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# Effect of addition of brown coal and microbe vaccine to litter on bedding quality and production results in turkey farming

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The study was conducted to investigate the effect of applying of two different additives to the straw litter on the physical and chemical characteristics of bedding and on turkey broiler performance. In building B-1 birds were kept on straw supplemented with brown fine coal, in building B-2 litter was sprayed with EM-1 microbe vaccine solution (200 ml/10 l water) twice a day. Building B-3 was a control object. During the experiment thermo-vision photographs of the surface and the inside layers of litter were taken. Once a week determined were the extent of litter surface encrustation and the health status of legs in turkeys. After rearing, final mortality and body live weight were specified. Both tested additives led to the significant rise in litter temperature, which positively affected its physical parametres and contributed to the improvement of the birds' welfare and performance (lower mortality, higher final weight).

KEY WORDS: bedding / brown coal / litter / microbe vaccine / thermo-vision / turkey broilers

Environmental pollution caused by poultry manure production is a serious problem. To counteract this situation, different litter additives are used. The most popular are chemicals: sodium bisulfate, acidified clay, gypsum, alum (aluminum sulfate),

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lime (calcium hydroxide) hydrochloric acid, and zeolite. Bioenzymatic additives as well as various commercial products (e.g. Ammoblast, SOP C POULTRY, Bio-Kat) are also known [Châtillon 1999, Kadam *et al.* 2003, Shah *et al.* 2007]. Many studies were conducted to evaluate the effect of such additives on dry matter, nitrogen, soluble phosphorus content of manure, its pH, free ammonia and carbon dioxide and nitrogen losses as well as on broiler performance [Rudzik 1998, Praca Zbiorowa 2000, De Oliveira *et al.* 2003, Miles *et al.* 2003, Choi and Nahm 2004, Nahm 2004ab, Pourreza *et al.* 2004]. Effects of chemical additives to the litter on subsequent soil quality and plant absorption after manure application are also discussed [Miles 2003, Nahm, 2005].

Little information is available about the effects of additives in question on litter temperature [Pintaric and Dobeic 2000, Allamamaly and Drive 2001] and animal welfare. Sometimes only viability, mortality and leg score were investigated [Maurice *et al.* 1998, McWard and Taylor 2000, Eleroğlu and Yalçın 2005].

The aim of the study was to determine the influence of brown coal and microbe vaccine added to straw litter on litter temperature and encrustation extent as well as on welfare and performance of turkey broilers.

## Material and methods

The research was conducted in three buildings (B-1, B-2, B-3) where BIG-6 turkey female broilers were fattened for 15 weeks in the autumn-winter period. In each building the area of the production sector was 550 m<sup>2</sup> and the number of birds was 6400. The type of feed and the way it was applied, as well as the watering system and the microclimate parametres (automatically controlled) were the same in all buildings. In B-1 the birds were kept on straw with the additive of fine brown coal from Brown Coal Maine, Sieniawa. In B-2, starting from week 4 of fattening, litter was sprayed twice a day with EM-1 (EMRO, Japan) microbe vaccine solution (200 ml/10 l water). B-3 was a control object and no litter additives were applied.

During the experiment thermo-vision photographs of the surface and the inner layers of litter were taken. Photos of the first series were taken 24 hours after spreading litter in the buildings, but before starting heating (term "0"). Next, the series of photos were taken once a week (terms I-XV). During each shooting session the surface photos were taken of four places and photos of the exposure of two places in each building. Photos were taken on the same day of the week and at the same hour to avoid the effects of works conducted on farm, mainly related to spreading the fresh litter.

In each building, two hours before the shooting session, four hardboard rings (1.9 m in diameter) were placed on the litter. Their role was keeping birds outside, and thus stabilizing the litter temperature.

The photos were taken from the height of 1.3 m with thermo-graphic camera V–20 II, (VIGO System S.A) with thermal division NETD 0,05°C. Temperature values for the areas covered by the photo  $[0,9 \text{ m}^2]$  were read using photographs programme THERM V-20 (Tab. 1).

Once a week the extent of surface encrustation of beddings was determined. The evaluation was done on six chosen plots, 1 m<sup>2</sup> each, according to the following scale:

0 - no encrustation;

- 1 ring of crust around watering and feeding troughs, about 20 cm wide;
- 2 as above and the zone of crust along gable walls, about 50 cm wide;
- 3 flexible encrustation invisible, but detectable by feet (<1 cm thick) on 50% of the bedding surface;
- 4 as above, but on 80% of the bedding surface;
- 5 as above, but on 100% of the bedding surface;
- 6 encrustation still invisible, but firmly detectable by feet (about 5 cm thick);
- 7 visible patches of encrustation on 50% of the surface;
- 8 as above, but on 100% of the surface;
- 9-100% of the surface covered with compact crust about 7 cm thick;

10 - as above but the crust about 10 cm thick.

During the period of fattening the status of turkeys' legs was also assessed. The observations were carried out on 60 birds randomly chosen in each building once a week. The following pathological changes were taken under consideration: dystrophy of thigh muscles, deformations of toes, swellings and inflammatory statuses of joints, rachitic changes.

At the end of experiment material and fattening costs were compared among buildings as well as production results calculated (mortality, the final live weights).

#### Statistical analysis

A main aim of statistical evaluation of rough material was to identify the differences between three types of litter and to assess their effect on the litter surface temperature, extent of litter surface encrustation and the health status of birds' legs.

The experimental data of the litter surface temperature (a measurable variable of continuous type) were analysed using a one-way ANOVA method for each fixed week separately, where a type of litter as the examined factor arises at three levels: the litter with brown coal (B-1), the litter treated by microbe vaccine (B-2) and the litter without additives as control level (B-3) – Rao [1982].

Each of the  $H_i$  hypotheses about lack of litter effect (for i-th fixed week i=1,2,...12) can be verified under the established assumptions of the model, using adequate test statistics, which under true  $H_i$  hypotheses have the Snedecor's F-distribution with appropriate degrees of freedom [Rao 1982].

To obtain a correct and reliable result of statistical analyses the assumptions about the ANOVA model have to be verified by using a test for the normality of model residuals and by using a test for variance homogeneity [Scheffé 1959, Levene, 1960]. For more detailed differences between three types of litter we used Tukey's test of homogenous groups. The Tukey's test of multiple comparisons (HSD test as measure of Student range) makes it possible to separate groups of similar effects, so called, homogenous groups at fixed significance level (Tab. 2). The results obtained in the study for a qualitative feature (extent of litter surface encrustation, scale 1-10) and for a variable describing the health status of turkeys' legs (as % of "sick legs"), were analysed statistically using the methods of nonlinear regression and multiple comparisons [Dąbrowski *et al.* 1997; Encyclopaedia of Biostatistics, 1998]. Additionally, the methods of nonlinear regression and multiple comparison for all weeks simultaneously were applied. In this approach we compared the regression curves corresponding to the different types of litter with appropriate confidence regions at fixed confidence level (0.95).

The comparative analysis is somewhat analogous to Scheffé's method of multiple comparison [Scheffé 1959]. It was assumed that if the confidence regions of the corresponding curves of regression at fixed confidence level for a common interval of variable t (week) were not overlapping, then the examined traits must have been statistically dependent on types of litter (Fig. 2, 4, 5).

Numerical calculations were carried out using the STATISTICA ver. 8.0 software package, supplemented with procedures developed by the authors.

## **Results and discussion**

The analysis of thermo-graphic photos showed that in "0" series the mean temperatures of litter in B-1, B-2 and B-3 were very similar. The effect of coal addition to the litter on the value of litter temperature was noticeable even in the first week of fattening. Litter temperature in B-1 was by 5,8 K higher than in B-3. The increase of temperature is a favourable phenomenon from the rearing point of view, particularly in the early weeks of fattening when birds have higher thermal demands while their adaptation abilities are weak. In the succeeding weeks, until the end of fattening period, the mean values of the temperature of the upper layer of litter were still higher in B-1 than in B-3, but the differences were smaller and varied from 1.7 to 5.3 K (Fig. 1).

In B-2 the measurements started from series 4 (*i.e.* on day 6 after the microbe vaccine EM-1 was first used). In this object, the same as in B-1 throughout the whole period of fattening, bedding surface temperature was higher than in the control B-3, the differences ranging from 0.1 to 4.3 K.

The comparison of means of litter temperatures in buildings where additives were applied showed that in B-1 they were higher (except for week V) by 0.68-2.92K than in B-2. The results of one-way analysis of variance imply that we reject all hypotheses H<sub>i</sub> about lack of litter effects for i-th fixed week i=1, 2, ..., 12 (*i.e.* for succeeding weeks: IV, V, XV) at significance level P <10<sup>-6</sup> or weeks IV, V, VIII, X, XI , and next for week XIV – <10<sup>-5</sup>, for week IX – P<10<sup>-4</sup>, for weeks VI, XIII, XV – <10<sup>-3</sup>, for week VII – P< 0.006 and at significance level P<0.02 for week XII. It means that differences between effects of three types of litter were significant and for all weeks the mean surface temperatures of litter with brown coal (B-1) were significantly higher than in the control B-3 (Fig. 2, Tab. 1, 2). It was additionally proved by using

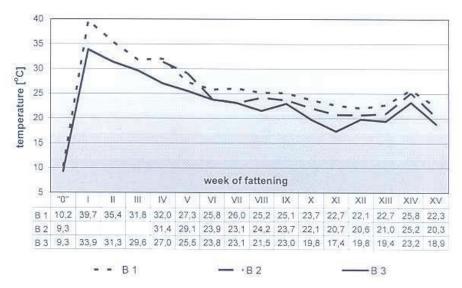


Fig. 1. Mean temperature for litter supplemented with brown coal (B-1), microbe vaccine EM-1 (B-2) and without additives (B-3) in succeeding weeks.

	Building								
Week	B-1			_	B-2		B-3		
	mean	SD	SE	mean	SD	SE	mean	SD	SE
IV	31.96	0.97	0.39	31.36	0.38	0.16	27.04	0.55	0.22
V	27.27	0.91	0.37	29.07	0.48	0.20	25.46	0.61	0.25
VI	25.77	0.37	0.15	23.86	1.23	0.50	23.83	0.38	0.15
VII	26.04	1.36	0.56	23.07	2.23	0.91	23.07	0.60	0.24
VIII	25.22	0.53	0.22	24.16	0.62	0.25	21.55	1.01	0.41
IX	25.07	0.91	0.37	23.66	0.39	0.16	22.96	0.26	0.11
Х	23.70	0.22	0.09	22.05	0.17	0.07	19.83	0.46	0.19
XI	22.66	0.53	0.22	20.71	0.52	0.21	17.37	1.41	0.57
XII	22.11	0.67	0.27	20.64	1.84	0.75	19.79	0.45	0.18
XIII	22.72	1.53	0.63	20.96	0.83	0.34	19.41	1.10	0.45
XIV	25.83	0.49	0.20	25.17	0.23	0.10	23.22	0.89	0.36
XV	22.32	0.26	0.11	20.27	1.04	0.42	18.92	1.85	0.75

 Table 1. Basic statistics of temperature (°C) for litter supplemented with brown coal (B-1), microbe vaccine EM-1 (B-2) and control (B-3) in each fixed week of experiment

SD- standard deviation, SE- standard error of mean (all statistics were calculated for independent measurements n = 6).

the polynomial regression and the multiple comparison of confidence regions which for the litter B-1 and for the litter B-3 are separate (a lack of common points) in the whole period of process (Fig. 2) at confidence level equal 0.95. The mean effects of the surface temperature of the litter sprayed with EM-1 (B-2) in comparison with the

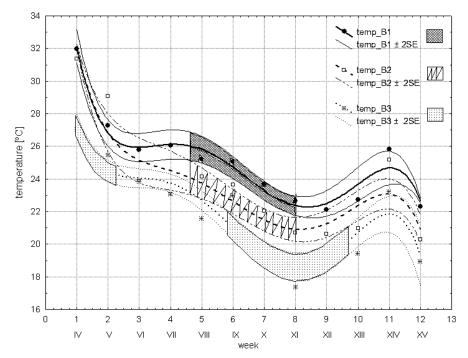


Fig. 2. Polynomial curves (5-th degree) with experimental data of the mean temperature for litter supplemented with brown coal (B-1), microbe vaccine EM-1 (B-2) and without additives (B-3) and their 95% - confidence regions - approx. delimited by intervals (temp -2SE; temp +2SE) for each week.

Week	Homogenous groups in descending order	Calculated significance level (so-called P-value)			
IV	(B1; B2) > B3	0.00018			
V	B2 > B1 > B3	0.00127; 0.00125 0.00018			
VI	B1 > (B2; B3)	(0.002; 0.0017)			
VII	B1 > (B2; B3)	0.0122			
VIII	B1 > B2 > B3	(0.066; 0.00023) 0,00018			
IX	B1 > (B2; B3)	(0.003; 0.00022)			
Х	B1 > B2 > B3	0.00018			
XI	B1 > B2 > B3	(0.0062; 0.00021) 0.00018			
XII	B1 > B3	0.0094			
XIII	B1 > B3 B1 > B2	0.0008 0.054			
XIV	(B1; B2) > B3	(0.0002; 0.0003)			
XV	B1 > (B2; B3)	(0.028; 0.0008)			

 Table 2. Homogenous groups for types of litters (B-1, B-2, B-3)

 by using HSD Tukey's test of multiple comparisons

litter without additives (B-3) were significantly higher only for weeks IV, V, VIII, X, XI, and XIV (Tab. 1 and 2). Similar results we obtained by using nonlinear regression method: 95% confidence regions for the control (B-3) are not overlapping with ones corresponding to the litter with microbe vaccine (B-2) in time intervals IV and V and IX and XII. The differences between B-1 and B-2 occurred significant in the following cases: in the building B-1 the average surface temperatures of litter were higher than in building B-2 for weeks: VI, VII, VIII, IX, X, XI, XIII, XV and essentially lower for week V (Tab. 2). From results of nonlinear regression models the differences were confirmed only for weeks VIII–XI.

The increase in litter temperature after applying mineral and bioenzymatic additives was also observed by Pintaric and Dobeic [2000]; chemical additives do not changed this factor [Allamamaly and Drive 2001, Pourreza *et al.* [2004].

Decay processes of organic matter are exothermic reactions. The values of temperatures read from the photos of the exposures gave the additional possibility of comparing the intensity of the processes taking place in the tested bedding (Fig. 3). The values of the maximum temperatures read from thermo-vision photos of litter in exposures had similar distribution to the values of mean temperatures of the surface of litter. During the whole production cycle, higher values were noted in exposures made in buildings B-1 and B-2 than in B-3 (differences between B-1 and B-3 ranged from 1.0 to 8.2 K, while those between B-2 and B-3 from 1.0 to 4.0 K). The comparison of maximum temperatures in exposures for litter with additives showed, that in B-1 (apart from weeks V, VI, IX) they were higher than in B-2 (differences ranging from 0.6 and 7.0 K).

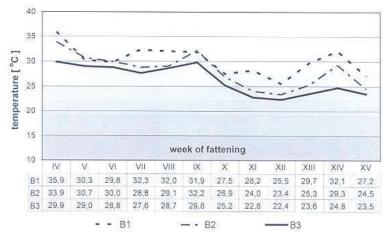


Fig. 3. Litter temperature maximum values in exposures for litter supplemented with brown coal (B-1), microbe vaccine EM-1 (B-2) and without additives (B-3) in succeeding series.

Higher temperatures of litter upper layers as well as exposures in buildings B-1 and B-2 compared to control object, indicated that mineralization processes were more intense in litter with additives to raise their quality as a fertilizer, in accordance with Słobodzian-Ksenicz *et al.* [2007]

In the buildings, where the experiment was conducted, microclimate parametres were automatically controlled and thus it was not possible to find how the rise in bedding temperature affected the temperature of air. The rise in litter temperature may also contribute to the lowering of the use of energy required to heat the building. Unfortunately, a heating system used on the farm was common for all buildings and it did not give a possibility to check differences between objects. It should be a subject of further studies.

Litter additives used in this experiment improved the microclimate parametres in the building. They lowered  $CO_2$  and  $NH_3$  air concentration as well as the relative air and litter humidity (full results will be given in forthcoming report).

The quality of litter affects the health status of birds and production results. A serious problem in turkey farms is fast "encrustation" of litter surface. The extent of encrustation is one of the factors causing toe and leg diseases [Weaver and Meijerhof 1991]. Introducing of additives to the litter slowed down the encrustation rate (Fig. 4) and thus improved the well-being of the birds. In the 10-degree scale of encrustment suggested by Słobodzian-Ksenicz [2002], litter with brown coal showed the slowest rate of crust formation (the 9th level in week XV). A little faster encrustation was noted in litter with EM-1 (the 10th level in week XIV) while in B-3 the 10th level of encrustation was noted in week X of fattening.

Type of litter	Model	Regression coefficient			Determination coefficient	Standard error of estimation	
		а	b	с	$(R^2, \%)$		
B-1	$f(t) = \frac{a}{1 + be^{-ct}}$	7.844	7.817	0.523	96.61	0.49	
В-2		9.974	3.972	0.367	98.55	0.32	
B-3		10.426	2.267	0.413	95.30	0.50	

 Table 3. Equations of logistic regression model describing the changes of litter surface encrustation for three types of litter: B-1, B-2 and B-3.

The changes in encrustation process of litter surface are of a two-stage character and can be described by logistic curves (determination coefficients above 95% and small standard errors of estimation – Table 3). The 95% confidence regions corresponding to the logistic curves explicitly show significant differences in dynamics of processes for litter of B-1 and B-3. The changes in degree of encrustation of litter surface of B-2 were slower than for control litter B-3 and statistically different in time from week V to week X. Besides, the changes for the litter B-2 were slightly faster than for the litter B-1 and statistically greater starting from week XIV (Fig. 4).

The evaluation of health status of turkeys' legs showed that earliest pathologies occurred in B-3 broilers – in 100% of birds randomly chosen for assessment, in week 8

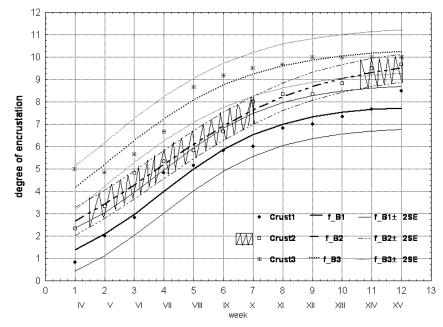


Fig. 4. Logistic curves with experimental data of extent of litter surface encrustation for three types of litter -B-1, B-2 and B-3 – with 95% confidence regions.

of fattening. In B-2 malformations in 100% of birds randomly chosen for observation were found in week 13 of fattening, while in B-1 the same happened only in week 15.

The evaluation of health status of turkeys' legs was carried out in consideration of character of this process by using logistic regression similarly to degree of encrustation of litter surface (Tab. 4). With respect to higher standard errors of estimation than for the encrustation process we obtained only significant differences between the litter B-1 and the control litter B-3 in the interval [V and IX] and between the litter B-2 and the litter B-3 in the interval [VI and VIII] – Figure 5.

 Table 4. Equations of logistic regression model describing the changes of the health status of turkeys' legs (in %) for three types of litter: B-1, B-2 and B-3

Type of litter	Model	Regres	sion coe	fficient	Determination coefficient	Standard error of estimation	
		a	b	с	(R <sup>2</sup> ,%)		
B1		84.990	7.754	0.536	91.71	8.58	
B2	$f(t) = \frac{a}{1 + be^{-ct}}$	98.515	3.331	0.367	92.36	7.36	
В3		97.698	6.307	1.111	92.89	6.61	

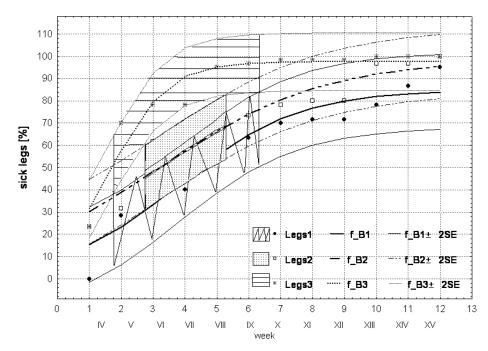


Fig. 5. Logistic curves with experimental data of health status of turkeys' legs for three types of litter: B-1, B-2 and B-3 with 95% confidence regions.

Suffering birds moved towards watering and feeding troughs with difficulties. This probably affected their final body weight – those kept on litter with coal (B-1) had the highest final weight (9.93 kg/individual) and those kept on litter with vaccine (B-2) had little lower (9.83 kg/individual) but it was still higher than for the control birds (B-3) kept on straw without additives (9.68 kg/individual). Final broiler live weight production achieved on bedding with coal was by about 650 kg, and on bedding with vaccine EM-1 by about 390 kg higher than on litter with no additive.

Improved production by applying litter additives was reported also by Kadam *et al.* [2003], Bennett *et al.* [2005], Eleroğlu and Yalçın [2005], and Tacconi *et al.* [2007], while Maurice *et al.* [1998], Allamamaly and Drive [2001], and Nahm [2004] did not notice any improvement.

During the whole production process, the lowest mortality occurred in B-1 (2.6%), a little higher in B-2 (3.2%) and the highest in B-3 (3.7%), which confirms the thesis that the introduction of additives to the litter had a positive effect on health and welfare of birds, reported also by Nahm [2005] and Tacconi *et al.* [2007].

The costs of purchasing and applying additives are difficult to calculate. Although brown fine coal is considered mining waste and its price is relatively low (less than  $27 \notin$ , with the demand of 1t/100 m<sup>2</sup> of area) the cost of freight must be calculated and

it may be substantial. The price of vaccine and medium is not high (about  $27 \notin$  per one production cycle) but there are additional costs: the cost of a spray canister and barrels (between 130 and  $270 \notin$ ) and labour costs – the preparation and application of vaccine (breeding bacteria, conditioning of vaccine, preparation of solution, spraying, washing the equipment) – it takes around 150 hours per one production cycle.

From the above it may be concluded that the use of EM-1 generates low costs, but is time-consuming. The profitability of the use of coal depends in practice mainly on the distance from the farm to the mine and the cost of applying this additive can be relatively high.

The results presented here can be summarized as follows.

Both additives introduced to straw litter – brown coal and microbe vaccine EM-1 – caused the significant rise in bedding temperature. Bedding with brown coal led to significantly higher litter temperature than bedding with microbe vaccine EM-1 from week VI to XI. Based upon results of nonlinear regression models the differences were confirmed only for weeks VIII-XI. The litter without additive reached the higher extent of encrustment sooner than litter with brown coal (significant differences in the whole production period) and than litter with EM-1 (significantly different during week V to week X). The use of additives decreased the incidence of pathological changes in birds' legs. The significant differences were found between the litter with brown coal and the control in the interval V and IX and between the litter with EM-1 and the control in the interval VI and VIII. Broilers kept on litter with coal reached the final body weight by 0.25 kg/bird higher, and those kept on litter with vaccine EM-1 by 0.15 kg/bird higher than birds kept on litter without additives. Mortality in building with brown coal was by 1.1 per cent points (pp) lower and with EM-1 by 0.5 pp lower than in control object.

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# Wpływ dodatku miału węglowego i szczepionki przeciwbakteryjnej do ściółki na jej jakość i wyniki produkcji broilerów indyczych

#### Streszczenie

Doświadczenie przeprowadzono na trzech grupach brojlerów indyczych utrzymywanych w trzech budynkach. W budynku B-1 ptaki utrzymywano na ściółce z dodatkiem miału węglowego, w budynku B-2 ściółkę zamgławiano dwa razy dziennie roztworem szczepionki bakteryjnej EM-1, a w budynku B-3 nie stosowano dodatków, traktując go jako kontrolny. Wartości temperatury wierzchniej i głębszych warstw podłoży odczytywano z wykonanych zdjęć termograficznych. Stopień zaskorupienia powierzchni podłoża i stan ptasich nóg oceniano raz na tydzień. Po zakończeniu tuczu określono wagę końcową i śmiertelność (%) ptaków. Oba testowane dodatki spowodowały istotny wzrost temperatury podłoża, co dodatnio wpłynęło na jego cechy fizyczne, podniosło dobrostan ptaków i poprawiło wyniki produkcyjne (niższa śmiertelność, wyższa końcowa masa ciała).