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ORIGINAL PAPER

EFFECT OF CALCIUM PEROXIDE (CaO_2) ADDITION TO POULTRY LITTER ON THE PARAMETERS OF ITS PHYSICOCHEMICAL, MICROBIOLOGICAL AND FERTILISING QUALITY

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Abstract

The quality of litter is an important environmental factor in poultry houses. Its proper physicochemical properties have a positive impact on poultry house microclimate and indirectly on poultry farm surroundings as well. Commercial preparations available on the market which disinfect and reduce losses of mineral compounds (primarily nitrogen) in litter are relatively expensive, and thus they are not widely used. Therefore, it is necessary to look for a cheaper option, such as CaO_2 addition seems to be. The research was carried out in farm conditions in two poultry houses, in each of which approximately 21 000 Ross 308 broiler chickens were reared. In order to maintain good physicochemical parameters of litter throughout the broiler chicken rearing time, the researchers applied (before bringing in the birds) a single CaO_2 addition in the amount of 2 g per 1 m^2 of poultry house floor area. During the 6-week chicken rearing period, the following parameters were monitored: temperature, relative humidity and pH value of the litter top layer. The total count of aerobic mesophilic bacteria and the total count of yeasts and moulds were estimated in the litter. Moreover, the content of mineral compounds (N, P, K, Mg, Ca) in fresh litter mass was checked in the 1st, 3rd and 6th week. The results prove that calcium peroxide application has no negative impact on the examined physicochemical parameters of litter (especially on temperature). The addition of calcium peroxide to poultry litter also influenced the stabilization of litter microflora. The content of examined mineral compounds in experimental poultry house litter, which was increasing with chicken rearing time, as shown in the experiment, proved the improving fertilising value of the produced manure. Moreover, due to the slow CaO_2 decomposition, it is sufficient to apply it only once during a 6-week broiler chicken production cycle.

Keywords: calcium peroxide, poultry litter, physicochemical parameters, litter microflora fertilising value.

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INTRODUCTION

In recent years, poultry manure handling has become a problem for poultry farm owners. As a result of short rearing time and a high concentration of birds within a small area, producers often encounter the problem of what to do with dung generated during bird rearing, and how to reduce unfavourable excessive release of mineral compounds, especially nitrogen, into the natural environment.

Currently, there are many methods used, which are intended to optimise the physicochemical parameters of poultry litter, and, as a consequence, to reduce losses of mineral compounds. They include: physical (Lewis, Gous 2009), biological (Mituniewicz 2012) and chemical methods (Cook et al. 2011). Nevertheless, regardless of the method employed, from the point of view of breeders, it is important that optimisation of the physicochemical parameters of litter is, on the one hand, cheap, and on the other hand, safe to use in the presence of birds. Considering this, it becomes very important to apply mineral-type additives in bedding material. Among these additives, calcium compounds have been proven to have a positive effect (Mituniewicz et al. 2008, Mituniewicz 2012).

Calcium peroxide belongs to the group of inorganic metal peroxides, called permanent sources of active oxygen. Besides calcium peroxide, the most popular compounds from this group include sodium and magnesium peroxides. The main advantage of these compounds is that while they undergo decomposition in contact with water, they form hydrogen peroxide, which then decomposes to form water molecules and oxygen radicals. However, an important factor is that the rate of oxygen release from calcium, sodium and magnesium peroxides is much slower than in the case of direct hydrogen peroxide application, which allows using these compounds on a larger scale (Walawska, Gluzińska 2006, Kostecki, Mazierski 2008). The advantage of calcium peroxide over other peroxides is the fact that its solubility in water is poorer than that of sodium peroxide; therefore, it can be used directly without the need of granulation. Whereas the economic factor is important when we compare options to use magnesium and calcium peroxides - the market price of CaO_2 is much lower than MgO_2. This results from a difference of production technology costs. In the case of calcium peroxide, it is simple and involves heating of calcium oxide with hydrogen peroxide. An important advantage in favour of using calcium peroxide in practice is also the fact that four times more oxygen is released from CaO_2 than from magnesium peroxide.

The practical use of calcium peroxide as a source of active oxygen concerned supporting the processes of bioremediation and oxygenation in lower layers of water in ponds and lakes. This contributed to oxygen deficit elimination and intensifying the water self-purification process. As a result, acceleration of mineralizing processes occurring in bottom sediments was achieved (Kostecki, Mazierski 2008). Moreover, research showed the satisfactory effects of using calcium peroxide to improve effectiveness in the biodegradation of petroleum products polluting the natural environment (Kostecki, Mazierski 2008) and biodegradation of polycyclic aromatic hydrocarbons in bottom sediments (Miksch 2009).

Completed studies (Walawska, Gluzińska 2006, Kostecki, Mazierski 2008, Miksch 2009) also indicate the possibility to remove organic matter and polycyclic organic fouling from water reservoirs and bottom sediments. They show that calcium peroxide addition positively affects the biochemical activity of bottom sediment. Consequently, this activity increases by ca. 20% at an optimum dose of CaO_2. At the same time, it was proven that, depending on natural environment components (soil, aquatic environment - bottom sediments), optimal calcium peroxide doses were different: between 0.05 and 5 g kg^{-1} in the case of bottom sediments, up to 100 - 200 g m^{-2} for a soil environment.

As has been demonstrated before, the primary applications of CaO_2 are connected with its slow decomposition in contact with moisture and gradual oxygen release. Additionally, due to its proven action (Kostecki, Mazierski 2008, Miksch et al. 2009): alkalifying and improving adsorption of chemical compounds, we can expect bonding mineral compounds in litter, which prevents their loss from organic material.

However, in available literature, it is easy to see that there are no results of studies on using calcium peroxide as a source of active oxygen in animal housing, and in particular in the presence of animals. Therefore, the purpose of the undertaken research was to determine the impact of calcium peroxide addition to litter on its physicochemical and microbiological parameters and fertilising value.

MATERIAL AND METHODS

The studies were carried out in production conditions in a commercial poultry farm. Each planned test group included one poultry house with full stock according to applicable regulations (OJ 2009 No 223, item 1784), reaching 21 000 birds (15 pcs m^{-2}). Ross 308 broiler chickens were reared for 6 weeks on litter consisting of rye straw (litter layer thickness ca. 150 mm), without adding any litter later on.

The farm buildings, each with a floor area of 1 300 m^2, were provided with mechanical ventilation, allowing fan productivity adjustment, and artificial lighting with light intensity control.

During 6 weeks of rearing in Poultry House 1, the birds were kept on litter without any addition and formed a control group (ExG1). At the same time, in Poultry House 2, birds were kept on litter with calcium peroxide added – dose 2 g CaO_2 m^{-2}. An experimental group (ExG2) was established in this way. Calcium peroxide was applied once, by spilling it over the litter before bringing in the birds.

Chickens of both groups were fed with standard industrial feeds: Prestarter (KW-1), Starter (KW-2), Premium Grower (KW-3) and Premium Finisher (KW-4), according to the manufacturers' instructions.

Basic physical parameters – temperature and relative humidity of straw litter top layer (down to 30 mm) – were measured with a HAY-METER HM 1625/02 type hytherograph in five spots of each poultry house, twice a week, throughout the chicken rearing time.

Litter reaction (pH) was determined according to the PN-ISO 103a90: 1997 standard. Litter was taken for tests in five spots in each poultry house, once a week, and then the samples were mixed in sterile containers so as to make an aggregate sample for each object.

Microbiological tests of litter for the total count of aerobic mesophilic bacteria were performed in accordance with PN-EN ISO 4833-2:2013-12 and for the total count of yeasts and moulds in accordance with PN-ISO 21527-1: 2009. Litter samples were collected at 5 points in each hen house, and subsequently the samples were mixed in sterile containers to form a pool sample for each facility.

The content of mineral compounds in litter was determined for fresh material directly before bringing in the birds (week 1), and in litter with manure, in the 3rd and 6th week of rearing. The tests were carried out in an accredited laboratory, using the following methods:

– nitrogen content (N) – PB 05 ed. 02 from 21.06.04 (g kg^{-1});
– phosphorus content (P) – PB 04 ed. 2 from 21.06.04 (g kg^{-1});
– potassium content (K) – PB 03 ed. 2 from 21.06.04 (g kg^{-1});
– magnesium content (Mg) – PB 06 ed. 2 from 21.06.04 (g kg^{-1});
– calcium content (Ca) – PB 03 ed. 2 from 21.06.04 (g kg^{-1}).

The results were analysed statistically with Statistica 12.5 computer software, with the use of single-factor analysis of variance, in the orthogonal design. The significance of differences between the average values of the features under study was determined with the Duncan's test.

RESULTS

In the first days after bringing in the chickens, litter temperature values were comparable to air temperature values registered in poultry houses (Figure 1), close to 30°C (Figure 2). These values matched the instructions given by the breeders of the Ross 308 broiler chickens, according to which the bedding temperature after bringing in the birds until their seventh day

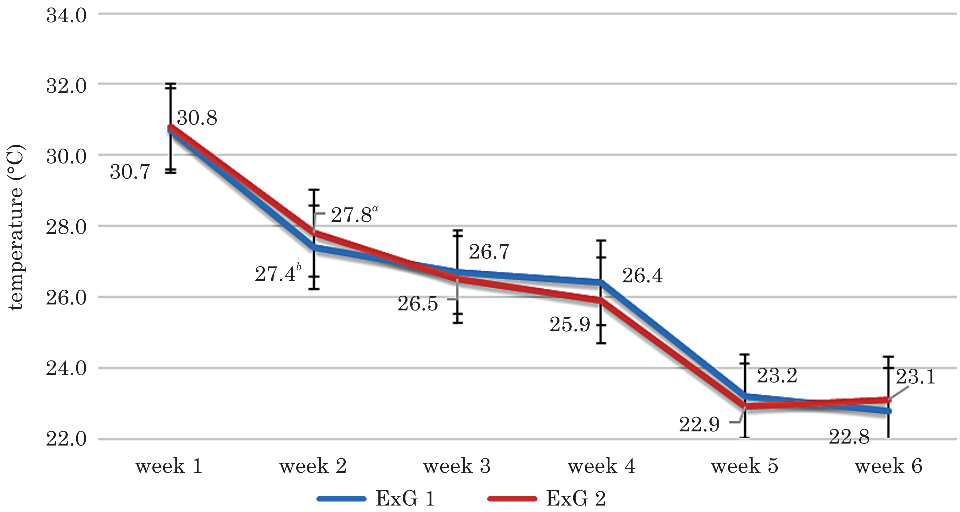

Fig. 1. Average values of poultry house air temperature: *a*, *b* – statistically significant difference between groups, for $P \leq 0.05$

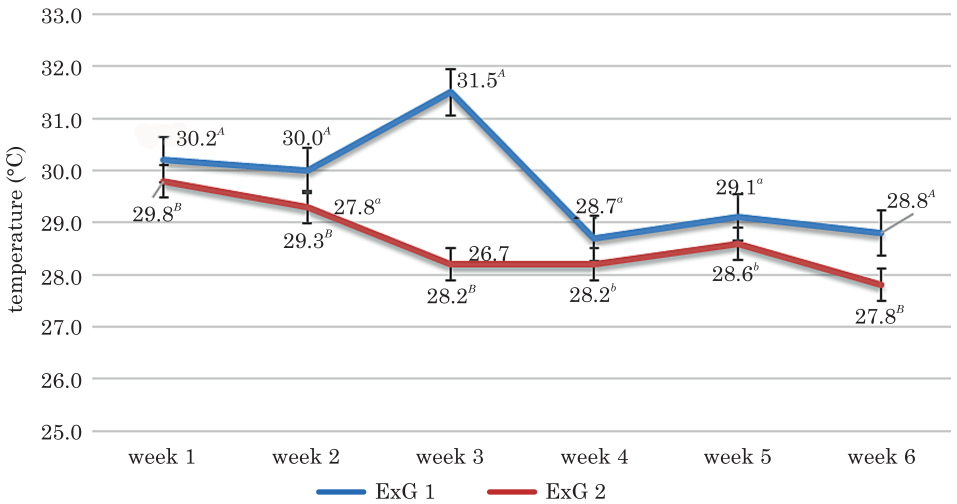

Fig. 2. Average values of litter top layer temperature in poultry houses. Values marked with various letters considerably differ from each other: *A*, *B*, *a*, *b* – statistically significant difference between groups, for $P \leq 0.01$ and $P \leq 0.05$, respectively

of life should range from 28 to 30°C. It was also observed that throughout the analysed period, litter temperature was statistically significantly lower in the experimental poultry house than in the control henhouse. This confirms that CaO_2 addition did not make the bedding temperature rise.

Figure 3 indicates that in successive weeks of broiler chicken rearing time, litter moisture content was successively growing, both in the control

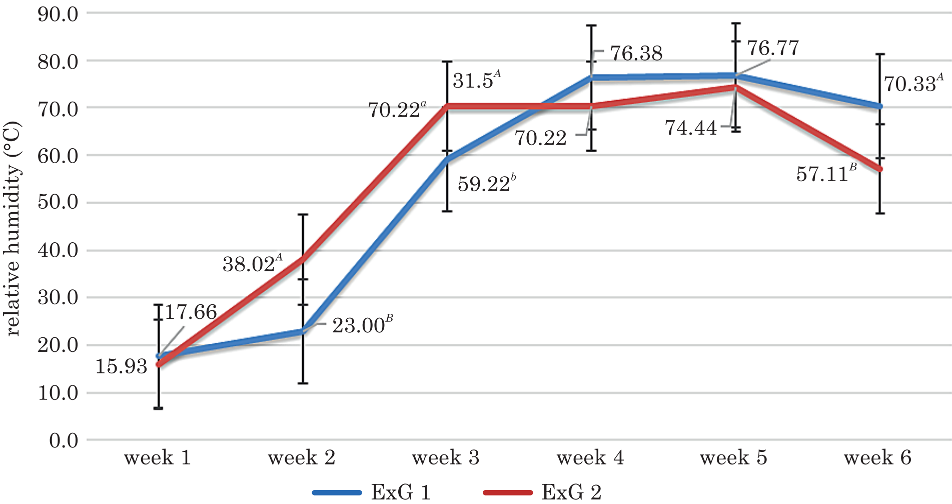

Fig. 3. Average values of litter top layer relative humidity in poultry houses. Values marked with various letters considerably differ from each other: *A*, *B*, *a*, *b* – statistically significant difference between groups, for $P \leq 0.01$ and $P \leq 0.05$, respectively

group and in the experimental group. The exception was the last week of research, when a drop in litter humidity level was observed in both test groups. Statistically significantly lower relative humidity of the litter top layer was registered in the group with CaO_2 added in weeks 2, 3 and 6. At the same time, no significant differences in the value of this parameter were observed for individual groups in the 4th and 5th week of the research.

Figure 4 shows the pH values observed in the respective weeks of research. Litter taken for analyses directly before bringing in the chickens in

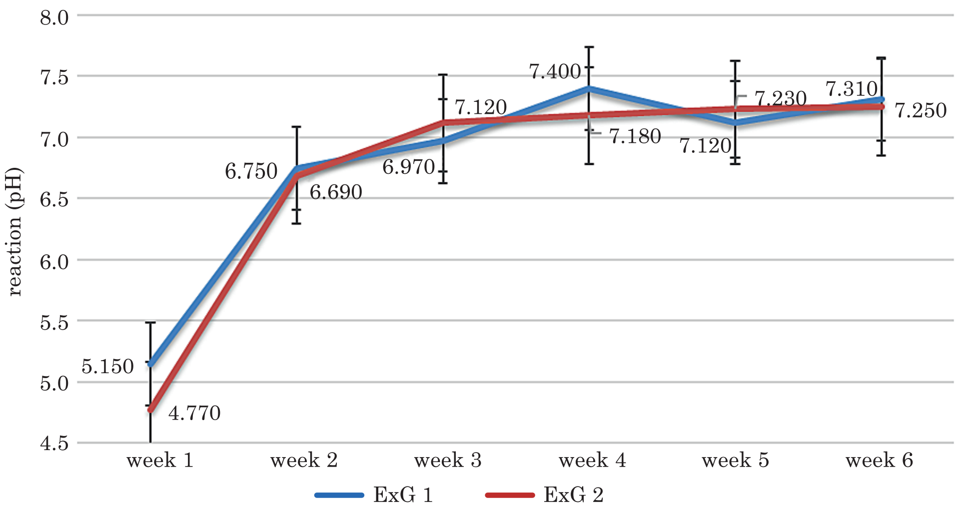

Fig. 4. Average pH values of litter in successive analysed weeks

the experimental group (after spilling calcium peroxide over the litter) proved to have a slightly higher pH value than the control group. In both poultry houses, from the second analysed week, the researchers observed a successive, constant increase in the litter pH value. This status remained until the end of the experiment. Although no significant differences were observed between the groups in the respective weeks of research, there was a visible tendency for higher stability of an estimated parameter in the group with calcium peroxide added (ExG2). However, at the end of the analysed period, the litter reaction value was much the same in both groups.

The dynamic increase in the total number of aerobic mesophilic bacteria in the litter in the control group was observed as early as in the second week of the study, while in the experimental group it was observed in the third week (Figure 5). This week was characterised by the highest count of bacte-

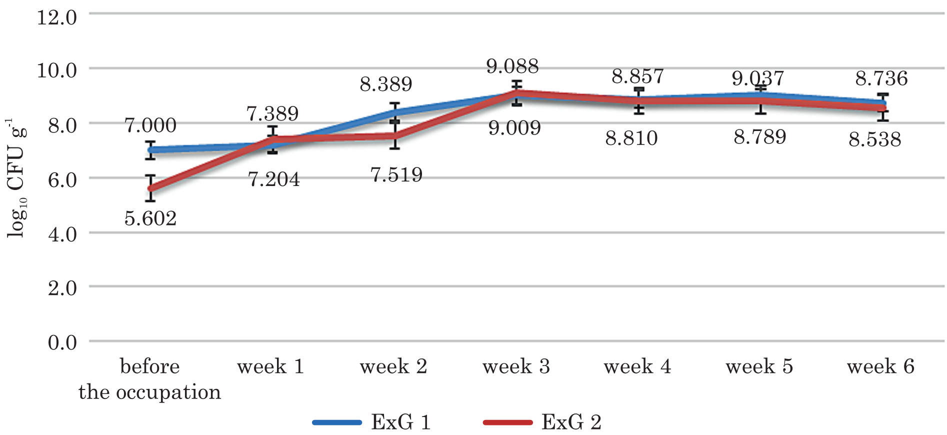

Fig. 5. The total number of mesophilic aerobic bacteria in the litter in successive analysed weeks

ria incubated from the litter, both in the experimental and control group. However, in ExG2 the total bacterial count was slightly higher. In the fourth week of the study the bacterial count in both groups decreased. In the group where calcium peroxide was added to the litter this trend persisted until the end of rearing. After completion of the study the titer of aerobic bacteria in this group was 8.538 $\log_{10}$ CFU g^{-1}. In the control group a higher variability in the count of incubated bacteria was observed, which reached a level of 8.736 $\log_{10}$ CFU g^{-1} at the end of the study period.

In the performed experiment, the addition of calcium peroxide significantly limited the development of yeasts and moulds in the litter (Figure 6). The highest level of mycological contamination in the litter was recorded in the second week of the study and it equalled 7.290 $\log_{10}$ CFU g^{-1} in ExG1 and 6.908 $\log_{10}$ CFU g^{-1} in ExG2, respectively. However, in this week, despite intensive growth in the count of incubated yeasts and moulds, mycological contamination of the litter with the addition of calcium peroxide was signifi-

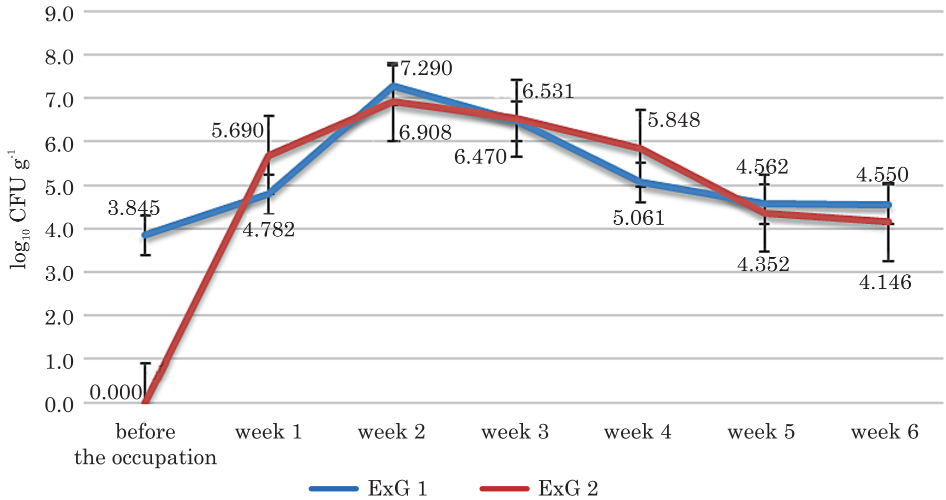

Fig. 6. The total number of yeasts and molds in the litter in successive analysed weeks

cantly lower than in the control group. The mean count of these organisms throughout the study was at a level of 5.223 $\log_{10}$ CFU g^{-1} in the control group and of 4.782 $\log_{10}$ CFU g^{-1} in the experimental group. Therefore, the application of CaO_2 to the litter effected the reduction in the amount of mycological contamination also in the entire period of the study.

The amount of minerals contained in the litter increased with the growing manure volume in the successive weeks of rearing birds (Table 1). Poul-

Table 1

Concentration of selected elements (g kg^{-1}) in litter with chicken manure in successive analysed weeks.

Parameter	Week of research	Experimental Group 1 (ExG 1 - control)	Experimental Group 2 (ExG 2 - 2 g CaO_2 m^2)
N	1	6.200	7.400
	3	31.90	36.50
	6	33.10	34.80
P	1	0.567	0.741
	3	8.284	9.418
	6	7.761	9.418
K	1	8.964	11.29
	3	21.58	24.24
	6	21.25	24.90
Mg	1	0.663	0.543
	3	5.367	5.246
	6	4.945	6.211
Ca	1	5.861	3.289
	3	5.720	9.152
	6	7.436	12.30

try manure coming from the poultry house with CaO_2 added (ExG2) showed a higher concentration of all analysed minerals compared to the control group (ExG1). In the control group, in the last (6th) week of research, a reduced content of P, K and Mg was observed in litter with manure, whereas in the experimental group (ExG2), a similar tendency was observed for nitrogen content. However, the content of this element was higher than in litter without CaO_2 added.

DISCUSSION

In the industrial production of boiler chickens in Poland, the most popular animal housing system is the deep litter one (Atapattu, Wickramasinghe 2007). Maintaining the right physicochemical parameters during the rearing period, on the one hand, positively affects the microclimate of a livestock building, and thus the birds (Duncan 1998, Shepherd, Fairchild 2010) and, on the other hand, is beneficial for the natural environment, as it reduces losses of minerals in litter.

The problem of maintaining correct values of litter thermal and humidity parameters has been raised by many authors (Atapattu, Wickramasinghe 2007, Mituniewicz 2012). The results of our own studies proved that throughout the bird rearing time, the temperature of the litter top layer remained within the limits recommended by the breeder of Ross broiler chickens 308 (28 - 30°C). Moreover, the researchers have not observed any effect of calcium peroxide consisting of increased litter temperature, which should be considered as an advantage. This is particularly important, as this parameter may reach up to 40ºC as a result of changes induced by microorganisms living in litter. This may also generate a rise in the temperature in the area where the birds live (Seedorf et al. 1998, Chernaki-Leffer et al. 2007).

The research outcome was different for litter relative humidity. This is a particularly important issue in the rearing of birds, and for many years now, researchers have been looking for solutions which would effectively allow keeping the RH value near 40%. The results obtained during our own studies proved that the relative humidity of the litter top layer (Figure 3) oscillated between 70 - 75% for both test groups, and these values remained the same from the 3rd (ExG2) until the 6th week (ExG1) of broiler chicken rearing time. Studies by other authors (Fidanci et al. 2010, Witkowska et al. 2010, Mituniewicz 2012) do not show this rapid increase in litter top layer humidity as early as in the 3rd analysed week, and the average value of this parameter remained within 30 - 40%. The abrupt acceleration of the litter damping process shown in our own research should be connected with colibacillosis (*Colibacteriosis gallinarum*) found in poultry houses. This disease is induced in birds by *Escherichia coli* (APEC) serotypes, which are pathogenic for them. Infected birds show the following symptoms: loss of appetite, dejection, oppression, growth inhibition and diarrhoea (OSEK 2000, WITKOWSKA et al. 2010). Increasing water content in litter material is a consequence of a higher volume of watered manure penetrating the bedding, which is induced by the disease. According to the recommended treatment method for birds, antibiotic therapy was administered. However, unequivocal regress in the litter damping process observed during the 6th week in the experimental group deserves attention, as this may suggest a drying effect of the CaO_2 addition applied. It seems that this was the outcome of its slow decomposition, effected by increasing litter humidity (WALAWSKA, GLUZIŃSKA 2006, KOSTECKI, MAZIERSKI 2008, MIKSCH et al. 2009, TUREK-SZYTOW et al. 2012). The increasing volume of manure and water delivered with it to the litter, and changes in the bedding reaction, could affect the calcium peroxide decomposition rate. This is so because a drop in the litter pH value is one of the factors that accelerate CaO_2 decomposition and, consequently, the volume of oxygen being released increases (as a result of the chemical reactions involved) (WANGA et al. 2010). These properties would explain the delayed calcium peroxide impact on moisture content in litter bedding and its drop occurring just at the end of the analysed period. Additionally, the results of the research carried out by WANGA et al. (2010) have proven that calcium peroxide has properties inhibiting *E. coli* growth. Supporting the effect of the antibiotic therapy administered in a poultry house with CaO_2 added could be a consequence of this action. However, in order to confirm this point, more thorough analyses using tests for *E. coli* serotypes which are pathogenic for birds would be required.

Although calcium peroxide is a strongly alkaline compound (KOCIOŁEK-BALAWEJDER, ŻEBROWSKA 2001, WALAWSKA, GLUZIŃSKA 2006), no statistically significant effect of CaO_2 application on litter reaction (pH) was observed. A successive increase in litter pH reaction was observed in both poultry houses as early as from the 2nd week of research; this tendency remained until the end of rearing time. In our own research, it was also observed that in the last week of bird rearing time, the litter reaction was lower (ExG2 - 7.250) than in the studies (MITUNIEWICZ 2012) where calcium oxide forms (CaO and CaOMgO; 8.870-9.180, respectively) were applied as litter addition. This is particularly important, since a high alkalifying level establishes favourable conditions in litter for growth of microorganisms responsible for the change of nitrogen compounds into ammonia, which is an adverse effect as regards the welfare of both birds and the environment (higher emissivity of this gas). Many authors specify (AL HOMIDAN et al. 2003, LIU et al. 2007, LAHAV et al. 2008, SISTANI et al. 2009) that the highest level of ammonia production in litter material takes place when the pH reaches a value of 8 or higher. The pH values obtained in our own research also prove that the calcium peroxide dose applied in the experiment (2 g m^{-2} CaO_2) does not enhance litter alkalifying in production conditions.

An important element in the assessment of the quality of litter is its degree of microbial contamination, as it is closely related to the presence of uricolytic microorganisms responsible for the production of ammonia.

The mean count of aerobic mesophilic bacteria incubated from the litter samples throughout the study was 8.319 $\log_{10}$ CFU g^{-1} for ExG1 and 7.962 $\log_{10}$ CFU g^{-1} for ExG2, respectively. These values were lower than those obtained by Witkowska et al. (2010) and Mituniewicz (2012). According to many authors (Thaxton et al. 2003, Fries et al. 2005, Omeira et al. 2006), the count of microorganisms present in the bedding largely depends on the applied litter. These authors also emphasise a high variability in the count of bacteria incubated from the litter which equalled from 3 to 10 $\log_{10}$ CFU g^{-1}.

According to Miksch et al. (2009), owing to the oxidizing properties of calcium peroxide, the intensification of biological processes in the environment is possible. The addition of this compound to bottom sediment stimulated is biological activity and restores its biological balance. Moreover, CaO_2 supports of the processes of soil bioremediation.

The aim of the experiment was to shift the process of biological transformations in the direction of aerobic transformations, at the same time creating disadvantageous conditions for the development of microflora responsible for putrefaction. However, based on the results obtained in a production experiment, which indicate a lower microbial count in the bedding, it can be expected that calcium peroxide applied to the litter has a disinfecting effect. An additional evidence for this suggestion is a huge reduction in the count of incubated bacteria from the third to the last week of the study, and the lack of, in contrast to the control group, another outbreak of intensive proliferation of microorganisms in the fifth week. After an intensive growth of aerobic mesophilic bacteria recorded during the third week of the study, the addition of calcium peroxide probably caused the restoration of biological balance in the litter, and oxygen produced in the reactions of decomposition of this compound had a disinfecting effect. However, in this study no differential diagnosis of microorganisms was performed and the count of anaerobic organisms was not determined either, hence the above-mentioned suggestion would require further studies to evaluate the ratio of counts of anaerobic and aerobic bacteria.

The results of the studies for the presence of yeasts and moulds differed from the results obtained by other authors. Witkowska et al. (2010) report that the amount of mycological contamination in the litter tends to increase with the growth of chickens. The results obtained by the above-mentioned authors also indicated a higher contamination of the litter by yeasts and moulds in the last week of the study. In a study conducted by Mituniewicz (2012), who used calcium compounds in the oxide form (CaO and CaMgO), a relationship between the applied dose and the rearing period, and the count of incubated fungi was found. It increased proportionally throughout the time of bird rearing. Moreover, Witkowska et al. (2010) emphasise that the increase in the count of yeasts and moulds may bear evidence of the poor quality of the litter.

However, in the authors' own research, no increase in mycological contamination of the litter dependent on the length of litter use was found. At the same time, the results of the present study can indicate that the dose of calcium peroxide added to the poultry litter in the conducted experiment was characterised by very good properties, which limited the development of yeasts and moulds in the litter throughout the bird rearing period.

All over the world, poultry manure is considered to be valuable natural fertiliser characterized by a high content of nitrogen, phosphorus, potassium and micronutrients. Owing to these properties, it is often used by farmers (Chan et al. 2008, Warren et al. 2008, Magagula et al. 2010). However, prolonged use of poultry manure may adversely affect the natural environment, primarily because of the leaching of minerals (especially phosphorus) into surface waters (Warren et al. 2008). Maguire et al. (2006) and Ruiz et al. (2008) confirmed the positive effect of calcium compounds (CaO) used in poultry manure on reducing the rate of the leaching of minerals, especially phosphorus, from the soil solution. This results from the formation of a stable calcium phosphate compound, which leaches to ground waters to a much smaller degree. Moreover, poultry manure with calcium compounds added to it, used as natural fertiliser, is characterised by a slower release of nitrogen compounds into the soil and also contributes to increasing the concentration of phosphorus, potassium and magnesium in the soil. A greater supply of mineral compounds to plants results in higher biomass production (Adegbidi et al. 2003). Moreover, due to the fact that calcium peroxide is a strongly alkaline compound, using poultry manure with CaO_2 added as a natural fertiliser may increase soil reaction. This is important because soils showing an acid reaction are the prevailing type in Poland. The optimal range of pH values for biological processes taking place in soil remains between 5.5 and 7.2. In soils with a pH value under 4.5, soluble aluminium forms appear in the soil solution, which are destructive to root hairs. The process of alimentation and water absorption by plants becomes disturbed. Therefore, reaction is the parameter which reduces yielding for the majority of plants in sour soils. However, separate and more detailed studies should be carried out in order to confirm unambiguously the impact of fertilisation with poultry manure containing calcium peroxide addition on soil acidity.

CONCLUSIONS

1. No CaO_2 impact on litter temperature increase has been observed, which is a desirable phenomenon as regards the temperature comfort of birds and poultry house microclimate.

2. Owing to slow calcium peroxide decomposition, it is sufficient to apply it only once during a 6-week chicken rearing period; whereas it seems to be justified to carry out further studies on the potential to use it for poultry species requiring a longer rearing time.

3. The manure obtained from broiler chicken rearing with the addition of calcium peroxide, may be high quality natural dung, rich in nutrient components. Moreover, owing to strongly alkaline properties, it may contribute to increasing the reaction of sour soils, which prevail in Poland.

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