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Survivability of *Salmonella senftenberg W_{775}* in Cattle Slurry under Various Temperature Conditions

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The effect of varied temperatures of cattle slurry storage conditions on the rate of elimination of *Salmonella senftenberg W_{775}* bacilli was studied. It was demonstrated that a temperature of 4°C had a stabilizing effect on the bacteria, which resulted in the longest time of *Salmonella* bacilli survivability. A regression analysis defined the daily loss of the bacteria. The rate of elimination of *Salmonella* amounted to 0.26 log/day at a temperature of 4°C, which resulted in a theoretical time of bacteria survivability of 42 days. At a temperature of 20°C, faster inactivation of *Salmonella* cells was shown, which was defined as 0.30 log/day. *Salmonella senftenberg W_{775}* bacilli at 30°C were able to survive 13 days, at a daily population loss of 0.87 log. The *Salmonella* cells died at 40°C (10 days). A significant relationship between the survival period of *Salmonella senftenberg W 775* in cattle slurry and the temperature of its storage was noted.

Key words*: Salmonella senftenberg*, survival, temperature, cattle slurry.

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A rational management of slurry in agriculture due to the potential of spread of pathogenic microorganisms is one of the most important problems of environmental protection. Slurry is defined as liquid organic fertilizers of universal application on agricultural acreage and fertilization of grassland. The application of this fertilizer exerts a considerable effect on soil microflora, constituting a very important source of biogenic elements, such as carbon, nitrogen and phosphorus (DORN & SCHLEIFF 1997; SMOLIŃSKI *et al.* 2004; STRAUCH 1997). However it should be remembered that slurry carries a large load of pollution in the form of fungi, bacteria and viruses as well as parasite eggs posing a threat for the health of people and animals. The application of excessive doses of this fertilizer, accompanied by negligence in sanitary regimes, can lead to the biological contamination of soils, surface and ground waters as well as a deterioration in the phytosanitary status of the plants grown (OLSZEWSKA 2000). Slurry contains a sufficient amount of nutrients indispensable for the development of non-specific bacteria. When accumulated at high amounts in areas of intensive farming, it does not undergo self-heating which, as a result, leads to the maintenance of viability even for a few months of the microorganisms inhabiting it. Slurry can contain up to 10^9-10^{10} aerobic bacteria or relatively anaerobic bacteria per 1 ml. Pathogenic bacteria penetrate through secretions or excretions originating from diseased animals or vectors. Depending on the health status of the herd and its origin, microorganisms isolated include the following genera: *Salmonella*, *Leptospira, Treponema*, *Erysipelothrix*, *Mycobacterium*, *Brucella*, *Bacillus*, *Riteksje*, *Chlamydia* and others. *Salmonella,* showing a long period of survivability, is an especially dangerous bacterium living in slurry (CZERNOMYSY-FUROWICZ & FUROWICZ 1995; KLUCZEK 1996; SMOLIŃSKI *et al.* 2004). In Poland food poisoning and contamination defined as salmonelloses account for about 90% of all the cases of this type. Such great spread of salmonelloses is affected by three factors, such as the biology of the bacteria itself, human susceptibility to infections, as well as conditions favorable to the spread of the disease.

The aim of the present paper was to define the effect of varied slurry storage temperature conditions on the rate of elimination of *Salmonella senftenberg W_{775}* bacilli.

Material and Methods

Research was carried out from March to November 2004. The research material constituted cattle

slurry. A suspension was prepared, introducing pure cultures of *Salmonella senftenberg W_{775}* into nutrient broth. The culture was exposed to incubation at 37°C for 24 hours. Afterwards four cylinders of 3000 ml were filled with 2000 ml cattle slurry each, for which at the beginning of the experiment the physical and chemical composition was defined. Each cylinder was filled with 100 ml of the earlier prepared suspension. In such samples the count of *Salmonella senftenberg* per 1 ml of contaminated material was determined. At the same time the slurry samples were exposed to four temperatures: A-4°C, B-20°C, C-30°C, D- 40°C. Every 3-7 days samples were taken from material at 4°C and 20°C, and every 1-3 days from the material at 30 and 40°C. Bacterial count in the samples was determined with the MPN method. The *Salmonella* bacteria were isolated from cattle slurry at four stages; the first stage constituted precultivation of microorganisms in buffered 1% peptone water (incubation at 37°C for 24 h). Selective cultivation of microorganisms was carried out on liquid medium following Rappaport with tetrathioniate and malachite green added (incubation at 41°C for 48 h). The next stage involved transferring the culture onto BPLA agar medium with brilliant green, phenol red and lactose and onto XLD agar medium with xylose, lysine and deoxycholate. Incubation took place at 37°C for 24 h. On BPLA medium typical *Salmonella* colonies grew in the form of pale pink colonies which were surrounded by a characteristic agar color, while XLD medium showed typical colonies of these bacteria growing in a form of minute colonies with a black centre, surrounded by a light-red zone. The last stage involved identification by applying serological tests: polyvalent serum HM and the API 20 E microtest.

The results obtained were statistically verified. The statistical procedure was based on changes in the count of *Salmonella* bacilli in time with the following formula:

$$\mathrm{Log}(N) = ax + b$$

where:
N – bacterial count at a given time in slurry
x – time in days
a – direction coefficient accounting for a mean change in the count of bacteria as a log per day
b – free term theoretically corresponding to log of the bacterial count involved in a given process at "zero" time.

Based on the course of regression lines, a maximum time of bacteria survival under specific temperature conditions was calculated.

Results and Discussion

The physico-chemical composition of cattle slurry is shown in Table 1. The subject slurry contained 59943.4 mg of dry matter in 1 dm^3, 5945.9 mg of total nitrogen and 1125.9 mg of ammonium nitrogen. The content of phosphorus amounted to 2247.8 mg and 298.7 mg of potassium. The quantity of heavy metals was low, while the level of cadmium and mercurium was below threshold determination. The value of the pH reaction was 7.2.

The results of the bacteriological assay are given in Table 2 and Figs 1-6. The survivability of *Salmonella senftenberg W_{775}* bacilli was determined in this study. This serotype due to its high thermoresistance is most applicable to research of this type. In case of its full inactivation, one can assume that the other pathogenic bacteria, which usually demonstrate a lower resistance to environmental factors, have been eliminated (PALUSZAK *et al.* 1995). The survivability assay of *Salmonella senftenberg* was carried out at four temperatures: 4, 20, 30 and 40°C. After the bacterial suspension was introduced into cattle slurry at the initial stage of the experiment, the count of *Salmonella senftenberg* amounted to 9.5×10^9 cfu/ml. The experiment shows that bacteria were identified for the longest period at 4°C. The number of *Salmonella senftenberg* isolated in the cattle slurry after 7 days decreased by 1 log unit, as compared with the control, which amounted to 9.98 log, after 21 days – it amounted to 5.98 log, while after 30 days it got reduced to 2.04 log. On the other hand, at 20°C *Salmonella senftenberg*, as compared with the control (9.98 log) on the 7th day of the experiment decreased by 0.8 log, after 21 days it was reduced to 6.40 log, and on the 28th day it was only 1.6 log. At 30°C the bacteria were eliminated after 5 days by 1.58 log, as compared with the control, after 10 days they were reduced to 5.4 log, however on the 16th day the value amounted to 0.6 log. In the experiment at 40°C, after 5 days the count of *Salmonella senftenberg* bacilli decreased by 2.54 log, as compared with the initial sample (9.98 log), after 13 days it amounted to 1.04 log.

A clear effect of temperature as well as the composition of slurry on the course of the process of eliminating bacteria from slurry was revealed. EREBO and MUNCH (1983) demonstrated that to reduce the count of *Salmonella* bacilli by 5 log units in aerated slurry over winter, 8 weeks are needed, and 30 weeks for the non-aerated. PHILIPP *et al.* (1997) observed that in cattle slurry mixed with food leftovers, *S. senftenberg* exposed to 55°C was isolated for 6-8 days. STRAUCH (1993) investigated the survivability of different strains of *Salmonella* in animal excretions under farm conditions and reported that the longest period of sur-

Table 1

Chemical composition of cattle slurry

Specification	Units	Slurry
Dry matter	mg · dm^{-3}	59943.4
Organic matter	mg · dm^{-3}	46546.3
Residue after roasting	mg · dm^{-3}	22987.2
Total nitrogen	mgN · dm^{-3}	5945.9
Ammonium nitrogen	mgNH_4 · dm^{-3}	1125.9
Phosphorus	mgP · dm^{-3}	2247.8
Magnesium	mgMg · dm^{-3}	274.2
Calcium	mgCa · dm^{-3}	750,8
Sodium	mgNa · dm^{-3}	361.6
Potassium	mgK · dm^{-3}	298.7
Lead	mgPb · dm^{-3}	18.8
Cadmium	mgCd · dm^{-3}	b.t.d.
Mercury	mgHg · dm^{-3}	b.t.d.
Copper	mgCu · dm^{-3}	0.07
Nickel	mgNi · dm^{-3}	0.02
Zinc	mgZn · dm^{-3}	44.7
Chlorides	mgCl · dm^{-3}	847.5
Sulphates	mgSO_4 · dm^{-3}	465.4
Reaction, pH		7.2

b.t.d : below threshold determination

Table 2

Coefficients of the inactivation rate of *S. senftenberg* in cattle slurry

Medium	Coefficient (a)	Coefficient (b)	Correlation coefficient	Maximum tim eof survival in days
A	-0.26	10.84	-0.93**	42
B	-0.30	10.76	-0.97**	36
C	-0.87	11.36	-0.96**	13
D	-0.95	10.01	-0.97**	10

** P<0.01

(a) regression coefficient corresponding with mean drop of bacteria number (in log) during one day,

(b) theoretical number of bacteria from given system in "0" time

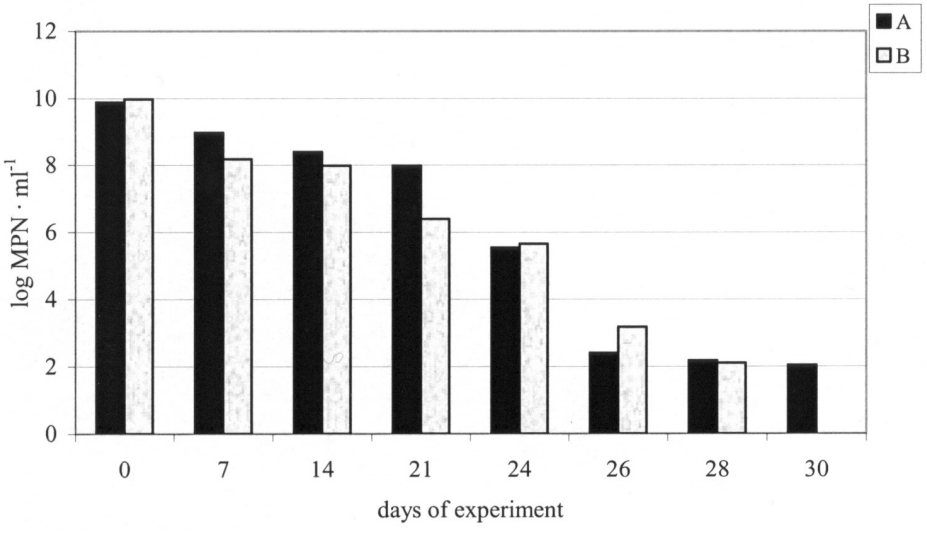

Fig. 1. Number of *Salmonella senftenberg W775* in investigated slurry expressed in log MPN · ml^{-1} at the temperature of 4 and 20°C during the experiment.

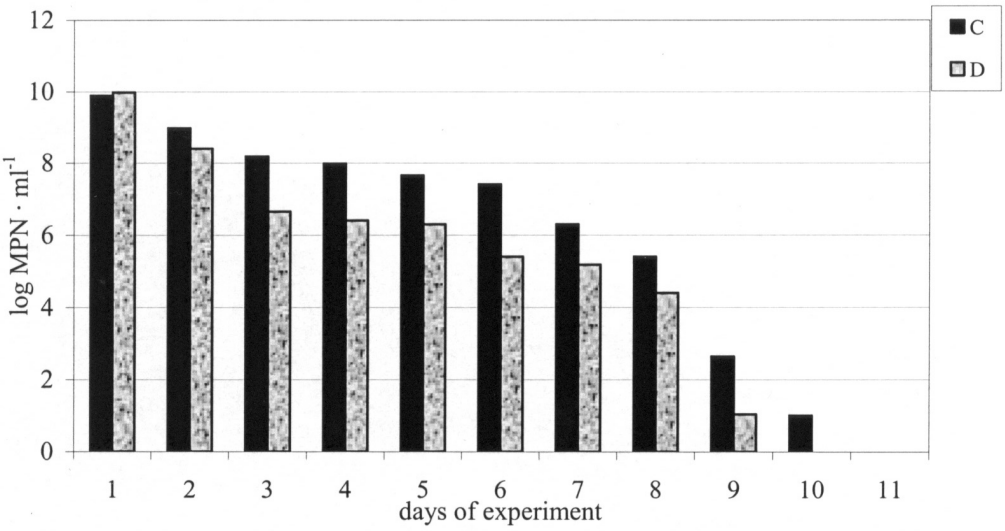

Fig. 2. Number of *Salmonella senftenberg* W_{775} in investigated slurry expressed in log MPN · ml^{-1} at the temperature of 30 and 40°C during the experiment.

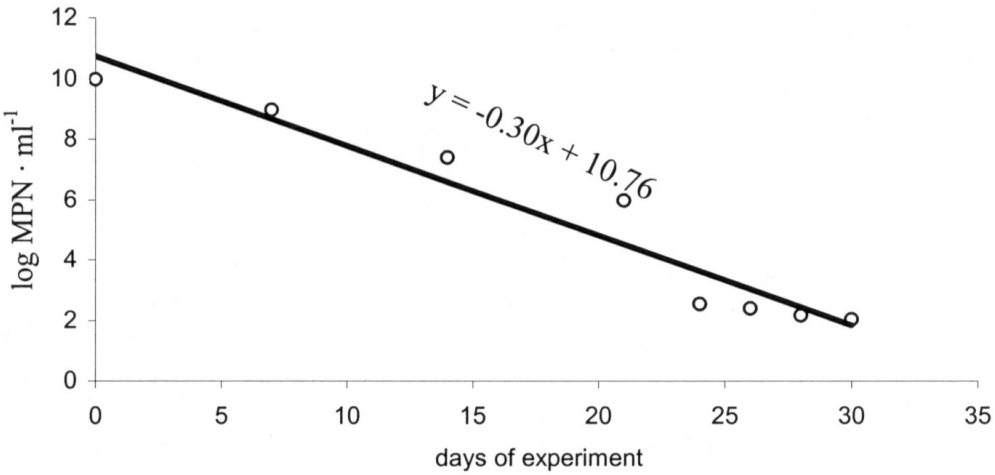

Fig. 3. Regression lines for survival *S. senftenberg* W_{775} in slurry at the temperature of 4°C.

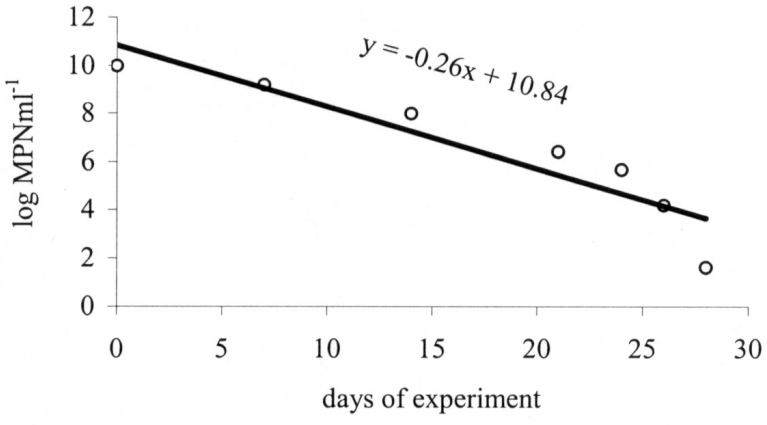

Fig. 4. Regression lines for survival *S. senftenberg* W_{775} in slurry at the temperature of 20°C.

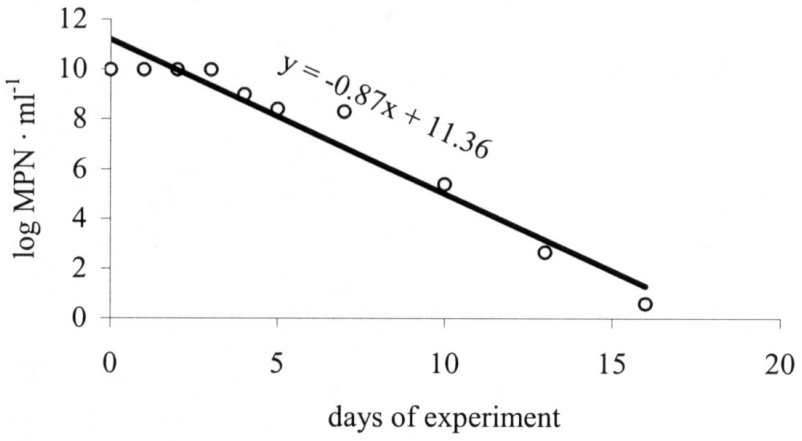

Fig. 5. Regression lines for survival *S. senftenberg W775* in slurry at the temperature of 30°C.

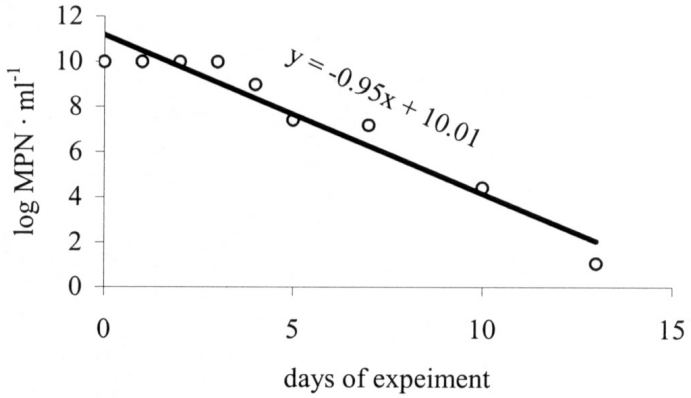

Fig. 6. Regression lines for survival *S. senftenberg W775* in slurry at the temperature of 40°C.

vival for *S. dublin* was 65 days in stale, *S. typhimurium*, *S. paratyphi B, S.anatum, S, manchester* survived for 177, 157, 210 and 180 days. respectively, in cattle fertilizer.

In the present research the rate of elimination of the bacteria investigated in slurry differed clearly. The longest time of survival was identified at 4°C. Bacteria were still isolated after 30 days of the experiment, and their count amounted to 1.1 x 10^1 cfu/ml (Fig. 1). The daily rate of bacterial cell elimination was 0.26 log, at a highly significant correlation coefficient 0.93 ($P<0.01$) (Fig. 3). A regression equation was used to calculate the maximum time of *Salmonella* bacilli survival, which was 42 days (Table 2). OLSZEWSKA *et al.* (1999) showed that *S. enteritidis* isolated in municipal sewage at 4°C were isolated for 28 weeks, while in slurry – 10 weeks. However the survivability of these bacilli at 20°C was 7 weeks in municipal sewage, and 6 weeks in slurry.

At 20°C the survival time of the bacteria was shorter as compared with the experiment carried out at 4°C. On the 28th day of the experiment the count of *Salmonella* was 1.0 x 10^2 cfu/ml, while on the 30th day these bacteria were not identified. The rate of *Salmonella* bacilli population loss was 0.30 log/day, at a high correlation coefficient r=-0.97 ($P<0.01$) (Fig. 4). The maximum survival time of *Salmonella* bacilli was 36 days (Table 2). According to WACHNIK (1976), at 10°C *Salmonella* bacteria survive from 80 to 180 days, while at 20°C – they live only for 35 days. This author explains the differences by the fact that at 10°C the saturation of slurry with a specific microflora decreases and the decomposition of organic substances is slower, while at 20°C a strong cultivation of thermophilic microorganisms occurs; showing a strongly antagonistic effect, i.e. they destroy the pathogenic bacteria, including *Salmonella.* In the present research *Salmonella* bacilli survived shortest at 30

and 40°C. On the 16th day of the experiment bacteria were isolated from slurry stored at 30°C, their count was 1.0 x 10^1 cfu/ml. However, on the 17th day no bacteria were found. At 40°C, *Salmonella* cells were identified on the 13th day of the experiment at the amount of 1.1 x 10^1 cfu/ml, after 16 days of the experiment no bacteria were recorded (Fig. 2). The rate of *Salmonella* cell death at 30°C was 0.87 log/day, however at 40°C it was 0.95 log/day, at highly significant correlation coefficients (Figs 5, 6). The maximum time of bacterial survival calculated based on regression equations were, respectively, 13 days at 30°C and 10 at 40°C. The data analysis shows that the differences recorded between the count of *S. senftenberg* bacilli at respective temperatures were highly significant. Highly significant correlation coefficients between thermal slurry storage conditions and the rate of elimination of bacteria confirm the present results obtained (Table 2). The literature reports on the survivability of *Salmonella* bacilli from 13 days even up to one year (MUNCH *et al.* 1987). PROVOLO *et al.* (1997), over summer in slurry without solids, noted a 100% elimination of *S. dublin* after 60 days of storage. STRAUCH (1993), however, reports on an average elimination time of *Salmonella* bacilli in slurry from 49 (*S. dublin*) to 310 days (*S. anatum)*. PLYMM-FORESHELL (1988) demonstrated the survivability of *S. dublin* and *S. typhimurium* bacilli in cattle slurry at 7-20°C on the 70th day, and at 55-60°C on the 7th day.

MÖLLER (1984) claims that in swine slurry the survivability of respective *Salmonella* strains differed both across species and between field and laboratory conditions. Under field conditions at 18-23°C the survivability of *Salmonella* strains was 39 days for *S. typhimurium*, *S. parathypi B* and *S. dublin*; *S. anatum, S.manchester* and *S. senftenberg* ssurvived for 47 days. However, under laboratory conditions *S. enteritidis* was isolated for 4 days, *S. typhimurium* – for 11 days, *S. anatum* and *S. manchester* – 34 days. KLUCZEK (1996) reports on the survival time of different species of *Salmonella* in stored cattle slurry. The following strains were identified: *S. anatum* – 286 days, *S. dublin* – 27 days, *S. parathypi* B – 55 days, while in the stored calf slurry *S. dublin* and *S. manchester* strains lived for 34 and 11 days, respectively. According to KWIATEK (1999), the survivability of *Salmonella* bacilli in slurry and faeces is high and considerably depends on temperature and moisture; in slurry *Salmonella* bacilli survive for 11-12 weeks.

Both the literature reports and the present research show that *Salmonella* bacteria live longer in slurry sampled in spring and at a low temperature (4°C), while the shortest survival period occurred in the slurry sampled in summer at a high temperature (40°C). A significant relationship was recorded between *Salmonella senftenberg* W_{775} survival time in cattle slurry, and its storage temperature.

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