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HYGIENIC ASPECTS OF CATTLE SLURRY STORAGE AS THE MOST POPULAR AND CHEAPEST METHOD OF HANDLING LIQUID ANIMAL EXCREMENTS*

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Abstract

Slurry is a very valuable natural fertilizer, but its improper use in agriculture poses a serious sanitary threat. Therefore its treatment before use for fertilization is advisable. One of such methods, which is still the most popular, is storage of liquid excrements. The aim of this study was to estimate the sanitization effectiveness of storage at 4ºC and 20ºC based on parameters describing the kinetics of changes in the population of some indicator bacteria in cattle slurry with different dry matter content. The material for the study was fresh cattle slurry. The liquid excrements used in the experiment had a dry matter content of 2, 6 and 14%. Slurry was stored at 4ºC and 20ºC. Bacilli of *Salmonella* Dublin, *E. coli* and enterococci were used as indicator bacteria. Number of microorganisms was determined based on MPN method in a 3-tube design. Basic parameters of the bacteria inactivation kinetics were calculated and statistical analysis was made using the program SAS 9.2 PL. In stored slurry a gradual elimination of all the studied microorganisms was observed. Hygienization effect of storage was smaller at 4ºC than at 20ºC and in excrements with a high dry matter content. Depending on storage temperature and dry matter proportion, the theoretical times of survival ranged from 81.85 to 220.80 days for bacilli of *Salmonella* Dublin, from 74.93 to 199.36 days for *E. coli*, and from 118.67 to 335.84 days for enterococci. The study showed explicitly that statutory time of slurry storage is insufficient to ensure its complete hygienization.

Key words: cattle slurry, hygienization, storage, bacteria survival

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Storage of slurry is the simplest and cheapest method of its treatment for agricultural purposes, and the main factors limiting the effectiveness of this process are time and temperature.

Time of the storage process is different in individual European countries. It depends mainly on the climate prevailing in a given region, in particular on the length of non-growth period and the annual course of temperature (Burton and Turner, 2003). In most cases a 4-month period of storage is sufficient for safe management of slurry in agriculture (Mohaibes and Heinonen-Tanski, 2004). Before spreading slurry over pastures it is necessary to store it for at least 60 days in summer and 90 days in winter. In north countries with severe climate, e.g. in Finland, it may be necessary to prolong the storage period even to 1 year (Mohaibes and Heinonen-Tanski, 2004; Park et al., 2002). In Poland, the Act of 10 July 2007 on fertilizers and fertilization (J. of Laws 2007 No. 147 item 1033) imposes an obligation to store slurry in tight, closed containers with a volume which allows storage of at least 4-month production of this fertilizer. Regulation of the Ministry of Environment of 23 December 2002 on the detailed requirements to be met by action programmes aimed at reducing nitrogen runoff from agricultural sources (J. of Laws 2003 No. 4 item 44) extends the minimal time of slurry storage up to 6 months.

Physico-chemical properties of slurry cause that it is does not self-heat during storage and it is characterized by a stable temperature of about 6°C in winter and 18°C in summer. This reduces the hygienization effectiveness of the storage process (Olszewska et al., 1997; Romaniuk, 1995). It is of particular importance due to a possible presence in slurry of pathogenic microorganisms, as well as eggs and oocysts of gastrointestinal parasites (Guan and Holley, 2003). Using improperly treated slurry for fertilization may pose a threat to human and animal health and limit agricultural production.

During storage of slurry most pathogenic bacteria and parasites present in it are not multiplied, and they are subjected to a gradual elimination (Burton and Turner, 2003). This is possible if during this process new portions of slurry are not introduced into the container. The survival time of microorganisms during storage is varied and depends on many factors: the species of microorganism, the source of slurry, temperature, dry matter content, redox potential, presence of antagonistic natural microflora and abundance in nutrients (Böhm, 2005). Bacterial survival during slurry storage is also dependent on the C:N ratio in liquid excrements, which indicates availability of nutrients for bacteria and may show the occurrence of considerable amounts of proteins (organic nitrogen), the decomposition of which, among other things, results in production of toxic ammonia (Burton and Turner, 2003).

The aim of this study was to assess the hygienization effectiveness of storage at 4ºC and 20ºC based on parameters describing the kinetics of changes in population of selected indicator bacteria in cattle slurry with different dry matter content.

Material and methods

The study used fresh cattle slurry, which was collected according to the Instructions of slurry sampling based on the standard PN-B-12098:1997.

Slurry samples delivered to the laboratory were used directly in the study as a fertilizer with an average, typical dry matter content (6%). In successive two variants use was made of slurry with low (2%) and high (14%) dry matter content, obtained by centrifuging liquid excrements and mixing their fractions in suitable proportions.

Slurry was stored at 4ºC and 20ºC, which corresponded to conditions prevailing in the winter and summer periods. Samples were stirred twice a day for 30 min. Then slurry was contaminated with suspensions of chosen tested bacteria in a volume of 25 cm^3 per dm^3 of liquid excrements.

Samples for determination, irrespective of the storage temperature and dry matter content, were collected after 1 hour (null sample), after 1 day and then once a week for 70 days from inoculation.

Bacilli of *Salmonella* Dublin, *Escherichia coli* and bacteria of the genus *Enterococcus* were used in the study. To prepare bacterial suspensions, the studied microorganisms were inoculated in a standard nutrient broth, and after 24-hour incubation at 37ºC, on standard nutritive agar. After incubation under the above conditions, a bacterial suspension in saline solution with a concentration of 4.50×10^9 cfu/cm^3 was prepared from the material obtained. Prior to inoculation of slurry samples, the suspensions were centrifuged, the supernatant was removed, and bacterial sediment was poured with liquid cattle excrements in a volume corresponding to the saline solution removed.

The number of studied bacteria was determined based on the MPN method in a 3-tube design. In the process of isolation of bacilli of the genus *Salmonella* 1% buffered peptonic water was used for initial multiplication (24 h at 37ºC) and for selective liquid medium according to Rappaport (24 h at 43ºC). For growth on solid medium BPL agar was used (24 h at 37ºC). Final identification included using diagnostic sera according to the scheme of antigenic structure of *Salmonella* following White – Kauffman – Le Minor.

To determine *E. coli* (environmental isolate) MacConkey's liquid medium was used (24 h at 43ºC). Then the material was inoculated on agar with tergitol with an addition of 1% solution of 2,3,5-TTC and ENDO agar (24 h at 43ºC). Final identification was carried out with the API 20E test.

Enterococci were determined using glucose azide broth (48 h at 37ºC) and kanamycin esculin azide agar (48 h at 37ºC). Final identification was carried out based on the serological Phadabac-D-test.

Statistical analysis of the results of microbiological analyses was performed using SAS 9.2 PL program. Theoretical times of survival, times of decimal elimination and the rate of test bacteria elimination were calculated. Significance of differences was evaluated between parameters determined based on the regression line equation for the given bacteria, depending on the variant of thermal storage and thickness of slurry, as well as for the given temperature and thickness of slurry depending on the

bacteria species. In order to do that, the normality of distribution was tested and the multiple analysis of variance was performed on the basis of GLM model. Also the significance of differences was analysed at $P \leq 0.05$ and $P \leq 0.01$ between the values of studied indices based on the Tukey's multiple comparison test.

Results

Gradual elimination of all the studied microorganisms was observed in stored slurry. It proceeded much faster at 20°C, as well as in liquid excrements with a lower dry matter content (Table 1). Initial concentration of indicator bacteria, irrespective of the thickness and storage temperature of slurry, remained at the level of 10^8 MPN×ml^{-1} (Table 1).

Calculated values of parameters describing the inactivation kinetics of tested microorganisms during storage of slurry were presented in Table 2.

Number of *Salmonella* Dublin rods in thin cattle slurry stored at 4°C decreased by 3.94 log (from 4.47×10^8 to 5.13×104 MPN×ml^{-1}), by 3.18 log (from 7.59×10^8 to 5.01×10^5 MPN×ml^{-1}) in slurry with an average content of dry matter and by 2.75 log (from 7.59×10^8 to 1.35×10^6 MPN×ml^{-1}) in thick slurry (Table 1). By contrast, in liquid cattle excrements stored at 20°C these decreases were clearly higher and amounted to 8.19, 6l.61 and 5.58 log, respectively (Table 1).

Based on calculations it was found that these bacilli were theoretically able to survive from 162.63 to 220.80 days in cattle slurry stored at 4°C and from 81.85 to 99.53 days at 20°C (Table 2), and differences were highly significant irrespective of the thickness of excrements. Whereas in respect of the dry matter content, survival times were highly significantly shorter in thin slurry than in medium and thick slurry stored at 4°C (Table 2).

Of all the tested microorganisms, the highest falls in the number of bacteria population in stored slurry were observed in the case of *E. coli*, irrespective of the temperature and thickness of excrements. Number of *E. coli* bacilli in cattle slurry stored at 4°C decreased during 70 days from 4.47×10^8 to 2.63×10^4 MPN×ml^{-1} in thin excrements, from 7.59×10^8 to 1.91×10^5 MPN×ml^{-1} in slurry with the medium proportion of dry matter, and from 7.59×10^8 to 6.46×10^5 MPN×ml^{-1} in thick slurry (Table 1). In liquid cattle slurry with low and medium thickness stored at 20°C a decrease in number of *E. coli* bacilli by 8.63 and 7.30 log, respectively, was found and in thick slurry their number decreased by 6.25 log (Table 1).

Theoretical survival times and times of decimal elimination calculated based on regression line equations were the shortest of all the studied microorganisms (Table 2). Highly significant differences in the theoretical survival time and time of decimal elimination were found in both variants of storage temperature in relation to enterococci, whereas in respect of *Salmonella* Dublin highly significant or significant differences were observed in slurry with medium and high proportion of dry matter stored at 4°C (Table 2). For the elimination rate of *E. coli*, in turn, statistically significant differences were found in comparison with enterococci (Table 2).

Table 1. Changes in number of bacteria [MPN×ml^{-1}] during storage of cattle slurry

Bacteria	Time of analysis	Number of bacteria [NPL×ml^{-1}]					
		thin slurry		medium slurry		thick slurry	
		4ºC	20ºC	4ºC	20ºC	4ºC	20ºC
Salmonella Dublin	1 hour	4.47×10^{8}	9.55×10^{8}	7.59×10^{8}	4.47×10^{8}	7.59×10^{8}	4.47×10^{8}
	1 day	3.31×10^{8}	7.59×10^{8}	4.47×10^{8}	1.55×10^{8}	4.46×10^{8}	3.02×10^{8}
	7 days	2.34×10^{8}	4.17×10^{8}	2.40×10^{8}	3.63×10^{7}	2.57×10^{8}	1.78×10^{7}
	14 days	1.51×10^{8}	2.00×10^{8}	1.20×10^{8}	8.91×10^{6}	7.62×10^{7}	1.15×10^{6}
	21 days	7.59×10^{7}	3.55×10^{7}	6.31×10^{7}	2.09×10^{6}	3.80×10^{7}	2.19×10^{5}
	28 days	3.47×10^{7}	5.62×10^{6}	3.16×10^{7}	5.13×10^{5}	2.00×10^{7}	6.92×10^{4}
	35 days	1.55×10^{7}	7.94×10^{5}	1.58×10^{7}	1.29×10^{5}	1.15×10^{7}	2.63×10^{4}
	42 days	5.89×10^{6}	1.00×10^{5}	7.76×10^{6}	3.16×10^{4}	6.92×10^{6}	1.15×10^{4}
	49 days	2.00×10^{6}	1.00×10^{4}	3.89×10^{6}	7.94×10^{3}	4.37×10^{6}	5.37×10^{3}
	56 days	6.17×10^{5}	9.55×10^{2}	1.95×10^{6}	1.91×10^{3}	2.88×10^{6}	3.02×10^{3}
	63 days	1.86×10^{5}	7.94×10^{1}	1.02×10^{6}	4.68×10^{2}	1.95×10^{6}	1.86×10^{3}
	70 days	5.13×10^{4}	0.62×10^{1}	5.01×10^{5}	1.10×10^{2}	1.35×10^{6}	1.17×10^{3}
E. coli	1 hour	4.47×10^{8}	9.55×10^{8}	7.59×10^{8}	9.55×10^{8}	7.59×10^{8}	9.55×10^{8}
	1 day	3.39×10^{8}	7.59×10^{8}	4.37×10^{8}	3.72×10^{8}	5.01×10^{8}	6.17×10^{8}
	7 days	2.29×10^{8}	5.01×10^{8}	2.04×10^{8}	8.32×107	2.82×10^{8}	1.41×10^{7}
	14 days	1.26×10^{8}	1.00×10^{8}	9.55×10^{7}	1.70×10^{7}	7.94×10^{7}	8.91×10^{5}
	21 days	5.37×10^{7}	1.26×10^{7}	4.47×10^{7}	3.47×10^{6}	3.31×10^{7}	1.74×10^{5}
	28 days	2.24×10^{7}	1.51×10^{6}	2.14×10^{7}	6.92×10^{5}	1.78×10^{7}	5.37×10^{4}
	35 days	8.51×10^{6}	1.70×10^{5}	9.77×10^{6}	1.35×10^{5}	9.33×10^{6}	1.90×10^{4}
	42 days	2.88×10^{6}	1.86×10^{4}	4.47×10^{6}	2.88×10^{4}	5.01×10^{6}	7.24×10^{3}
	49 days	9.33×10^{5}	2.00×10^{3}	2.04×10^{6}	5.75×10^{3}	2.88×10^{6}	3.16×10^{3}
	56 days	2.95×10^{5}	2.09×10^{2}	9.12×10^{5}	1.15×10^{3}	1.70×10^{6}	1.55×10^{3}
	63 days	9.12×10^{4}	2.19×10^{1}	4.17×10^{5}	2.34×10^{2}	1.02×10^{6}	8.91×10^{2}
	70 days	2.63×10^{4}	0.22×10^{1}	1.91×10^{5}	4.79×10^{1}	6.46×10^{5}	5.37×10^{2}
Enterococcus spp.	1 hour	9.55×10^{8}	9.55×10^{8}	9.55×10^{8}	7.59×10^{8}	9.55×10^{8}	7.59×10^{8}
	1 day	7.59×10^{8}	6.31×10^{8}	6.31×10^{8}	4.07×10^{8}	8.91×10^{8}	5.01×10^{8}
	7 days	5.79×10^{8}	3.31×10^{8}	4.07×10^{8}	1.55×10^{8}	4.27×10^{8}	8.51×10^{7}
	14 days	4.07×10^{8}	1.70×10^{8}	2.57×10^{8}	5.89×10^{7}	2.14×10^{8}	1.48×10^{7}
	21 days	3.02×10^{8}	7.94×10^{7}	1.62×10^{8}	2.24×10^{7}	1.29×10^{8}	5.62×10^{6}
	28 days	1.82×10^{8}	2.34×10^{7}	1.00×10^{8}	8.32×10^{6}	8.51×10^{7}	2.29×10^{6}
	35 days	1.05×10^{8}	5.89×10^{6}	6.17×10^{7}	3.02×10^{6}	5.75×10^{7}	1.00×10^{6}
	42 days	5.13×10^{7}	1.45×10^{6}	4.07×10^{7}	1.10×10^{6}	3.89×10^{7}	4.79×10^{5}
	49 days	2.40×10^{7}	3.31×10^{5}	2.63×10^{7}	3.80×10^{5}	2.88×10^{7}	2.34×10^{5}
	56 days	1.02×10^{7}	6.92×10^{4}	1.66×10^{7}	1.32×10^{5}	2.24×10^{7}	1.26×10^{5}
	63 days	4.07×10^{6}	1.41×10^{4}	1.00×10^{7}	4.37×10^{4}	1.78×10^{7}	6.92×10^{4}
	70 days	1.58×10^{6}	2.51×10^{3}	6.46×10^{6}	1.51×10^{4}	1.38×10^{7}	4.37×10^{4}

Table 2. Parameters of inactivation kinetics of tested bacteria

Temperature	Bacteria thickness	*S.* Dublin	*E. coli*	*Enterococcus* spp.
		theoretical time of survival (days)		
4°C	Thin	162.63 $^{A, a}_{A, a}$ (±12.53)*	148.12 $^{A, a}_{A, a}$ (±11.37)	241.97 $^{B, a}_{A, a}$ (±25.13)
	Medium	200.32 $^{A, a}_{B, a}$ (±16.82)	176.55 $^{B, a}_{B, a}$ (±11.49)	300.13 $^{C, a}_{B, a}$ (±15.68)
	Thick	220.80 $^{A, a}_{B, a}$ (±7.27)	199.36 $^{A, b}_{C, a}$ (±12.47)	335.84 $^{B, a, b}_{C, a}$ (±34.60)
20°C	Thin	81.85 $^{A, a}_{C, a}$ (±3.14)	74.93 $^{A, a}_{D, a}$ (±4.07)	118.67 $^{B, a}_{D, a}$ (±2.55)
	Medium	92.04 $^{A, a}_{C, a}$ (±3.87)	86.14 $^{A, a}_{D, a}$ (±2.51)	134.89 $^{B, a}_{D, a}$ (±3.06)
	Thick	99.53 $^{A, a}_{C, a}$ (±1.28)	92.19 $^{A, a}_{D, a}$ (±3.43)	142.10 $^{B, a}_{E, a}$ (±3.46)
		elimination rate (log/day)		
4°C	Thin	0.05 $^{A, a}_{A, a, c, e}$ (±0.004)	0.06 $^{A, a}_{A, a, c, e}$ (±0.005)	0.04 $^{A, a}_{A, a, c, e}$ (±0.004)
	Medium	0.04 $^{A, a}_{A, b, d, f}$ (±0.003)	0.05 $^{A, a}_{A, b, d, f}$ (±0.003)	0.03 $^{A, b}_{A, b, d, f}$ (±0.001)
	Thick	0.04 $^{A, a}_{A, b, d, f}$ (±0.001)	0.04 $^{A, a}_{A, b, d, f}$ (±0.002)	0.03 $^{A, b}_{A, b, d, f}$ (±0.003)
20°C	Thin	0.12 $^{A, a}_{B, c, d}$ (±0.004)	0.13 $^{A, a}_{B, c, d}$ (±0.008)	0.08 $^{A, b}_{B, c, d}$ (±0.001)
	Medium	0.09 $^{A, a}_{B, e, f}$ (±0.002)	0.10 $^{A, a}_{B, e, f}$ (±0.002)	0.06 $^{A, b}_{B, e, f}$ (±0.001)
	Thick	0.08 $^{A, a}_{B, e, f}$ (±0.001)	0.09 $^{A, a}_{B, e, f}$ (±0.001)	0.06 $^{A, b}_{B, e, f}$ (±0.000)
		decimal elimination time (days)		
4°C	Thin	18.38 $^{A, a}_{A, a}$ (±1.47)	16.78 $^{A, a}_{A, a}$ (±1.25)	26.53 $^{B, a}_{A, a}$ (±2.65)
	Medium	22.94 $^{A, a}_{B, a}$ (±1.74)	20.24 $^{B, a}_{B, a}$ (±1.17)	33.90 $^{C, a}_{B, a}$ (±1.34)
	Thick	25.64 $^{A, a}_{C, a}$ (±0.58)	23.09 $^{B, a}_{C, a}$ (±1.26)	33.17 $^{C, a}_{C, a}$ (±3.72)
20°C	Thin	8.56 $^{A, a}_{D, a}$ (±0.32)	7.94 $^{A, a}_{D, a}$ (±0.50)	12.87 $^{B, a}_{A, a}$ (±0.12)
	Medium	11.07 $^{A, a}_{B, a}$ (±0.21)	9.88 $^{A, a}_{B, a}$ (±0.17)	15.46 $^{B, a}_{E, a}$ (±0.16)
	Thick	12.90 $^{A, a}_{A, a}$ (±0.10)	11.74 $^{A, a}_{B, a}$ (±0.19)	17.04 $^{B, a}_{E, a}$ (±0.09)

A, B, C, … (different letters in superscript) – highly significant differences ($p \leq 0.01$) between individual bacteria within the same temperature and thickness of slurry.

a, b, c, … (different letters in superscript) – significant differences ($p \leq 0.05$) between individual bacteria within the same temperature and thickness of slurry.

A, B, C, … (different letters in subscript) – highly significant differences ($p \leq 0.01$) between temperature and slurry thickness variant within the same bacteria.

a, b, c, … (different letters in subscript) – significant differences ($p \leq 0.05$) between temperature and slurry thickness variant within the same bacteria.

* – standard deviation.

Theoretical survival rate of *E. coli* ranged from 148.12 to 199.36 days in cattle slurry stored at 4°C and was highly significantly longer than at 20°C, where it ranged from 74.93 to 92.19 days (Table 2). The effect of dry matter content on the time of survival was highly significant only at a lower storage temperature (Table 2).

Of all the studied microorganisms, the smallest reduction in numbers during 70 days of storage was observed for *Enterococcus* bacteria. In thin cattle slurry stored at 4°C a decrease in the number of these microorganisms by 2.78 log was observed (from 9.55×10^8 to 1.58×10^6 MPN×ml^{-1}), and on 56th day enterococci population still remained at the level of 10^7 MPN×ml^{-1} (Table 1). In liquid cattle excrements with medium thickness stored at 4°C the number of these bacteria also decreased by 2.17 log (from 9.55×10^8 to 6.46×10^6 MPN×ml^{-1}), but on 63rd day of the study it was still equal to 1.00×10^7 MPN×ml^{-1} (Table 1). In thick cattle slurry stored at 4°C the number of enterococci decreased only by 1.84 log (from 9.55×10^8 to 1.38×10^7 MPN×ml^{-1}) (Table 1). In liquid cattle excrements stored at 20°C the observed declines in the population of these bacteria were greater than at 4°C and amounted to 5.58 log in thin slurry, 4.70 log in medium slurry and 4.24 log in thick slurry (Table 1).

Theoretical times of survival and times of decimal elimination calculated for enterococci based on regression line equations were highly significantly longer in comparison with the other studied microorganisms, and elimination rate was statistically significantly lower (Table 2).

Depending on the thickness of excrements, theoretical survival time of *Enterococcus* bacteria ranged from 241.97 to 335.84 days in cattle slurry stored at 4°C and from 118.67 to 142.10 days in liquid cattle excrements stored at 20°C (Table 2). Differences resulting from temperature of slurry storage were statistically highly significant (Table 2). In respect of dry matter content, highly significant differences in the theoretical survival rate were found between all the variants of thickness of liquid excrements stored at 4°C, as well as between thin and thick slurry at 20°C (Table 2).

When analysing changes in the number of individual microorganisms occurring in the course of slurry storage, it was observed that falls recorded between the successive times of analyses were high in the second half of the research cycle in thin excrements, relatively even during 70 days of storage in medium slurry, and higher in the first half of the experiment in thick slurry (Table 1).

Based on the analysis of theoretical times of survival, elimination rates and times of decimal elimination, in the majority of cases it was found that differences between values of these parameters established for thin and medium liquid excrements were higher than between thick and medium slurry, irrespective of their storage temperature (Table 2).

Discussion

Storage of animal excrements is the basic method for their treatment, allowing the elimination of pathogens. Its effectiveness depends largely on climatic condi-

tions, and first of all on the temperature (Berggren et al., 2005; Bicudo and Goyal, 2003).

In the present study a distinct effect of temperature on survival rate of all the studied microorganisms was found. It was shown that a lower temperature has a stabilizing effect on the bacteria population, allowing prolongation of their survival rate, and a higher one increases the hygienization effectiveness of storage, shortening their survival times.

Relationships observed in the present study confirm the results of works by other authors (Berggren et al., 2005; Himathongkham et al., 1999; Jones, 1976; Kudva et al., 1998; Olszewska, 2005; Plachá et al., 2001).

Jones (1976) proved that the survival rate and elimination rate of *Salmonella* Dublin bacilli from cattle slurry was clearly dependent on the storage temperature. These bacteria survived for 13 days at 30°C, for 57 days at 20°C and for 132 days at 5 and 10°C. According to Jones (1976), a decrease in concentration of *Salmonella* Dublin bacilli in slurry was higher during the first 2 weeks of storage, with elimination rate growing along with the temperature. In the present study, *Salmonella* Dublin bacilli were theoretically able to survive for 162.63–220.80 days at 4°C and for 81.85–99.53 days at 20°C (Table 2), at a higher elimination rate in the first half of the storage cycle only in thick slurry (Table 1). Also the study by Olszewska (2005) proved that the theoretical survival time of *Salmonella* Dublin bacilli was longer at 4°C than at 20°C and amounted to 21 and 16 weeks, respectively, at the weekly elimination rate of these bacteria of 0.38 and 0.50 log MPN. In the present study, the elimination rate of *Salmonella* Dublin was also greater at a higher temperature (Table 2).

Literature reports also clearly indicate the effect of slurry storage temperature on the survival rate of *E. coli* and bacteria of the genus *Enterococcus*.

Olszewska (2005) reports that the theoretical survival time of *E. coli* in stored liquid excrements amounted to 22 weeks at 4°C and 19 weeks at 20°C, at the weekly elimination rate equal to 0.36 and 0.44 log MPN, respectively. In the present experiment, these bacteria also underwent a considerably slower elimination at 4°C (0.04–0.06 log units/day) than at 20°C (0.09–0.13 log units/day), hence their theoretical time of survival amounted to 148.12–199.36 days and 74.93–92.19 days, respectively (Table 2). Also other studies (Berggren et al., 2005; Kudva et al., 1998) confirmed a clear effect of temperature on the elimination rate of *E. coli* O157:H7 during storage of cattle excrements. In cattle slurry, fresh or processed, stored at 23°C, Kudva et al. (1998) observed the complete elimination of *E. coli* O157:H7 bacilli on 5th day of the experiment, whereas at 4°C the process proceeded more slowly. In the study by Himathongkham et al. (1999) *E. coli* O157:H7 underwent 90% reduction from stored cattle slurry within the range from 21.51 to 38.76 days at 4°C and from 7.67 to 14.75 days at 20°C. Similarly, in the present study, the time of decimal elimination of *E. coli* was also longer at a lower temperature (Table 2). Wang et al. (1996) proved that a linear decrease in population of *E. coli* O157:H7 occurs at 5°C, whereas at 22 and 37°C the bacteria elimination rate decreases in time. These observations were reflected in our own results concerning changes in the number of *E. coli* bacilli during storage of thick slurry (Table 1).

In the present study enterococci survived 241.97–335.84 days in liquid excrements stored at 4°C and 118.67–142.10 days at 20°C (Table 2). Effect of storage temperature on the survival rate of enterococci was also indicated in the studies by Olszewska (2005) and Olszewska et al. (2011). According to the literature reports, the time of decimal elimination of enterococci in the solid fraction of stored pig slurry was 36.89 days in the summer period and 95.38 days in the winter-spring period, whereas the daily elimination rate was equal to 0.03 and 0.01 log cfu, respectively (Plachá et al., 2001). In the present experiment 90% elimination of enterococci proceeded more rapidly and a daily elimination rate was higher, but the effect of slurry storage temperature on both parameters was equally clear (Table 2).

There are several different phenomena which determine the effect of high and low temperature on the survival of the bacteria. In spite of the fact that a temperature of 37°C remains within the range of growth optimum of intestinal bacilli, storing slurry in mesophilic conditions results in an increased production of free ammonia (Park et al., 2002) and higher nitrogen losses (Pratt et al., 2002). Increasing ammonia production in a higher temperature is connected with the intensification of metabolism of proteolytic bacteria in slurry and with increase in amount of oxygen dissolved in stored liquid excrements, which results in changing conditions prevailing in the container to anaerobic, optimal for protein putrefaction, with ammonia as one of the final products.

Temperature is closely connected with the activity of autochthonous microflora of slurry, which while decomposing organic matter contributes to production of ammonia, volatile fatty acids and sometimes even antibiotics, exerting a strong negative pressure on pathogenic microorganisms (Kołacz and Dobrzański, 2006). The higher temperature increases activity of natural microflora antagonistic towards pathogenic bacteria, shortening the survival time of intestinal bacteria (Arrus et al., 2006). Optimal conditions for development of slurry autochthonous microflora antagonistic towards pathogens prevail in mesophilic range of temperatures, whereas under psychrophilic conditions only such psychrotrophs can develop (with the growth optimum within mesophylic range) which show the ability to adapt to lower temperatures. However, in this case their metabolism is substantially slower, and a negative effect on pathogenic microorganisms is poorer (Burton and Turner, 2003; Zeemann et al., 1998). According to reports of Motz and Kokociński (1977), microorganisms dominating among autochthonous bacteria are able to decompose organic connections of nitrogen, whereas microorganisms using carbohydrates as a nutritional substrate are relatively rare, and their population is subject to substantial changes in a short time. Wachnik (1976) proves that bacteria of the genus *Salmonella* can survive at 10ºC for 80–180 days, whereas at 20ºC they are isolated on average for 35 days. According to this author the cause of the above situation is the fact that natural microflora of slurry shows a considerably higher living activity in a higher temperature and owing to that, its antagonistic effect towards rods of *Salmonella* is stronger. This phenomenon may be caused by the fact that slurry does not provide the optimal environment for development of pathogens, which are adapted to survive in intraorganic conditions. Moreover, rods of the genus *Salmonella* are typical heterotrophs and are not able to slow down the rate of metabolizing nutrients contained in

slurry. As a result they lose competition to autochthonous microorganisms (Plachá et al., 2001). The essential role of natural microflora of slurry in elimination of *Salmonella* is also proved by Jones et al. (1977), who determined the survival rates of *S.* Dublin in sterilized slurry, sterilized slurry infected with the addition of raw slurry, and raw slurry. In the first environment, bacteria of the genus *Salmonella* did not have to compete for nutrients with other microorganisms and were not affected by toxic substances produced by these organisms. As a result, they survived as long as 290 days. In the other two environments, the survival time of *S.* Dublin was the same and amounted to 74 days, and the elimination rate in the initial period of the study was considerably higher in raw slurry.

Beside the temperature, one of the main factors determining survival rate of bacteria in slurry is the content of dry matter. The overwhelming majority of literature reports show a longer time of microorganisms survival in excrements with a higher dry matter content. According to Jones (1980), an increase in solid particles content in excrements from 10 to 50 g/dm^3 results in prolongation of survival time of *Salmonella* by 40%. Jones (1976) also showed that in cattle slurry with the dry matter content amounting to 1% stored at 10ºC *Salmonella* Dublin bacilli survived from 86 to 93 days, whereas an increase in dry matter proportion up to a level of 4.8% caused a prolongation of survival time up to 114–142 days. In the present study an increase in dry matter content from 2.0 to 6.0% during the process of cattle slurry storage resulted in prolongation of the theoretical survival time of *Salmonella* Dublin at 4°C by more than 37 days, whereas at 20°C by more than 10 days (Table 2). Analogous tendency was observed by Provolo et al. (1997) who reported that *Salmonella* Dublin bacilli survived for 70–80 days in thin slurry with dry matter content of 1–2%, up to 120 days in stored excrements with dry matter proportion of 6–7%, and as long as 150 days in cattle slurry containing 9% dry matter.

Also in the case of *E. coli* O157:H7 McGee et al. (2001) proved that they survived longer in slurry with a higher dry matter content. Reduction in the number of these bacteria during 12 weeks of storage in fertilizer containing 10.57% dry matter was lower by 2 log than that in slurry with a dry matter proportion equal to 6.67%. The difference observed in the present study in elimination of *E. coli* between liquid excrements with extreme thicknesses amounted to about 1 log in both temperature variants.

Conclusions drawn from the study by Burrows and Rankin (1970) are helpful in explaining the above regularities, showing that a higher content of solid substances can exert a protective effect on pathogens. It may be assumed that the factor prolonging the survival rate of bacteria is probably their aggregation or adsorption on the surface of solid particles present in slurry. Higher content of dry matter, and particularly of organic matter, enables pathogenic bacteria to adhere to solid particles (Kearney et al., 1993). Dry matter proportion determines abundance of slurry in nutritive substances for bacteria, which is one of the most important factors determining the survival rate of fecal bacteria, which are unable to slow the rate of metabolism with the shortage of nutrients. However, although an increase in proportion of organic matter prolongs the survival time of intestinal bacteria, it also has a positive effect on development and activity of competitive autochthonous microflora, intensifying an-

tagonistic effects, which shortens the survival time of allochthonous bacteria (Gerba and Bitton, 1984).

The conducted study explicitly showed a low hygienization effectiveness of storage, and the statutory time of slurry storage is not sufficient for ensuring its complete decontamination. Moreover, usefulness of the studied microorganisms for constant monitoring of slurry processing methods was confirmed.

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Higieniczne aspekty składowania gnojowicy bydlęcej jako najpopularniejszej i najtańszej metody postępowania z płynnymi odchodami zwierzęcymi

STRESZCZENIE

Gnojowica stanowi bardzo wartościowy nawóz naturalny, jednak jej niewłaściwe zastosowanie w rolnictwie stwarza poważne zagrożenie sanitarno-higieniczne. W związku z powyższym celowa jest jej obróbka przed nawozowym wykorzystaniem. Jedną z metod, cieszącą się wciąż największą popularnością, jest składowanie płynnych odchodów.

Celem badań była ocena skuteczności higienizacyjnej składowania w temperaturze 4 i 20ºC w oparciu o parametry opisujące kinetykę zmian populacji wybranych bakterii wskaźnikowych w gnojowicy bydlęcej o różnym udziale suchej masy.

Materiał do badań stanowiła świeża gnojowica bydlęca. W badaniach wykorzystano płynne odchody o zawartości suchej masy: 2, 6 i 14%. Gnojowicę składowano w temperaturze 4 i 20ºC. Jako bakterie wskaźnikowe wykorzystano pałeczki *Salmonella* Dublin, *E. coli* oraz enterokoki. Liczbę drobnoustrojów

ustalono w oparciu o metodę NPL w układzie 3-probówkowym. Obliczono podstawowe parametry kinetyki inaktywacji bakterii oraz przeprowadzono analizę statystyczną w programie SAS 9.2 PL.

W składowanej gnojowicy obserwowano stopniową eliminację wszystkich badanych drobnoustrojów. Efekt higienizacyjny składowania był słabszy w temperaturze 4 niż 20ºC oraz w odchodach o wysokim udziale suchej masy. W zależności od temperatury składowania i udziału suchej masy teoretyczne czasy przeżycia wahały się dla pałeczek *Salmonella* Dublin od 81,85 do 220,80 dnia, dla *E. coli* – od 74,93 do 199,36 dnia, a dla enterokoków – od 118,67 do 335,84 dnia.

Badania jednoznacznie wykazały, że ustawowo przewidziany czas składowania gnojowicy jest niewystarczający dla zapewnienia jej pełnej higienizacji.