 4). The bacterial count reduction at 25-m and 50-m distance from the barn was by far less pronounced. A higher bacterial count was recorded on the west.

CONCLUSIONS

• Total airborne bacterial count is directly influenced by air temperature, relative humidity and air flow velocity, as demonstrated by Wilcoxon matched pair test at the level of $p<0.05$.

• The mean values of total bacterial count in the barn ranged from 2.82×10^4 cfu/m^3 at noon to 7.76×10^4 cfu/m^3 in the evening.

• Total bacterial count was observed to increase in the evening, which could be attributed to daily animal and human activities in the barn.

• Total bacterial count in the outdoor air depends on their barn concentration, weather conditions influencing bacterial survival, topographic properties of the area, and distance from the barn.

• Bacterial emission into the outdoor air depends on the source of contamination, position of air outlet on the barn roof or wall, ground configuration, atmospheric events, air flow, air temperature, humidity, sunlight, and biologic age of the bacteria.

• Bacterial count in the air outside the barn decreased several-fold at 5-m distance from the barn, as demonstrated by Wilcoxon matched pair test at the level of $p<0.05$. This was explained by the rapid dilution and air flow effect which was not precluded by any nearby building. At 25 m and 50 m from the barn, total bacterial count decreased at a considerably slower rate; however, it was noted that the airborne bacterial count may have also depended on the vicinity of barns belonging to other neighbouring farms.

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